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Heterogeneity of Genome Rearrangements in Rotaviruses Isolated from a Chronically Infected Immunodeficient Child

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Rotaviruses with genome rearrangements, isolated from a chronically infected immunodeficient child, were adapted to growth in BSC-1 cells. Preparations of viral RNA from fecal extracts showed a mixed atypical rotavirus RNA profile, which was due to the presence of at least 12 subpopulations of viruses grossly differing in genotype. Besides various forms of genome rearrangements involving segment 8-, 10-, and 11-specific sequences, reassortment in vivo was likely to have occurred during the emergence of these viruses. The protein products of viral genomes with various forms of segmental rearrangements seemed to be largely unaltered. Genome rearrangement is proposed to be a third mechanism directing the evolution of rotaviruses.

Human rotaviruses, the main viral cause of infantile gastroenteritis, occur as numerous genomic variants (electropherotypes) which are either isolated sequentially or circulate at the same time (13, 14, 17, 46). Rotaviruses have a genome consisting of 11 segments of double-stranded RNA (dsRNA) which have been separated on polyacrylamide gels to yield characteristic profiles (13, 14, 46). Most human rotaviruses are group A rotaviruses (39), and at least two, or possibly three, subgroups and five serotypes have been differentiated (25, 30, 33). Rotaviruses lacking the group A antigen and showing "atypical" RNA profiles have also been described and have been classified as groups B to E (6, 39, 40). Group B rotaviruses have been found to cause major outbreaks of acute diarrhea among adults in different parts of China (7, 27, 44, 52).

Within group A rotaviruses atypical RNA profiles have also been observed. Pedley et al. (41) described the genomes of rotaviruses obtained from chronically infected, immunodeficient children; in these viruses normal RNA segments were missing from the profile, and various bands of dsRNA were found which probably are concatemeric forms of segment-specific sequences. Similar observations were reported by others (8, 12), and rotaviruses with genome rearrangements also emerged in vitro after serial passage at a high multiplicity of infection (26). Rearranged bands of genomic RNA were found to replace the normal RNA segments both structurally and functionally (2). In addition, group A rotaviruses with atypical RNA profiles have been found in immunocompetent children, either accompanied by diarrhea (1, 33) or without clinical symptoms (5), and in calves (43; G. S. Scott, M. A. McCrae, and D. H. Pocock, personal communication) and rabbits (50). Genome heterogeneity was observed in rotaviruses isolated from cattle (47) and from monkeys (42) and found to be due to the presence of different subpopulations of viruses in individual isolates.

The evolution of viruses exhibiting this high degree of genome variability is not clear. Several mechanisms can be considered to explain all these findings. (i) Rotaviruses possess a virus-coded, virion-associated, RNA-dependent RNA polymerase which may be error prone like other viral RNA polymerases and could provide numerous mutants in a short period of time (9, 23, 24, 48). In support of this, sequential point mutations have been demonstrated in isolates obtained within months of a local outbreak (16). (ii) Since rotaviruses have segmented RNA genomes, reassortment of the segments of different strains after coinfection of cells occurs readily in vitro (21, 36) and in vivo (19). (iii) Rotaviruses can mutate by genome rearrangements (2, 26, 41), the mechanism(s) of which is under investigation. (iv) A combination of these mechanisms can be operative (2, 20).

In this report we describe extensive genome heterogeneity of rotaviruses in a single individual, immunocompromised host and show that this heterogeneity is mainly due to various forms of genome rearrangement (and possibly reassortment) events. The genomic variants seem to coexist in a constantly varying (dynamic) equilibrium (9, 23, 24).

MATERIALS AND METHODS

Cells and viruses. BSC-1 cells (obtained from Microbiological Associates) and MA104 cells (obtained from Flow Laboratories) were grown in roller cultures in Eagle minimum essential medium supplemented by 10% fetal calf serum.

Rotavirus specimens were obtained from a chronically infected child suffering from cartilage hair hypoplasia (35) which was accompanied by a cellular immunodeficiency (22, 31). At the age of 11 months the child developed a thrombocytopenic purpura and autoimmune hemolytic anemia with leukopenia. The T lymphocytes (OKT3) were proportionally decreased and responded very poorly to phytohemagglutinin and pokeweed mitogen. Poliovirus immunization was followed by shedding of poliovirus type 2 during a period of 4 months. Although the numbers of B lymphocytes and immunoglobulin levels seemed to be normal, the child was receiving weekly injections of gamma globulins. The symptoms of chronic diarrhea persisted from January 1985, and a fecal specimen obtained in February 1985 contained rotavirus. Feces were regularly collected at 2- to 3-week intervals for over 1 year; all were positive for rotaviruses and astroviruses by electron microscopy. The rotaviruses shared the common group A antigen (as shown by positive reaction in the enzyme-linked immunosorbent assay of Abbott Lab-

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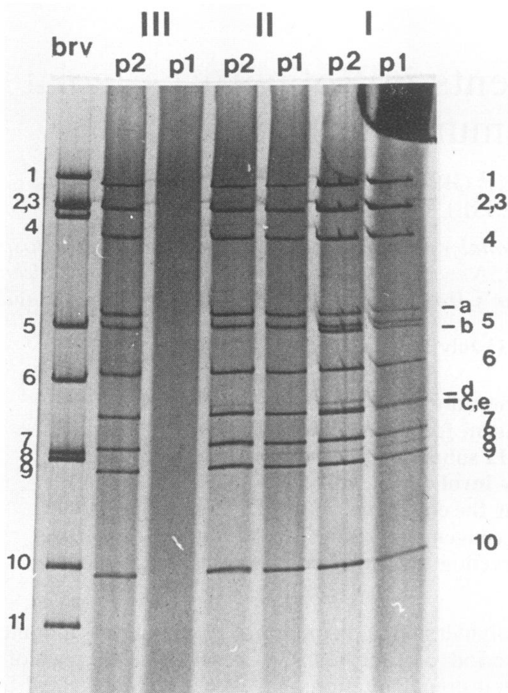

FIG. 1. RNA profiles of human rotaviruses obtained from two consecutive passages (p1, p2) on BSC-1 cell roller cultures of fecal specimens I, II, and III, collected on days 272, 301, and 364, respectively, after onset of chronic infection in an immunodeficient child. The RNAs of tissue culture supernatants were extracted and separated on a 7.5% polyacrylamide gel with the Laemmli buffer system. The gel was silver stained. The RNA of tissue culture-adapted bovine rotavirus (brv) was coelectrophoresed as a control. RNA segments of 1 through 11 of bovine rotavirus are indicated on the left, and RNA segments 1 through 10 and RNA bands a through e of human rotaviruses are indicated on the right.

oratories). More details of the case history have been published elsewhere (3).

Virus was obtained in the supernatant of 10% suspensions of feces in phosphate-buffered saline after low-speed centrifugation and semipurified by ultracentrifugation through a 30% sucrose cushion as described previously (15). Growth experiments on roller cultures of BSC-1 cells were made with semipurified virus after activation with trypsin and filtration through Millipore 0.45-μm filters as described previously (53). Plaque-to-plaque isolation of viruses was as described previously (2, 26).

Electron microscopy. Virus particles were pelleted from preclarified tissue culture supernatants by ultracentrifugation at 100,000 × g for 30 min, suspended in a small volume (10 to 15 μl) of water, mixed with an equal volume of 3% phosphotungstic acid (pH 6.5), mounted on carbon-collodium grids, and examined in a Philipps 201C electron microscope.

Subgroup and serotype determination. Subgroup and serotype of isolates were determined by using subgroup-specific monoclonal antibodies and type-specific polyclonal antisera as specific reagents (17).

RNA extraction and polyacrylamide gel electrophoresis. The RNA was extracted either from semipurified virus or from 0.5-ml samples of crude tissue culture supernatants (15). Polyacrylamide gel electrophoresis was either on 2.8% polyacrylamide–6 M urea slab gels with Loening buffer (15) or on 7.5% polyacrylamide slab gels with the Laemmli (29) discontinuous buffer system (2).

Northern blotting (RNA blotting) and hybridization. Viral RNAs were separated on polyacrylamide gels and electroblotted, and the blots were hybridized to radiolabeled segment-specific cDNA probes obtained by random priming of isolated rotavirus RNA segments (49) in a reverse transcriptase reaction as described previously (2, 32).

Radiolabeling of virus-coded proteins. Virus-infected cells were labeled with [^{35}S]methionine (Amersham International Ltd.; specific activity, 900 Ci/mmol) and separated on 15% polyacrylamide gels as previously described (26).

RESULTS

Genome heterogeneity of individual rotavirus isolates. Of a series of 16 isolates, 3, obtained on days 272, 301, and 364 after the onset of illness, were concentrated and semipurified by differential and ultracentrifugation, activated with trypsin, and inoculated onto BSC-1 cell roller cultures. After two passages all three isolates grew well and showed atypical and mixed RNA profiles (Fig. 1). RNA segment 11 was missing from all profiles, and additional bands of dsRNA (bands a through e, Fig. 1) were found in abnormal positions and in different intensities. The RNA patterns differed between isolates with respect to segments 8 and bands b and d (Fig. 1). The atypical RNA profiles of rotavirus of the second

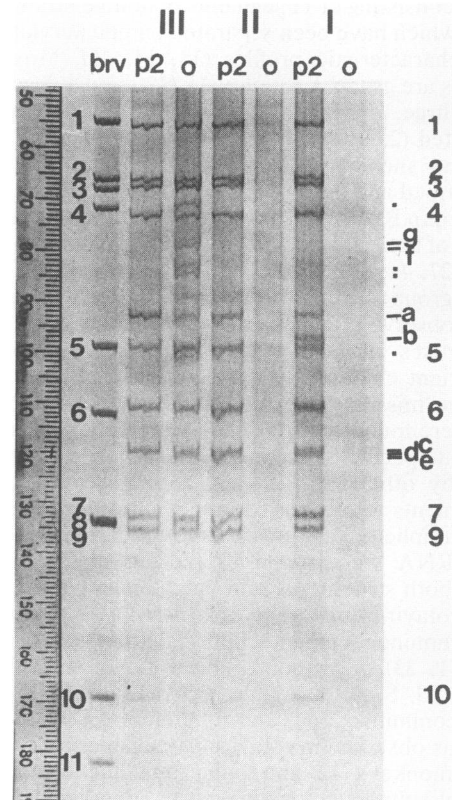

FIG. 2. RNA profiles of rotaviruses of original fecal extracts (O) and passages 2 (p2) of specimens I, II, and III. Separation was on a 2.8% polyacrylamide–6 M urea slab gel with Loening buffer. The gel was silver stained. RNA segments 1 through 11 of bovine rotavirus (brv) are indicated on the left, and RNA segments 1 through 10 and additional RNA bands a through g of human rotaviruses are indicated on the right. Additional RNA bands never observed in plaque isolates are labeled (●).

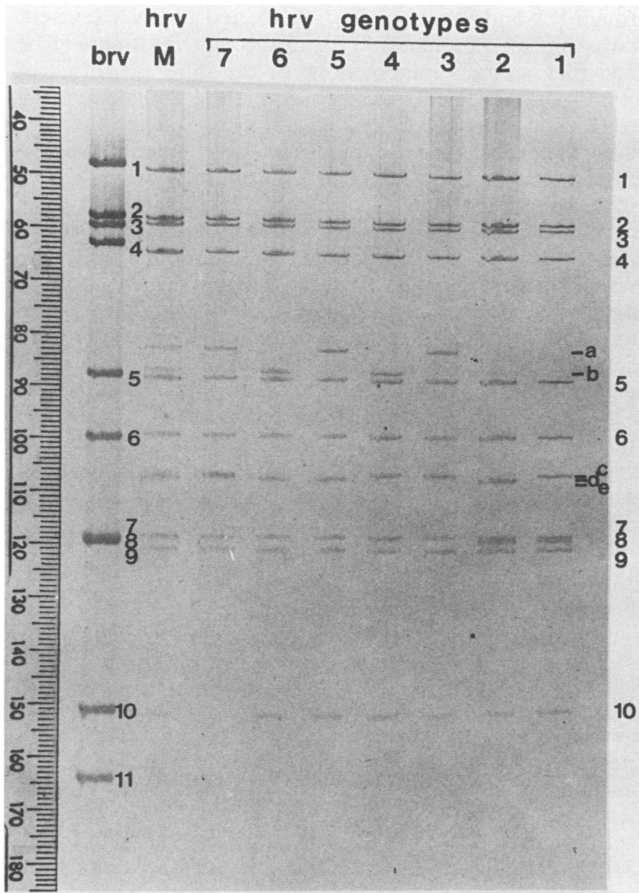

FIG. 3. RNA profiles of human rotavirus (hrv) mixture (M) in the supernatant of the second passage of isolate I and of seven rotavirus clones of genotypes 1 through 7 purified by plaque-to-plaque isolation three times from that passage. Plaque isolates were amplified in roller cultures of BSC-1 cells before RNA extraction. Separation on gel and staining was as described in the legend to Fig. 2. RNA segments 1 through 11 of bovine rotavirus (brv) RNA (control) are indicated on the left, and RNA segments 1 through 10 and bands a through e of human rotaviruses are indicated on the right.

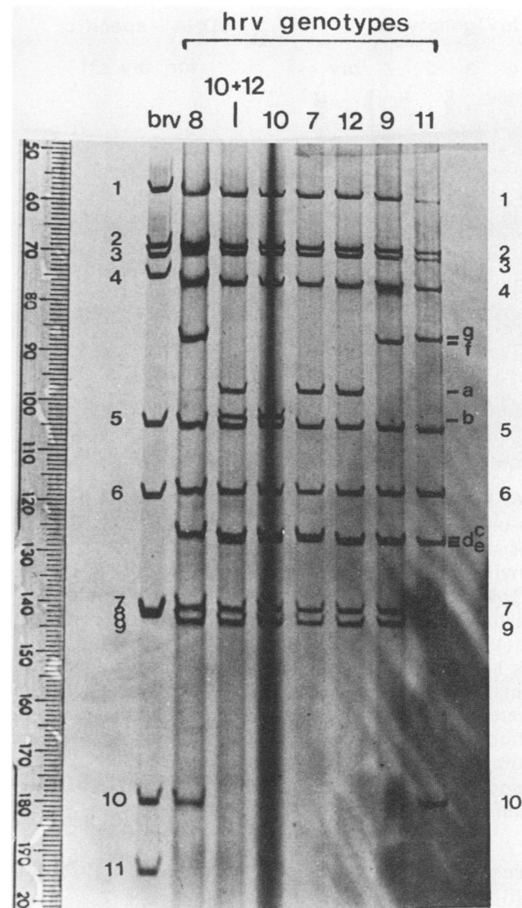

FIG. 4. RNA profiles of human rotavirus (hrv) genotypes 7 through 12. RNA extraction, gel electrophoresis, and staining were as described in the legend to Fig. 3.

passage were similar although not identical to those obtained from original fecal extracts (Fig. 2). The RNA profiles of the second passages presented fewer additional bands than the original extract (isolate III, Fig. 2). The chronological series of RNA profiles of original fecal extracts was characterized by periodic changes in the positions and intensities of rearranged bands (results not shown) and in this respect was very similar to the series published earlier by Pedley et al. (41).

Plaques were produced from serial 10-fold dilutions of the first and second passages of the three isolates. Viruses obtained from single plaques were twice more plaque-to-plaque purified and then amplified under liquid overlay, and the progeny were analyzed by RNA polyacrylamide gel electrophoresis described previously (15). Numerous different genotypes were found (Fig. 3 through 5).

The gene derivation of rearranged RNA bands a through g (Fig. 3 and 4) was established by Northern blot analysis (Fig. 6 and 7). Segment 11-specific sequences were contained in rearranged bands c and e (Fig. 6), segment 10-specific sequences were in band d (Fig. 7), and segment 8-specific

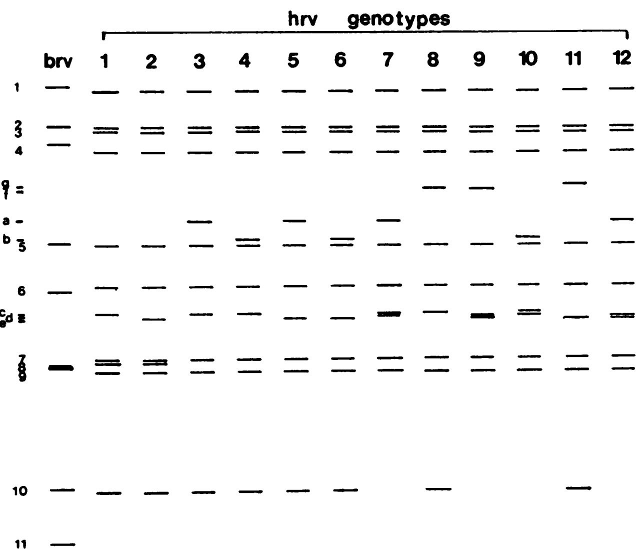

FIG. 5. Diagram of RNA profiles of the human rotavirus (hrv) genotypes 1 through 12 and of bovine rotavirus (brv) (as a control) shown in Fig. 3 and 4.

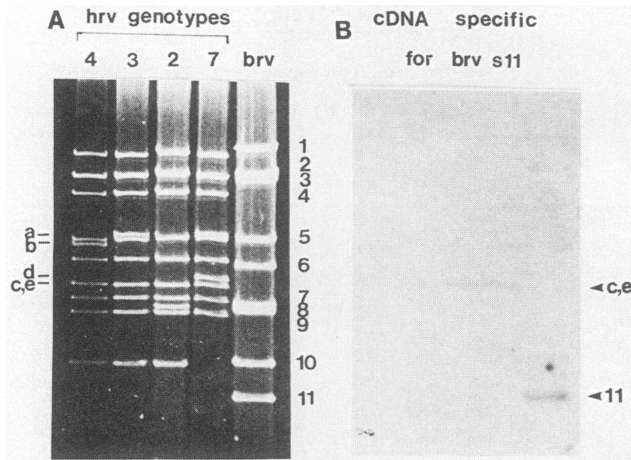

FIG. 6. Segment derivation of additional bands of dsRNA in the genomes of human rotavirus (hrv) genotypes as tested by hybridization of segment-specific radiolabeled cDNA probes to Northern blots. Bovine rotavirus (brv) and human rotavirus RNAs of genotypes 2, 3, 4 and 7 were separated on a 7.5% polyacrylamide gel with Laemmli buffer. In panel A, the gel was stained with ethidium bromide (1 μg/ml). The positions of RNA segments (designating segments 1 through 11 of bovine rotavirus and segments 1 through 10 of human rotavirus) and of additional RNA bands (a through e) are indicated. The gel was electroblotted onto Biodyne membrane and hybridized to ^{32}P-labeled cDNA specific for RNA segment 11 of bovine rotavirus (2, 32). Hybridization occurred to RNA segment 11 of bovine rotavirus (homologous control) and to RNA bands c and e of the human rotavirus genomes.

sequences were in bands a, b, f, and g (Fig. 7; data not shown for bands b and g).

From these data we concluded that there was extensive genome heterogeneity in the isolates. In clones that were plaque-to-plaque purified, 12 different genotypes were found (Fig. 3 through 5). RNA segment 11 was always replaced by band c or e; RNA segment 8 was either maintained (genotypes 1 and 2) or replaced by band a, b, f, or g (genotypes 3 through 12); and in genotypes 7, 9, 10, and 12 RNA segment 10 was replaced by band d (Fig. 3 through 7). It should be noted that various combinations of the different forms of genome rearrangements were observed (e.g., in genotypes 3 through 6 or genotypes 3, 4, 7 and 10; Fig. 5), suggesting that reassortment of viruses possessing rearranged bands of genomic RNA is likely to have occurred in vivo.

The sizes of the rearranged bands of genomic RNA were determined from Fig. 4 by using a plot of the sizes of the bovine rotavirus RNA segments (4, 28, 45) against the distances of migration as a calibration curve; thus band g contained approximately 1,930 base pairs (bp), band f contained 1,900 bp, band a contained 1,680 bp, band b contained 1,550 bp, and bands c, d, and e contained 1,250 bp. This indicated that the rearranged bands were approximately 1.56 (segment 11), 1.67 (segment 10), and 1.46 to 1.82 (segment 8) times the size of the original segments. Rotavirus particles carrying one or several rearranged segmental RNAs had between 450 and 1,790 additional bp packaged, but they were indistinguishable in size and shape (Fig. 8).

The serological characterization gave an interesting result: rotaviruses of all genotypes were unambiguously subgroup I, but none of the serotype-specific reference antisera (directed against types 1 to 4) reacted with the viruses. However, we assumed that the genotypes of these viruses were derived from a short electropherotype virus, since natural human subgroup I rotavirus isolates so far almost always had a slower migration of segments 10 and 11 (short electropherotype). We therefore considered bands c and e to be concatemeric forms of a segment 10 of the short electropherotype which is equivalent to segment 11 of the long electropherotype (10) and is approximately 800 bp in length (17), determined as described above for the rearranged bands a through g.

A total of 281 plaque isolates obtained from passages of the different isolates were genotyped, and the frequencies of genotypes in different isolates and passages were determined. Viruses with different rearrangements of segments 11, 10, and 8 and with different combinations of these rearrangements were all found to be part of the total popu-

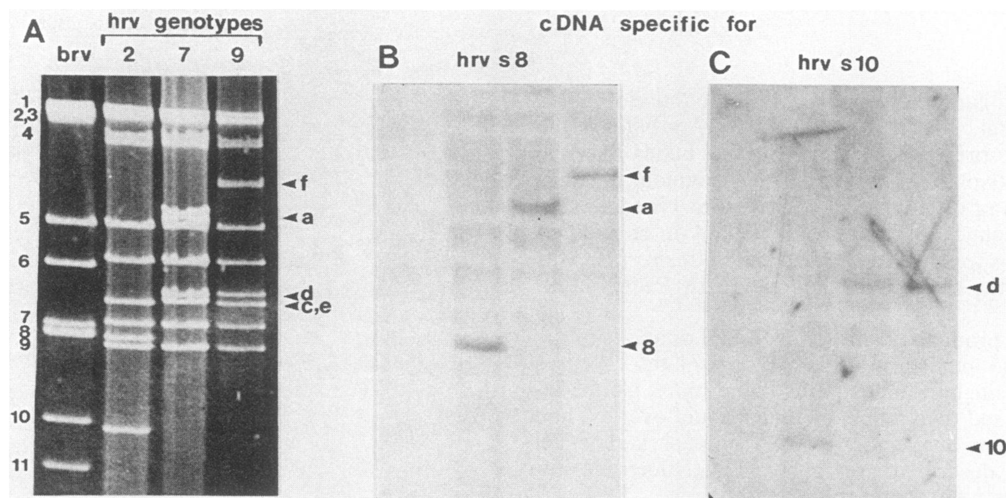

FIG. 7. Bovine rotavirus (brv) RNA and human rotavirus (hrv) RNAs of genotypes 2, 7, and 9 were separated on a 7.5% polyacrylamide gel which was stained with ethidium bromide (A). Positions of RNA segments and bands are indicated. The RNAs were electroblotted onto Biodyne membranes and hybridized to ^{32}P-labeled cDNA specific for RNA segments 8 (B) and 10 (C) of the human rotavirus genome. Segment 8-specific cDNA hybridized to itself (genotype 2) and to bands a and f of genotypes 7 and 9, respectively (B), and segment 10-specific cDNA hybridized to itself (genotype 2) and to RNA band d of genotypes 7 and 9 (C).

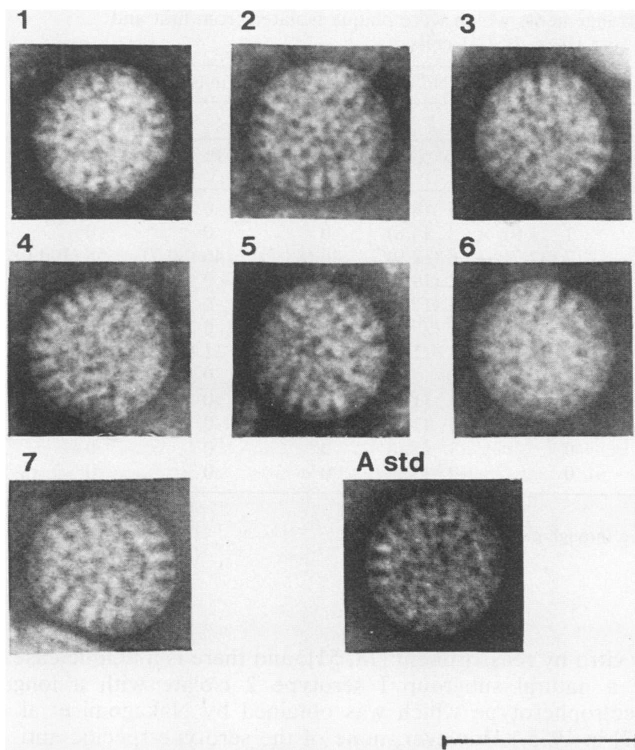

FIG. 8. Electron micrographs of double-shelled particles of human rotaviruses of genotypes 1 through 7 and of a human rotavirus with a standard genome of the long electropherotype (subgroup AII, serotype 1 [A std]). Bar, 100 nm.

lation of isolate I (Fig. 6 and 7 and Table 1). Isolates I and II also contained viruses with normal RNA segment 8 (genotypes 1 and 2, Fig. 6 and Table 1), and most viruses had a normal RNA segment 10 (Table 1). The result of the subpopulation analysis of Table 1 agrees well with the RNA profiles in Fig. 1: genotypes with normal RNA segments 8 were only found in passages 1 and 2 of isolate I and in passage 1 of isolate II (faint RNA segments 8, Fig. 1); band d rearrangement was only seen in passages of isolates I and II; band b rearrangements were only seen in passages of isolate I; and bands f and g were not seen in the RNA profiles of Fig. 1 and 2 (clones containing them were only very rarely found [Table 1]).

Of the 12 genotypes, 9 were exclusively found in isolate I; isolate II contained only 3 genotypes (nos. 1, 3, 7), and isolate III only a single one (no. 3) (Table 1). However, the original feces of isolate I showed no clear RNA pattern (i.e., only a hardly visible one on the gel presented in Fig. 2), and that of isolate II was weak (clearly visible on the original gel presented in Fig. 2), whereas isolate III contained all of the abnormal RNA bands that were found in plaques of isolate I and additional abnormal RNA bands (marked by dots in Fig. 2) that were never found in any of the viable plaque isolates. Therefore the distribution of genotypes in the different isolates reflects the amount of rescuable virus in the original specimens rather than a change in frequencies of certain genotypes in vivo.

It was estimated that frequencies of different genotypes in passages 1 and 2 of different isolates were not significantly different with the single exception of genotype 3, which was less frequently found in passage 2 of isolate I compared with passage 1 (Table 1). This suggests that the frequencies of the different genotypes in passages to a certain extent reflect their frequencies as viable particles in the original fecal specimen. Attempts to plaque purify trypsin-activated rotaviruses directly from pellets of fecal extracts were repeatedly made but remained unsuccessful.

Intracellular protein synthesis. Monolayers of BSC-1 tissue culture cells were infected at a multiplicity of infection of 2 to 5 with the 12 genotypes of human rotavirus (Fig. 5) and bovine rotavirus (as a control) and labeled with [^{35}S]methionine, and the infected cell proteins were separated on 15% polyacrylamide gels as described previously (26). The pattern of virus-coded proteins was very similar for all genotypes, with the exception of reproducible minor migrational differences of VP5 (Fig. 9).

DISCUSSION

Group A rotavirus with atypical RNA profiles have been repeatedly detected in feces of chronically infected immunodeficient children (8, 12, 41). Certain RNA segments are decreased in concentration or completely missing from the profile on gels, but there are additional bands of dsRNA containing segment-specific sequences in concatemeric forms (41). Despite many attempts these viruses grew very poorly on their own in MA104 cells, but they readily reassorted with superinfecting bovine rotavirus (tissue culture-adapted UK Compton strain; 2). Several rearranged bands of the human rotavirus genome replaced normal RNA segments of bovine rotavirus structurally and functionally in reassortants (2). We had noticed that reassortants which had RNA segment 11 of bovine rotavirus replaced by a rearranged band of human rotavirus grew very well on BSC-1 cells (20). This observation prompted us to make a further attempt to adapt human rotaviruses with genome rearrangements to growth in cultures of BSC-1 cells. We have been successful with several rotavirus isolates obtained from a chronically infected child, and the genomes of these rotaviruses were analyzed in this paper.

It turned out that the mixed RNA profiles of these viruses as seen in extracts from the original clinical specimens represented a composite pattern derived from extremely heterogeneous populations of rotaviruses. Due to the fact that the tissue culture-adapted rotaviruses plaqued well they could be distinguished as different subpopulations; 12 different genotypes were obtained which remained genomically stable after repeated plaque-to-plaque purification. The differences in genotypes involved sequence rearrangements of segments 11, 10, and 8 into RNA bands designated a through g. The RNA bands were 1.56 (segment 11), 1.67 (segment 10), and 1.46 to 1.82 (segment 8) times the size of the original RNA segments. These numbers are similar to those obtained earlier (26, 41). Thus virions had between 450 and 1,790 additional bp amounting to 2.4 to 9.6% of the standard genome size of 18,600 bp (4, 28, 45) packaged, resulting in no apparent morphological changes but in differences of the biophysical properties of virions (M. McIntyre, V. Rosenbaum, M. Desselberger, D. Wood, and U. Desselberger, J. Gen. Virol., in press). Genome rearrangements seem to occur mainly in genes encoding nonstructural proteins; however, rearrangements of segment 6-specific RNA sequences have been described (41). The rearranged bands probably contain no mosaic structures (F. Hundley and U. Desselberger, manuscript in preparation; unpublished data); work on cloning and sequencing rearranged bands of genomic RNA of selected isolates is in progress.

Since rearranged bands of three segment specificities were observed in various combinations in the different genotypes,

TABLE 1. Genotypes of 281 rotavirus clones with genome rearrangements which were plaque isolated from first and second passages of isolates I, II, and III on BSC-1 cells

Genotype no.[a]	Replacement[b] of RNA segment:							No. of clones (% of total) in isolate				
	11 by band:		10 by band d	8 by band:				I		II		III, passage 2[c]
	c	e		a	b	f	g	Passage 1	Passage 2	Passage 1	Passage 2	
1	+							5 (9.6)	5 (8.1)	2 (3.8)	0	0
2		+						1 (1.9)	3 (4.8)	0	0	0
3	+			+				17 (32.7)	8 (12.9)[d]	46 (88.5)	46 (80.7)	58 (100.0)
4	+				+			5 (9.6)	12 (19.4)	0	0	0
5		+		+				5 (9.6)	11 (17.7)	0	0	0
6		+			+			15 (28.8)	17 (27.4)	0	0	0
7	+		+	+				1 (1.9)	1 (1.6)	4 (7.7)	11 (19.3)	0
8	+					+		2 (3.8)	0	0	0	0
9	+		+			+		1 (1.9)	1 (1.6)	0	0	0
10	+		+		+			0	1 (1.6)	0	0	0
11		+					+	0	1 (1.6)	0	0	0
12		+	+	+				0	2 (3.2)	0	0	0

[a] Genotypes were numbered 1 through 12 (Fig. 5).
[b] +, Rearrangement event (replacement of RNA segments 8, 10, and 11 by bands a through g).
[c] No plaques could be isolated from first passage of isolate III.
[d] Frequency significantly lower than in first passage (χ^2 test, $P < 0.02$).

it is likely that various reassortment events had occurred in vivo in addition to and simultaneously with the rearrangements. This assumption is reasonable because numerous reassortants have been isolated at high frequencies after coinfection of mice with two different rotaviruses (19).

The serological results were very interesting: all genotypes were of subgroup I with subgroup-specific monoclonal antibodies in an enzyme-linked immunosorbent assay (17). Thus one would expect them to be serotype 2, which so far has been found in almost all naturally occurring human subgroup I viruses. (Human subgroup I serotype 1 and subgroup II serotype 2 viruses have recently been obtained in vitro by reassortment [18, 51], and there is a unique case of a natural subgroup I serotype 2 isolate with a long electropherotype which was obtained by Nakagomi et al. [37] in 1985.) However, none of the serotype-specific antisera (directed against types 1 to 4) reacted with the viruses described in this paper. Thus they could represent a new serotype, as was recently shown (51) for viruses of the suprashort electropherotype first described by Matsuno et al. (33). We are in the process of investigating this question.

Different genotypes occurred at different frequencies in several isolates; this finding reflected differences in viable viruses rather than the natural evolution of different virus

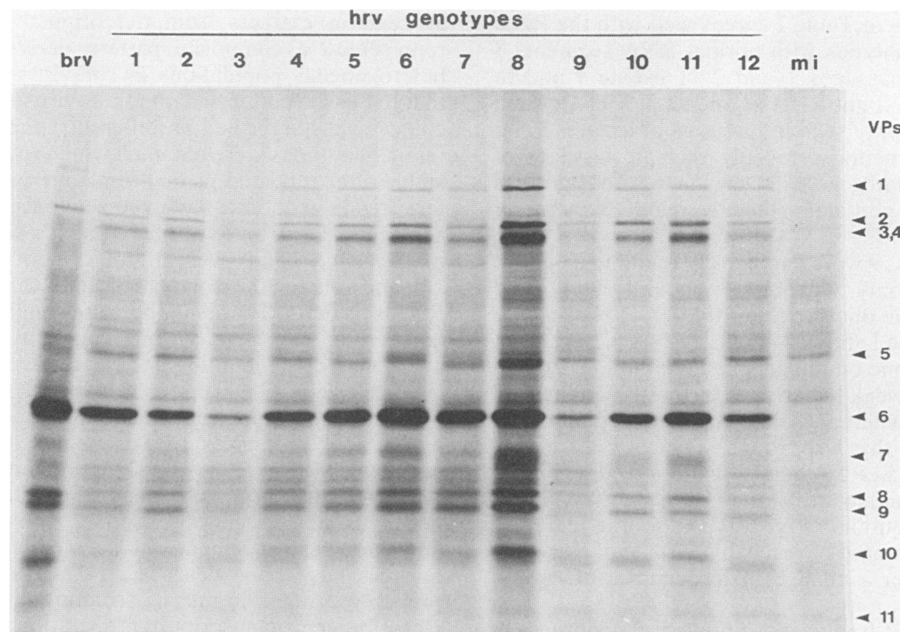

FIG. 9. Autoradiogram of a 15% polyacrylamide slab gel in which [^{35}S]methionine pulse-labeled protein lysates of BSC-1 cells infected with standard bovine rotavirus (brv) and with human rotaviruses (hrv) of genotypes 1 through 12 (tracks 1 through 12) were separated. Protein labeling and electrophoresis conditions were as described previously (26). The positions of virus-coded proteins (VPs) are indicated (the nomenclature of McCrae and McCorquodale [34] was used). Track mi contains a lysate of mock-infected, radiolabeled cells.

genotypes. However, from the chronological series of isolates obtained from several chronically infected hosts it was obvious that there was a periodical change in appearance and disappearance of rearranged RNA bands (see above) (41). From the data presented here it is now evident that mixed and varying RNA patterns represent the sum of various coexisting subpopulations of rotaviruses differing in genotype and changing in relative concentrations. It has been documented that populations of many RNA viruses do not have identical nucleotide sequences but show a distribution of genomic variants around a most abundant master sequence; the distribution may change in time, and continuously appearing variants are in transient dynamic equilibrium, competing with all variants of the population (9). Such combination of different but related molecular species has been called a "quasispecies" (9), and it can be argued that the quasispecies concept is also applicable to the emergence of a grossly varied heterogeneity of genome rearrangements in this collection of rotaviruses.

The profiles of virus-coded proteins in cells infected with rotaviruses which differed in genotype by rearrangement were very uniform. This indicates that rearrangement of segment-specific sequences apparently had left the normal reading frames and their expression unaltered. This result is in agreement with our previous findings on reassortants between bovine and human rotaviruses with genome rearrangements (2) and has also been reported in the first communication of a nucleotide sequence of a rearranged RNA band. Scott, McCrae, and Pocock (personal communication) found that a rearranged band of genomic RNA of a bovine rotavirus which replaced RNA segment 11 consisted of partial duplications of segment 11-specific sequences under maintenance of the open reading frame for the product VP11. The sequence data were confirmed by protein analyses of virus-infected cells (43). Other cases of rearrangements, however, have probably led to abolition or extension of the normal reading frame, with the consequence that no protein or an extended novel protein product was made (26). One possible mechanism compatible with the available data (2, 11, 26, 43) would be that at various stages of initial transcription the virion-associated RNA polymerase falls back on its template to reiterate part of it in the transcript.

The finding that a group A rotavirus replicates in vivo and grows and forms plaques in tissue culture without possessing the segment 11 equivalent in the normal position expected for long or short electropherotype viruses of that group has implications reaching beyond the fact that it was first observed as a peculiar characteristic of rotaviruses in immunodeficient hosts (which overall is a rare situation). Besselaar et al. (5) have described group A rotaviruses with atypical RNA profiles: segment 11 was missing from the profile, and an additional RNA band was found migrating ahead of segment 5; these viruses were spreading as a nosocomial infection over months in South African children who remained asymptomatic. Rotaviruses with ultrashort electropherotypes have been found in children with diarrhea in several parts of the world (1, 33, 51). Group A rotaviruses with rearrangements have also been isolated from apparently immunocompetent calves (43) and rabbits (50), and genome rearrangements have recently also been described in natural orbivirus isolates (11). We therefore propose that genome rearrangements, besides sequential point mutations and a reassortment continuum (38), are a further principle of evolution of rotaviruses (and possibly other dsRNA viruses) which might be operative to a much greater extent than envisaged at present.

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