JOURNAL OF VIROLOGY, July 1979, p. 104-111
0022-538X/79/07-0104/08/$02.00/0

Vol. 31, No. 1

Polymorphism of the Migration of Double-Stranded RNA Genome Segments of Reovirus Isolates from Humans, Cattle, and Mice

DANIEL B. HRDY,[1,2] LEON ROSEN,[3] AND BERNARD N. FIELDS[1,4]*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115,[1] Department of Anthropology, Harvard University, Cambridge, Massachusetts 02138,[2] Pacific Research Section, National Institute of Allergy and Infectious Diseases, Honolulu, Hawaii 96806,[3] and Division of Infectious Disease, Peter Bent Brigham Hospital, Boston, Massachusetts 02115[4]

Received for publication 20 December 1978

A series of 94 isolates of reovirus from humans, cattle, and mice showed extensive variability in the patterns of migration of the ten double-stranded RNA genome segments. This variation was found in all three serotypes, and involved all ten genome segments, including the segment responsible for serological specificity. Although a single pattern was present among several samples isolated from individuals and collected at a single time and place, there were often multiple genetic variants of a single serotype present in a population. Samples isolated from widely different geographic origins or different mammalian hosts showed different patterns; samples from a single species from the same area over a period of time showed more limited variations. Among most isolates, the migration of the slowest S segment, the segment that encodes the hemagglutinin and is responsible for serological specificity in laboratory strains, was similar to reference strains for type 1 and type 3 isolates. However, the type 2 isolates showed considerable variation in this segment.

Mammalian reoviruses are widely distributed in humans and a number of animal species (21). Although frequently isolated, their role in human disease is unclear. In most instances they appear to produce asymptomatic enteric or respiratory infections (8). In spite of this presumed minor role in human disease, reovirus has been the subject of intensive study primarily because of its segmented double-stranded RNA (dsRNA) genome. As the list of other segmented dsRNA-containing viruses has expanded, it has taken on additional significance as a model system. Other genera within the family Reoviridae include the insect-borne orbiviruses, phytoreoviruses of plants, cytoplasmic polyhedrosis virus of insects, and the medically important rotaviruses, which are currently refractory to detailed study in cell culture systems (15, 28).

The three serotypes of mammalian reovirus show considerable heterogeneity in electrophoretic migration among both the dsRNA segments and viral polypeptides (19). The ten dsRNA segments migrate at different rates on polyacrylamide gels and have been classified as L (large, three segments), M (medium, three segments), and S (small, four segments) (14, 17). Differences in the migration of these RNA segments between laboratory strains formed the basis for identifying viral recombinants derived from different serotypes (19). It was subsequently found that one of the dsRNA segments, S1, encodes the viral hemagglutinin (the σ1 outer capsid polypeptide) (17, 27). The hemagglutinin reacts with type-specific neutralizing antibody and is thus the antigen involved in serotype specificity (26).

Although traditional analysis of viral strains in nature has depended on serological studies, the availability of electrophoretic analysis of nucleic acids has begun to play an increasingly important role in epidemiological analyses. This method permits precise analyses of segmented RNA viruses such as reovirus (19), rotavirus (9), orbivirus (B. M. Gorman, J. Taylor, P. J. Walker, and P. R. Young, J. Gen. Virol., in press), and influenza (18), as well as a comparable analysis for the nonsegmented DNA virus herpes simplex, using restriction enzyme DNA fragments (13).

Access to a large number of isolates of reovirus collected during field investigations in the late 1950s and early 1960s from several parts of the world (L.R.) allowed us to evaluate several questions. (i) How much variability exists within a series of isolates of a single serotype from one mammalian species? (ii) How much variability

exists within a serotype among isolates that originate from different species? (iii) Do isolates within a single outbreak show variability? (iv) Is there evidence of consistent patterns defined by geographic areas? (v) Can the dsRNA pattern be used to reliably predict the serotype? The results of the study indicate striking polymorphisms of migration of dsRNA segments and have provided preliminary answers to these questions.

MATERIALS AND METHODS

Reovirus isolates. Human isolates of reovirus were collected from two sites: the respiratory tract (throat swab) and the gastrointestinal tract (anal swab or stool). Murine and bovine isolates were collected only from feces. Strains were originally isolated by inoculation in primary rhesus kidney cell cultures followed by a single passage in the same cell (22). Lysates of these cultures were stored at −70°C until use. The samples analyzed are listed in Table 1 with date and place of origin. A single recent strain was isolated as a contaminant from human kidney cell culture and was provided by Collaborative Research, Inc., Waltham, Mass.

The bovine samples were collected from three Maryland herds—Jessup, College Park, and Central Farm—the latter two of which exchanged members (23). Samples from Toluca, Mexico; Tahiti; the murine isolates; human isolate HT1b from Washington, D.C.; and the cell culture contaminant were typed by neutralization reduction by D.B.H. as outlined below. Samples typed as types 1 or 2 by neutralization were confirmed by hemagglutination inhibition by the method of Rosen (20). The balance of the samples were typed by hemagglutination inhibition by L.R. as previously reported (22) at the time of collection.

Since all but one of the samples were collected many years ago, all samples were titrated before passage to determine the content of infectious virus. The titers varied from $<10^1$ PFU/ml (in approximately one-third of samples) to $>10^7$ (two samples). One-half of the samples had titers of $\geq 10^4$ PFU/ml, attesting to the extraordinarily long-term stability of these viruses when stored at low temperatures.

TABLE 1. *Origin of reovirus isolates*

Source	Place	Date collected	No. of samples		
			Type 1	Type 2	Type 3
Human	Toluca, Mexico	8/59–12/59	1	3	
Human	Tahiti	12/59–2/60		3	1
Human	Washington, D.C.	10/57–7/59	22	2	15
Bovine	Maryland	5/59–3/61	31	1	12
Murine	France	10/61			1
Murine	New York, N.Y.	1960		1	
Cell culture	Waltham, Mass.	5/78		1	

Cells and preparation of virus stocks. Lysates of original virus isolates were passaged in mouse L cells. The L cells were maintained in suspension culture in Eagle minimal essential medium (MEM) as modified by Joklik (Industrial Biological Laboratories) supplemented with 5% fetal calf serum. To prepare stocks, lysates were inoculated onto confluent monolayers of mouse L cells in T_{25} flasks (Corning Glass Works, Corning, N.Y.) at 37°C, observed for cytopathic effect (CPE), and harvested by freeze-thawing. With lysates containing little virus, if CPE was not observed by 14 days, the flask was harvested. A second passage was made, harvested when CPE appeared, and used as stock for dsRNA labeling.

Preparation of labeled cytoplasmic dsRNA. A modification of the method of Ramig et al. (19) was used to prepare labeled cytoplasmic dsRNA. Briefly, L cells were inoculated with 1.0 ml of passaged viral suspension, and 200 μCi of ^{32}P was added in MEM with 0.25 μg of actinomycin D per ml. The cells were incubated for 2 days and harvested by scraping. The cells were then treated with Triton X-100 and centrifuged at 1,200 rpm for 5 min, and the supernatants were discarded. NaCl and ethanol were added to the pellets, and the mixtures were stored overnight at −20°C to precipitate dsRNA. The samples were then centrifuged at 12,000 rpm for 30 min in a Sorvall SS-34, dried under vacuum, and dissolved in 0.25 ml of gel sample buffer (10). Although the viral dsRNA in these samples has not been completely deproteinized, it has previously been reported that RNA extracted from purified virions and processed with extensive deproteinization, migrates to the same extent during electrophoresis as dsRNA processed from cytoplasmic extracts (19). Thus, the mobility of dsRNA when prepared by these methods is not affected by contaminating protein remaining in the sample.

Polyacrylamide gel electrophoresis of dsRNA. Slab gels were prepared to a final concentration of 10% acrylamide–0.267% bisacrylamide as described by Laemmli (10). Electrophoresis of samples which had been heated at 100°C for 1 m was carried out at a constant current of 20 mA for 18 h. Unfixed gels were dried onto filter paper in a Hoeffer Scientific Instruments gel dryer, and autoradiography was performed by exposing No-Screen X-ray film (Kodak) for 1 to 2 days.

Neutralization assay. Antisera against the three reovirus serotypes were obtained from the National Institute of Allergy and Infectious Diseases (catalog no. V-701-511-570; V-702-501-570; and V-703-501-570). Approximately 100 PFU of the unknown virus was incubated with twofold dilutions of antisera for 45 min at 37°C, then plated at 37°C on L cell monolayers by the method of Fields and Joklik (4). Greater than 80% reduction of plaques was considered neutralization of virus. Serological classification was determined by hemagglutination inhibition (20) or neutralization. Although not every sample was analyzed by both serological tests, samples displaying representative dsRNA patterns were tested with freshly grown stocks to verify the initial serological classifications.

RESULTS

Nomenclature of dsRNA segments. Previous studies have correlated the dsRNA segments between laboratory strains of the three serotypes of the mammalian reoviruses (25). Although in general the segments of the serotypes correspond in order of increasing electrophoretic mobility, this was not always the case (see fig. 6 in ref. 25). Because of this problem, we have arbitrarily labeled dsRNA segments of the isolates reported here by sequentially numbering from large to small segments in a size class. These segments are referred to in the text in parentheses. The segments from the laboratory strains are labeled without parentheses by prior nomenclature as shown in Fig. 1A, 2A, and 3A.

Type 1 reovirus. (i) Human isolates. Type 1 reovirus isolates, designated TM and WA, respectively, were isolated from human materials at Toluca, Mexico, and Washington, D.C. (Table 1). There was a single TM isolate and 22 WA isolates. Of these isolates, there were three RNA patterns: (i) TM (HT1a) (Fig. 1B), (ii) one WA isolate (HT1b) (Fig. 1C), and (iii) the remaining 21 WA isolates (HT1c) (Fig. 1D). Although the overall RNA patterns were similar, certain segments showed considerable variability. Although there were minor variations in migrations of the L segments [for example, note L(2) and L(3) of HT1a (Fig. 1B)] migrating slower than the corresponding segments of type 1 (Lang) (Fig. 1A), the major heterogeneity was noted in the M segments. The migrations of the M(1) segments, for example, were markedly different from each other (Fig. 1B, C, and D).

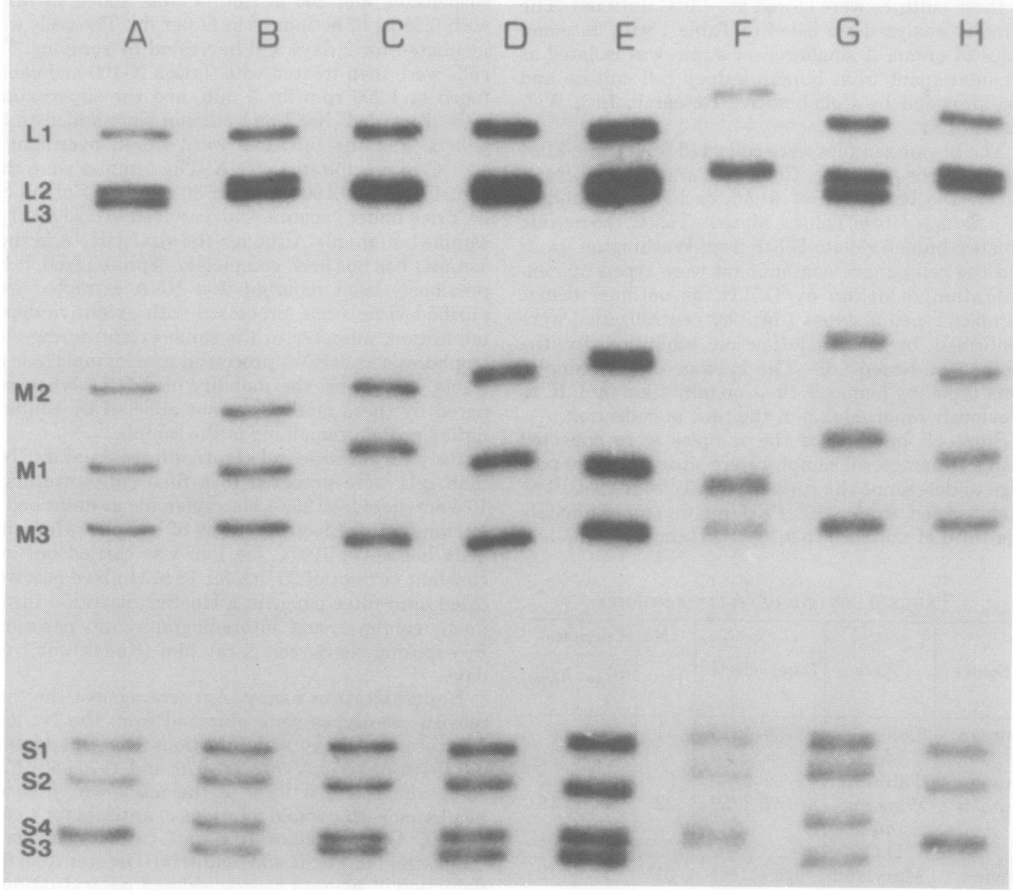

FIG. 1. *^{32}P-labeled dsRNA from type 1 reovirus isolates. Electrophoresis was from top to bottom in a single 10% acrylamide gel containing the Tris-glycine buffer system as described in the text. (A) Type 1 (Lang); (B) human type HT1a, TM; (C) human type HT1b, WA; (D) human type TH1c, WA; (E) bovine type BT1a, CP; (F) bovine type BT1b, CP; (G) bovine type BT1c, JE; (H) type 1 (Lang). RNA genome segments of type 1 (Lang) are identified (17).*

The 21 WA isolates showing identical HT1c patterns (Fig. 1D) were isolated in early 1957, whereas the single HT1b pattern was isolated in 1959. Thus, a virus isolated from one geographic area from a large number of individuals retained a distinct pattern. However, within this same geographic area a second clearly distinct pattern was seen at a later date (HT1b) (Fig. 1C). Furthermore, the single isolate from Mexico (Fig. 1B) was quite distinct from all the WA isolates.

(ii) Bovine isolates. Type 1 reovirus was isolated from three herds in Maryland over the 6-month period from November 1959 through May 1960 (Table 1). The College Park and Central Farm herds, which were separated by 25 miles, frequently exchanged members and are therefore treated as a single herd (CP). The Jessup herd (JE) did not exchange members with the other herds, and was about 15 miles distant from each of them. Of the 31 isolates, all samples displayed one of three patterns; BT1a, 15 isolates; BT1b, 11 isolates; BT1c, five isolates (Fig. 1E, F, and G). Note the comigration of the L(2), L(3) and M(2), M(1) dsRNA segments in the BT1b pattern (Fig. 1F). The comigration of segments results in the appearance of a more intense radioautograph band as well as the appearance of a "missing" segment(s) [L(3) and M(2) in Fig. 1F]. The 26 viral isolates showing BT1a and BT1b patterns were isolated from the CP herds; the BT1c pattern was seen exclusively among the JE isolates. As in the human type 1 isolates, there were marked variations in the migration of RNA segments. These included major variations in migration among segments of all size classes: L segments (Fig. 1F); M segments, especially M(1) and M(2) (Fig. 1E, F, and G); and S segments (compare Fig. 1F and G). Although the overall pattern resembled that of the human type 1 isolates, no consistent pattern that could identify an isolate as bovine in origin was detected. The smallest variations were present in the M(3), S(1), and S(2) segments. Although all of these 31 isolates were classified serologically as type 1, the RNA analysis provided additional data. Viruses classified as type 1 but containing two different patterns of RNA were present in the CP herds (Table 2). The BT1a pattern was predominant during November 1959, but by March 1960 there was a shift to the BT1b pattern (8 of 10 samples). By April 1960, six of seven samples were BT1a. The last isolate (May 1960) was BT1b. Although there is no way to study the precise origin of these distinct isolates from available data, it is possible that the transfer of animals between the College Park and Central Farm herds was important in exchanging these two distinct viruses. Studies of this type will be important in future analyses of such outbreaks and may provide important facts in the analysis of virus spread.

TABLE 2. *Place and date of origin of bovine type 1 patterns*

Date	Herd[a]	Pattern		
		BT1a	BT1b	BT1c
11/59	CP	7	1	
	JE			
3/60	CP	2	8	
	JE			2
4/60	CP	6	1	
	JE			3
5/60	CP		1	
	JE			

[a] See text.

Type 2 reovirus. (i) Human isolates. There were eight human isolates of type 2 reovirus obtained from Toluca, Mexico, Tahiti (designated TA), and Washington, D.C. (Table 1). Two patterns were noted among the three TM isolates (Fig. 2B and C), a single pattern among the three TA isolates (Fig. 2D), and a single pattern among the two WA isolates (Fig. 2E). As previously noted with the type 1 isolates, there was marked heterogeneity in the migration of several RNA segments. This was especially prominent among the M and S segments.

(ii) Nonhuman isolates. There were single isolates of bovine (CP herd, Fig. 2F), and murine origin (Fig. 2G) as well as a contaminant of unknown species origin from cell culture (Fig. 2H). Each of these isolates was distinct, with variations in migration seen in several segments.

Thus, although the number of type 2 isolates is quite small, it is apparent that there is a large number of distinct patterns with major differences in migration noted in each of the RNA size classes.

Type 3 reovirus. (i) Human isolates. Type 3 reovirus was isolated from human materials at Tahiti and Washington, D.C. (Table 1). There were a single TA isolate (Fig. 3B) and 15 WA isolates. The 15 WA isolates showed two patterns: HT3b in 2 isolates (Fig. 3C), and HT3c in 13 isolates (Fig. 3D). HT3c differs from type 3 (Dearing) mainly in the M(1) segment. The HT3b pattern is virtually identical to that of the Dearing strain, except for a slight difference in L(1) migration. The TA sample (HT3a) (Fig. 3B) differs from these strains in several segments, most notably the L(3), M(1), and S(3,4).

Thus the heterogeneity among type 3 samples

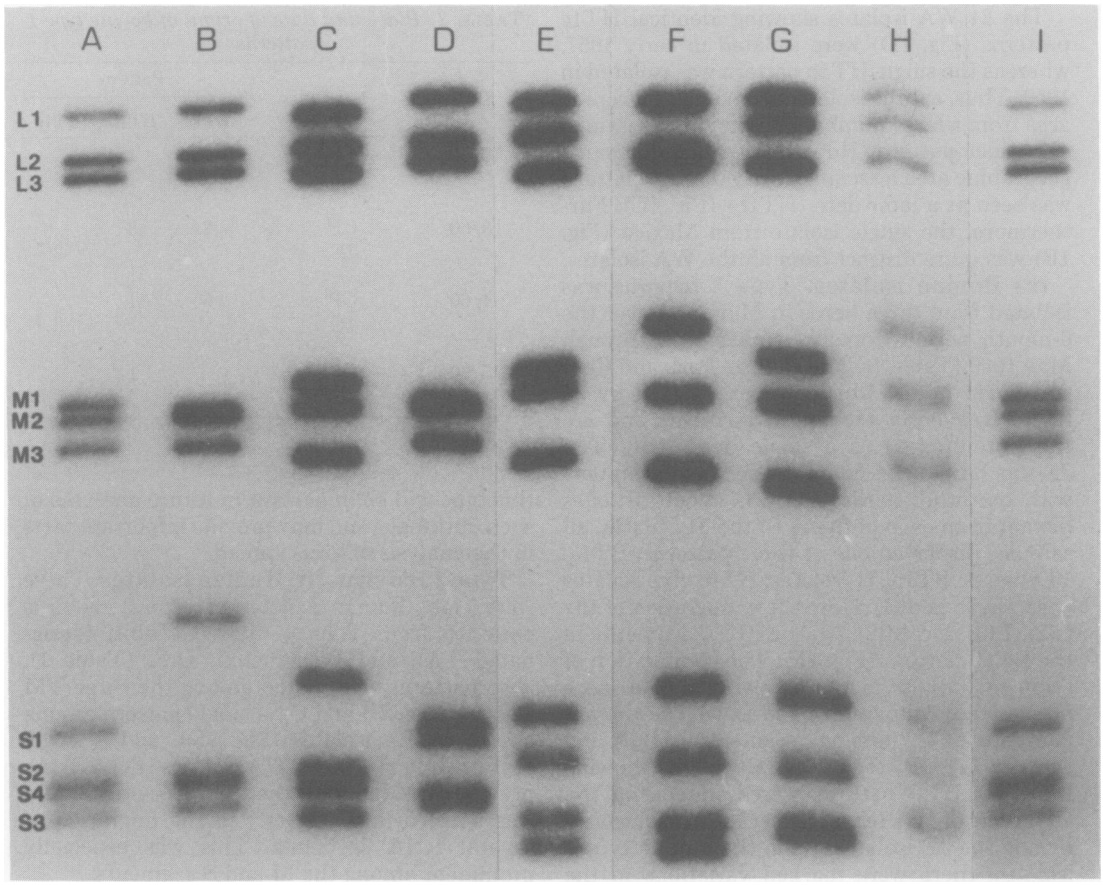

FIG. 2. *^{32}P-labeled dsRNA from type 2 reovirus isolates. Electrophoresis was from top to bottom in a single 10% acrylamide gel containing the Tris-glycine buffer system as described in the text. Lanes have been rearranged for comparison. (A) Type 2 (Jones); (B) human type HT2a, TM; (C) human type HT2b, TM; (D) human type HT2c, TA; (E) human type HT2d, WA; (F) bovine type BT2a, CP; (G) murine type MT2a, New York, N.Y.; (H) cell culture contaminant, type 2, Waltham, Mass.; (I) type 2 (Jones). RNA genome segments of type 2 (Jones) are identified (17). Note that the type 2 (Jones) strain used as a marker was slightly defective (containing reduced amounts of L1). The mobility of this and the other segments was, however, identical to that of nondefective preparations (19, 25).*

appears somewhat more restricted than that among the type 1 or 2 samples, but this may be due to small sample size.

(ii) Bovine isolates. There were 12 bovine isolates of type 3 reovirus: 6 from the CP herd and 6 from the JE herd. Three patterns of RNA were seen: BT3a (two isolates) (Fig. 3E) and BT3b (four isolates) (Fig. 3F) from the CP herd, and BT3c (six isolates) (Fig. 3I) from JE. Although there were minor variations in RNA migrations between the BT3a and BT3b isolates, such as in L(1,2), M(2), and S(1,4), the BT3c pattern was markedly different. The L(1,2) and M(1) RNA segment species among the latter isolates were especially different. Thus, the isolates from the CP herd were distinct but showed only slight variations, whereas those isolated from the second herd were considerably altered.

(iii) Murine isolates. A single murine isolate of type 3 (Fig. 3G) was distinct from the other isolates, with minor variations in migration of numerous segments.

Comparison of human respiratory and gastrointestinal isolates. Of the 47 human isolates, 42 were from gastrointestinal and 5 were from respiratory sources. Of these five, there were one type 1 and four type 3 isolates from WA. The patterns of these isolates were the same as viruses isolated at the same time from gastrointestinal sources. The type 1 isolate had the HT1c pattern (Fig. 1D), which was also found in all concurrently isolated type 1 gas-

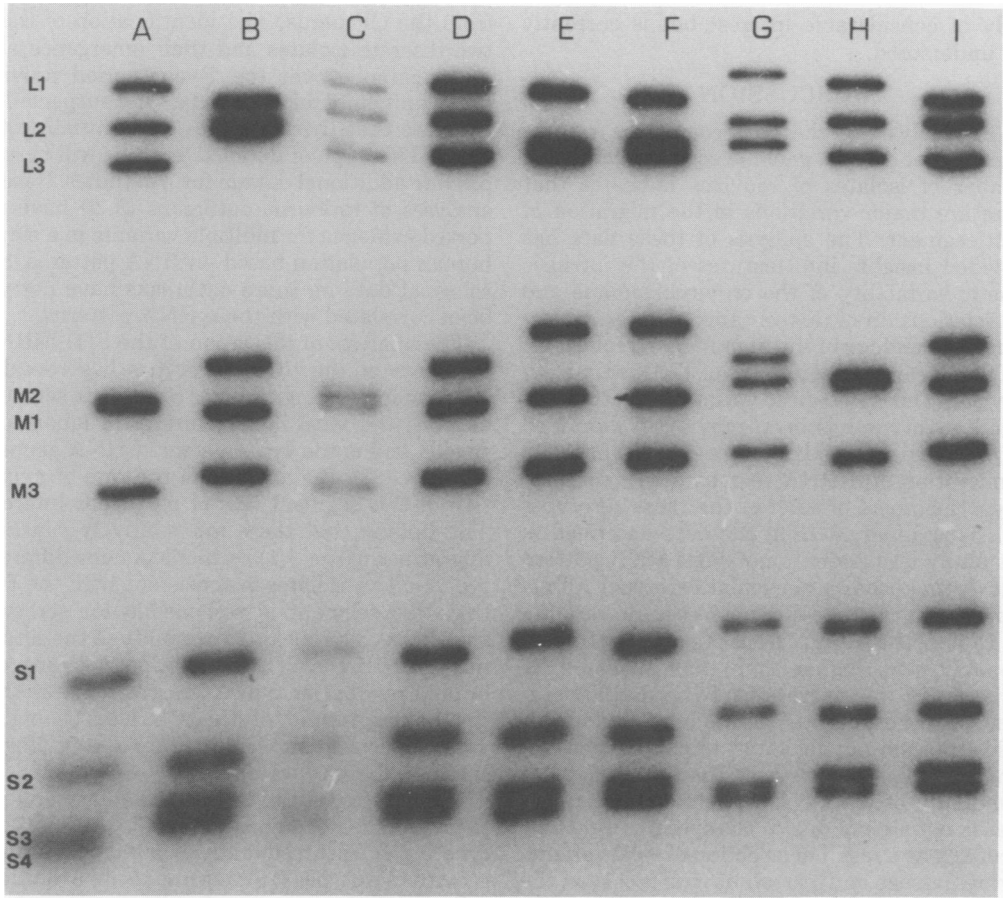

FIG. 3. *^{32}P-labeled dsRNA from type 3 reovirus isolates. Electrophoresis was from top to bottom in a single 10% acrylamide gel containing the Tris-glycine buffer system as described in the text. (A) Type 3 (Dearing); (B) human type HT3a, TA; (C) human type HT3b, WA; (D) human type HT3c, WA; (E) bovine BT3a, CP; (F) bovine type BT3b, CP; (G) murine type MT3a, France; (H) type 3 (Dearing); (I) bovine type BT3c, JE. RNA genome segments of type 3 (Dearing) are identified (17).*

trointestinal viruses. Similarly, all four type 3 respiratory isolates were HT3a (Fig. 3B). This was the pattern most commonly isolated from gastrointestinal type 3 samples from the same time and place. Thus, the site from which the viruses were isolated did not appear to play an important role.

Correlation of S(1) segment and serotype. The S1 dsRNA segment of type 3 (Dearing) reovirus migrates significantly slower than that of type 1 (Lang) or type 2 (Jones). The S1 segments from the latter two strains are difficult to distinguish from each other. This finding is of some potentially predictive importance, since the S1 segment encodes the viral hemagglutinin, the type-specific polypeptide (27). A preliminary determination of serotype could be made by analysis of the migration of S(1) segments in certain instances. The S(1) segment of type 1 strains migrates faster than that of type 3. Thus, in every instance, the S(1) segment of type 1 migrated close to the S(2) segment (Fig. 1), while that of type 3 migrated considerably slower (Fig. 3). In striking contrast to the relatively consistent pattern seen with type 1 or 3, the S(1) segment of type 2 migrates with enormous variability. For example, the S(1) segment of one isolate of TM migrates very slowly (Fig. 2B), whereas the S(1) of the TA isolate migrates as fast as the S1 of type 3 (Fig. 2D). Thus, although the migration of the S(1) segment has some predictive value for type 1 or 3 strains, the variability of migration of S(1) among type 2 isolates indicates the limitations of this approach. The significance of the marked variability in S(1) migration of type 2 strains is poten-

tially of considerable interest but is currently not understood.

DISCUSSION

The analysis of the patterns of the rate of migration of dsRNA genome segments among a number of isolates of reovirus indicates that there are major variations in the migration of each segment. The analysis of these data has provided insights into features of the intrinsic genetic variability of the reovirus genome and the relationship of this variability to the molecular epidemiology of the mammalian reoviruses.

There are three serotypes of the mammalian reovirus, and serological variability within the type 2 serotype has been previously described (6). We have reported differences in the patterns of migration of dsRNA segments between prototype members of each of the three serotypes (19). It is apparent from the data described in this study that there is no single RNA pattern that distinguishes a particular serotype. All genome segments appear to possess some variability. In fact, the actual extent of polymorphism is probably even higher than that indicated by variation in the migration of the dsRNA segments, since differences due to base pair substitution or limited deletions might not be detected. It should be stressed that the variability in mobility of the RNA segments between isolates is not simply due to changes in viral RNA occurring as a result of selection of viral variants during passage and growth of viral stocks in the laboratory. The pattern of migration of RNA segments remains stable throughout several passages (R. Ahmed, personal communication), and thus the differences between isolates seen in this study could not have been generated after the one or two passages required to develop high-titered stocks.

This variability in migration of dsRNA segments is seen among several isolates within a serotype, among the three serotypes, and among isolates derived from different species or geographic areas. Thus, in comparing the RNA pattern of isolates of reoviruses of different origins, the RNA pattern does not identify the serotype, host of origin, or geographic locale.

In spite of the extraordinary polymorphisms in the overall rates of migration of most of the dsRNA segments, two points of interest have clearly emerged. Although it had previously been noted that more than one serotype can exist in a population during a single outbreak (21), it is now apparent that multiple genetic variants of a single serotype may be present during a single outbreak. This was best illustrated by the two bovine type 1 isolates obtained from the CP herds. The identification of these two discrete isolates and their emergence and disappearance over the 2-year period provide important epidemiological data not suspected at the time that these samples were initially analyzed. This type of detailed analysis will clearly provide additional data in future studies. Recent analyses of rotavirus outbreaks (3, 9) have reported evidence for multiple variants in a single human population based on RNA patterns. Serological data on these outbreaks have not yet been correlated with these RNA patterns.

The analysis of migration of the S(1) dsRNA segments of the three serotypes has revealed another interesting finding. Since this segment encodes the viral hemagglutinin in laboratory strains and is the type-specific dsRNA genome segment (27), the analysis of the rate of migration of this segment was of particular interest. The finding that there are relatively constant migrations of the S(1) segments among different type 1 and 3 isolates is consistent with the fact that this segment is responsible for serotype specificity. The striking variability in the rate of migration of the S(1) segment seen among the limited number of type 2 isolates suggests that the hemagglutinin of these isolates is highly variable, consistent with the previous finding of serological subtypes among type 2 strains (6). The chemical nature of this variability and genetic basis is not understood. It is possible that type 2, the least frequently isolated of the three reovirus serotypes (23), represents an evolutionarily unstable serotype. Studies underway on the RNA sequence of these isolates as well as biological properties of the hemagglutinin of type 2 (such as cell and tissue tropism) may provide important insights into the biological significance of such variation.

It should be pointed out that variability in the genetic structure of the mammalian reoviruses is not unique. Other viruses whose genome structures have recently been analyzed have also shown considerable variability. For example, the DNAs of herpesviruses after endonuclease treatment have recently been reported to vary between isolates (13). Influenza virus has also been shown to vary in the mobility of genome RNA segments in various strains isolated in differing hosts and geographic areas (18, 24). Even laboratory-adapted viruses possess genetic variation; RNA phage $Q\beta$ has recently been shown to have large nucleotide sequence heterogeneity in a single population (2). The genetic variability of reovirus isolates and other viruses is the counterpart of protein variation reported for other organisms, including *Escherichia coli* (16), drosophila (12), and humans (5). Detailed study of

the genetic variability of viruses in a natural population should elucidate the amount of variability necessary to allow rapid evolutionary change while maintaining a stable virus population.

Finally, isolates from different sources do not reveal consistent differences in RNA patterns between species, although this does not prove that species-specific patterns might not exist. Rather, reovirus isolates seem to represent a genetically heterogeneous group in all species, as has been reported for influenza (7). Of more significance, we have not been able to determine whether genome segment exchange takes place between human and nonhuman species, as has been postulated to occur in influenza (11). Such gene exchange clearly could help rapid evolution by exchange of genetic material and assist in the maintenance of a nonpersistent virus in small host populations (1). In addition, the possibility that mammalian reoviruses infect multiple mammalian species under natural conditions could play a similar role in their maintenance in a population.

ACKNOWLEDGMENTS

This work was supported by research grant R01 AI 13178 from the National Institute of Allergy and Infectious Diseases. D.B.H. was the recipient of Public Health Service fellowship 1 F32 AI05673 from the National Institute of Allergy and Infectious Diseases.

We thank Susan L. Pevear, Linda Powers, R. Frank Ramig, Donald Rubin, Howard L. Weiner, and Roz White for invaluable assistance.

LITERATURE CITED

1. **Black, F. L.** 1975. Infectious diseases in primitive society. Science **187**:515–518
2. **Domingo, E., D. Sabo, T. Taniguchi, and C. Weissmann.** 1978. Nucleotide sequence heterogeneity of an RNA phage population. Cell **13**:735–744.
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. **Fields, B. N., and W. K. Joklik.** 1969. Isolation and preliminary genetic and biochemical characterization of temperature-sensitive mutants of reovirus. Virology **37**: 335–342.
5. **Harris, H.** Enzyme polymorphisms in man. 1966. Proc. R. Soc. Br. **164**:298–310.
6. **Hartley, J. W., W. P. Rowe, and J. B. Austin.** 1962. Subtype differentiation of reovirus type 2 strains by hemagglutination-inhibition with mouse antisera. Virology **16**:94–96.
7. **Hinshaw, V. S., W. J. Bean, R. G. Webster, and B. C. Easterday.** 1978. The prevalence of influenza viruses in swine and the antigenic and genetic relatedness of influenza viruses from man and swine. Virology **84**:51–62.
8. **Jackson, G. G., and R. L. Muldoon.** 1973. Viruses causing common respiratory infection in man. IV. Reoviruses and adenoviruses. J. Infect. Dis. **128**:811–822.
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. Virology **87**:247–255.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **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.
12. **Lewontin, R. C., and J. L. Hubby.** 1966. A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics **54**:595–609.
13. **Linnemann, C. C., I. J. Light, T. G. Buchman, J. L. Ballard, and B. Roizman.** 1978. Transmission of herpes-simplex virus type 1 in a nursery for the newborn. Identification of viral isolates of DNA "fingerprinting." Lancet **i**:964–966.
14. **McCrae, M. A., and W. K. Joklik.** 1978. The nature of the polypeptide encoded by each of the 10 dsRNA segments of reovirus type 3. Virology **89**:578–593.
15. **McNulty, M. S.** 1978. Rotaviruses. J. Gen. Virol. **40**:1–18.
16. **Milkman, R.** 1973. Electrophoretic variation in *E. coli* from natural sources. Science **182**:1024–1026.
17. **Mustoe, T. A., R. F. Ramig, A. H. Sharpe, and B. N. Fields.** 1978. Genetics of reovirus: identification of the dsRNA segments encoding the polypeptides of the μ and σ size classes. Virology **89**:594–604.
18. **Palese, P.** 1977. Genes of influenza. Cell **10**:1–10.
19. **Ramig, R. F., R. K. Cross, and B. N. Fields.** 1977. Genome RNAs and polypeptides of reovirus serotypes 1, 2, and 3. J. Virol. **22**:726–733.
20. **Rosen, L.** 1960. Serologic grouping of reoviruses by hemagglutination-inhibition. Am. J. Hyg. **71**:242–249.
21. **Rosen, L.** 1960. Reoviruses, p. 73–107. *In* S. Gard, C. Hallaver, and K. F. Meyer, (ed), Virology monographs. Springer-Verlag, New York.
22. **Rosen, L., and F. R. Abinanti.** 1960. Natural and experimental infection of cattle with human types of reovirus. Am. J. Hyg. **71**:424–429.
23. **Rosen, L., F. R. Abinanti, and J. F. Hovin.** 1963. Further observations on the natural infection of cattle with reoviruses. Am. J. Hyg. **77**:38–48.
24. **Scholtissek, C., W. Rhode, V. Von Hoyningen, and R. Rott.** 1978. On the origin of the human influenza virus subtypes H2N2 and H3N2. Virology **87**:13–20.
25. **Sharpe, A. H., R. F. Ramig, T. A. Mustoe, and B. N. Fields.** 1978. A genetic map of reovirus. I. Correlation of genome RNA's between serotypes 1, 2 and 3. Virology **84**:63–74.
26. **Weiner, H. L., and B. N. Fields.** 1977. Neutralization of reovirus: the gene responsible for the neutralization antigen. J. Exp. Med. **146**:1305–1310.
27. **Weiner, H. L., R. F. Ramig, T. A. Mustoe, and B. N. Fields.** 1978. Identification of the gene coding for the hemagglutinin of reovirus. Virology **86**:581–584.
28. **Wood, H. A.** 1973. Viruses with double-stranded RNA genomes. J. Gen. Virol. **20**:61–85.