Genetic Diversity in Natural Populations of Mammalian Reoviruses: Tryptic Peptide Analysis of Outer Capsid Polypeptides of Murine, Bovine, and Human Type 1 and 3 Reovirus Strains

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We have studied the structural relationships between the outer capsid polypeptides of eight murine, bovine, and human isolates of type 1 and 3 mammalian reoviruses. Our results show that the outer capsid polypeptides of reoviruses isolated from different mammalian species, in different years and different geographical areas, have both conserved and unique methionine-containing tryptic peptides. We found that tryptic peptides from μ1C polypeptides of two human, one murine, and two bovine type 3 isolates and one human and two bovine type 1 reoviruses are highly conserved. Our data show that only one tryptic peptide pattern of the μ1C polypeptide (encoded by the M2 gene) was present in reoviruses isolated from the three different mammalian species. The μ1C polypeptide of the type 3 Dearing strain contained one tryptic peptide not found in any other reovirus isolate examined. In marked contrast to the μ1C polypeptides, the σ3 polypeptides (encoded by the S4 gene) of three type 1 and three type 3 isolates were divided into two patterns based on significant differences in their tryptic peptides. In addition, at least seven tryptic peptides were conserved among the σ3 polypeptides of all virus strains examined. The σ3 polypeptide of the type 3 Dearing strain was distinguishable from the σ3 polypeptides of all other strains examined. The one μ1C and two σ3 tryptic peptide patterns were found to occur interchangeably in isolates of type 1 or type 3. About 1/3 of the tyrosine-containing tryptic peptides of σ1 polypeptides of four type 3 isolates examined were conserved. Comparison of peptide differences in σ1 polypeptides of these isolates showed that each had one or more unique tryptic peptides, suggesting that the S1 genes coding for these polypeptides had undergone genetic drift or, alternatively, that there are at least two tryptic peptide patterns present among the σ1 polypeptides of these isolates. Our results suggest that genetic drift and reassortment are the most likely explanation for the extensive genetic diversity found in natural populations of mammalian reoviruses.

The three serotypes of mammalian reoviruses contain a genome of 10 segments of double-stranded RNA (dsRNA) that is enclosed in an icosahedral, double-capsid protein shell (11). Genetic and biochemical studies have shown that each viral RNA segment encodes a single viral polypeptide species (13, 14).

The classification of reoviruses into three serotypes is based primarily on the capacity of immune sera to neutralize infectivity or to inhibit hemagglutination of erythrocytes (18). Genetic studies indicate that the antigen against which host type-specific neutralizing antibodies are directed is the reovirus hemagglutinin, the σ1 polypeptide (23, 24). Thus, the classification of a reovirus strain into serotype 1, 2, or 3 indicates that the virus possesses a type 1, 2, or 3 σ1 polypeptide (encoded by the S1 genome segment). This conventional classification scheme does not indicate the origins of the other nine reovirus genes.

It has been well documented that human prototype (type 1 Lang, type 2 Jones, and type 3 Dearing) and certain natural isolates of viruses of the three serotypes reassort genome segments after mixed infections in vitro to yield viruses with genome segments derived from two different parental viruses (10, 20). The demonstration of genome segment reassortment was made possible by finding that there are differences in the electrophoretic migration rates of the polypeptides and the dsRNA species of human prototype strains of types 1, 2,

and 3 (16). Recently, we have shown that there is an even greater degree of heterogeneity than previously suspected in the electrophoretic mobilities of the RNA segments of virus types 1, 2, or 3 isolated from human, bovine, and murine sources in nature (9). This heterogeneity occurs among viruses that are classified serologically as belonging to the same or different serotypes and among isolates from different species or different geographical areas (9). For example, among viruses that were isolated from a single bovine population of two herds which frequently interchanged members, both type 1 and 3 isolates were identified serologically, and the virus isolates within each serotype showed multiple RNA patterns (9). It is therefore likely that single herd members were simultaneously infected with both type 1 and 3 strains of reovirus. Since it has been demonstrated that reassortment occurs among prototype human isolates of type 1, 2, and 3 viruses or between human and natural isolates of strains in vitro (10, 20), some of the RNA heterogeneity present in viruses isolated from this bovine population may have been due to reassortment in the natural environment. However, as discussed above, classifying a given strain as type 1 or 3 indicates only the origin of the S1 genome segment. Therefore, due to the large degree of RNA heterogeneity among these viruses and the lack of serotypic markers for the polypeptides encoded by the other nine viral genes, we could not determine whether genome reassortment accounted for any of the observed RNA heterogeneity. It was possible, for example, that most or all of the RNA heterogeneity was due to point mutations, deletions, or insertions in viral RNA.

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We recently reported that the σ1, σ3, and μ1C outer capsid polypeptides of the three human prototypes, laboratory strains type 1 Lang, type 2 Jones, and type 3 Dearing, contain both conserved and unique tryptic peptides (8). Although the σ1 (type-specific antigen) and σ3 polypeptides of these three strains share approximately 50% of their tryptic peptides, the μ1C polypeptides share greater than 90% of their methionine-containing tryptic peptides. Neverᵢ theless, we were able to distinguish the σ1, σ3, and μ1C polypeptides from each laboratory isolate on the basis of their tryptic peptide maps. This result provided us with a potential method for determining the origins of the M2, S1, and S4 genes of type 1, 2, and 3 reovirus isolates by analyzing the tryptic peptides of their μ1C, σ1, and σ3 polypeptides, respectively.

The present more-detailed analyses indicate that the μ1C polypeptide is highly conserved in reoviruses isolated from several different mammalian species. The σ1 and σ3 polypeptides showed much greater sequence diversity as indicated by changes in their tryptic peptides. Several σ1 and σ3 peptides were conserved in viruses isolated from all human, bovine, and murine sources.

MATERIALS AND METHODS

Radioisotopes. L-[^{35}S]methionine (>1,200 Ci/mmol), sodium [^{125}I]iodide (17 Ci/mg), and ^{32}P$_i$ (carrier free) were obtained from New England Nuclear Corp., Boston, Mass. L-[^{3}H]lysine was obtained from ICN Radiochemical Corp., Irvine, Calif.

Human, bovine, and murine reovirus isolates. The origin of the type 3 Dearing strain used in this study was the same as that for other reports from this laboratory (16). Type 1 Lang was obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Md. (catalog no. N-701-001-010). All other strains used in this study were obtained from Leon Rosen (9). Each strain was originally identified as type 1 or 3 by neutralization or hemagglutination inhibition tests (18, 19). We also reconfirmed the serotype of each strain by plaque reduction neutralization tests, using hyperimmune rabbit anti-reovirus sera (data not shown). The RNA genotype of each strain was verified by discontinuous slab gel electrophoresis of ^{32}P-labeled viral RNA species and by comparison with previous results (9) (data not shown).

The serotype, place of origin, species of origin, and year of isolation of each strain are shown in Table 1. All of the bovine isolates were obtained from two adjacent Maryland cow herds which frequently interchanged members (9, 19). Each original isolate was cloned once and passaged twice to obtain working stocks.

Cells. The origin of L-cells and their maintenance in suspension cultures were described previously (16).

Virus purification. The methods used for infecting L-cell suspension cultures with reoviruses, radioactive labeling of viral polypeptides in infected cells, and virus purification from infected cells have been described in detail in previous reports (8, 16). [^{3}H]lysine-labeled purified virus was obtained under the same conditions as used before except that labeling was carried out in regular Eagle minimal essential medium containing normal amounts of amino acids. L-[^{3}H]lysine (40 to 60 Ci/mmol) was added at 18 h postinfection to a specific activity of 10 μCi/ml.

Preparation of virus-infected cell polypeptides or dsRNA segments. The preparation of [^{35}S]methionine-labeled viral polypeptides and their immunoprecipitation from infected cell extracts have been described previously (3, 8).

To prepare ^{32}P$_i$-labeled viral dsRNA, monolayers of L-

TABLE 1. Origins of mammalian reovirus isolates

Serotype	Origin	Place	Month and year
1 (Lang)	Human	Ohio	Mar. 1954
1 (clone 28)	Bovine	Maryland[a]	Nov. 1959
1 (clone 50)	Bovine	Maryland	Apr. 1960
3 (Dearing)	Human	Maryland	? 1955
3 (clone 8)	Human	Tahiti	?
3 (clone 9)	Murine	France	Oct. 1961
3 (clone 18)	Bovine	Maryland	Mar. 1961
3 (clone 31)	Bovine	Maryland	Apr. 1960

[a] The four bovine strains were isolated from two adjacent Maryland cow herds which frequently exchanged members (9).

cells were infected at 5 PFU per cell with each virus strain and overlaid with minimal essential medium containing 0.25 μg of actinomycin D per ml and 100 μCi of ^{32}P$_i$ per 60-mm dish. Plates were then incubated for 48 h at 37°C, and a virus-infected cell cytoplasmic extract was prepared (16). The viral dsRNA species were then ethanol precipitated and resolved on discontinuous polyacrylamide slab gels, and the gels were dried and autoradiographed as previously described (16).

Polyacrylamide gel electrophoresis. Viral polypeptides and RNA were resolved in polyacrylamide slab gels by the method of Laemmli (12) as described previously (8).

Tryptic peptide analysis. The methods used for analysis of [^{35}S]methionine-containing or [^{125}I]tyrosine-containing tryptic peptides were described in detail previously (8). L-[^{3}H]lysine-containing tryptic peptides from type 1, 2, or 3 μ1C polypeptides were analyzed by the same method with the following modifications. L-[^{3}H]lysine-labeled purified type 1, 2, or 3 virus was resolved by preparative slab gel electrophoresis, and the gel slabs were soaked for 10 min in a solution of 1 M sodium salicylate, pH 7.0 (E. J. Brown, Ph.D. thesis, McMaster University, Hamilton, Ontario, Canada, 1981). The gels were then dried at 80°C on a Hoeffer gel drier and fluorographed at −70°C (1). The μ1C bands were then excised from the gels, using the fluorograph as a template, and the proteins were eluted at 37°C in a solution of 100 mM NH$_4$HCO$_3$ containing 0.1% sodium dodecyl sulfate (7). Eluted protein samples were dialyzed against 100 mM NH$_4$HCO$_3$ to remove sodium salicylate and then ethanol precipitated twice in the presence of 50 μg of bovine serum albumin carrier protein. Samples were then suspended in 100 mM HN$_4$HCO$_3$, digested with trypsin, and resolved in two dimensions on cellulose thin-layer plates as described previously (8). After chromatography in the second dimension, plates were air dried and dipped into a solution of 2-methylnaphthalene containing 0.4% 2,5-diphenyloxazole and again dried (1) (Brown, Ph.D. thesis). Plates were then fluorographed at −70°C for 2 to 8 weeks (8).

RESULTS

Polypeptide species of type 1 and 3 reovirus isolates. Several diverse human, bovine, and murine virus strains were selected for study (Table 1). The RNA patterns of these strains have been reported previously by Hrdy et al. (9) and reconfirmed by us (data not shown). The important point of those experiments (9) as it relates to the present study is that there is a large degree of heterogeneity of electrophoretic mobility of the dsRNA segments found in different isolates of the same serotype or between serotypes.

To compare the intracellular polypeptides of these same isolates, L-cells were infected with each strain, and the viral
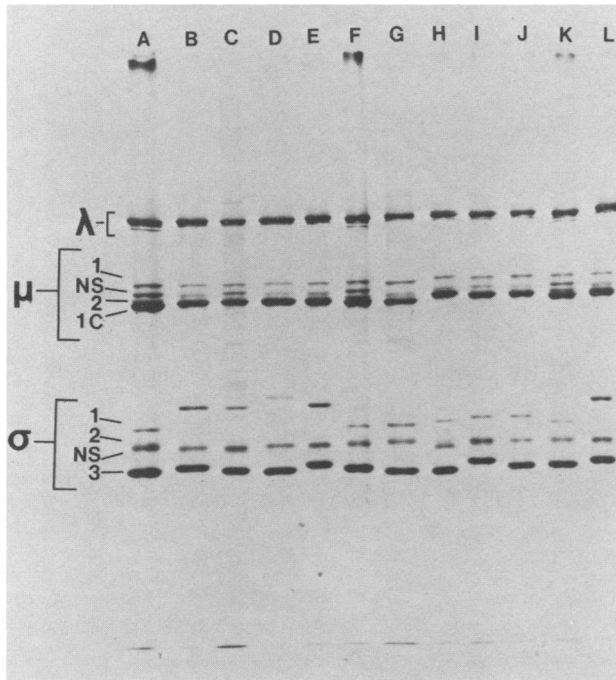
FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the polypeptides of reovirus natural-isolate strains. Viral polypeptides were labeled with [^{35}S]methionine in L-cell suspension cultures, immunoprecipitated from cell extracts, and then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography as described in the text. The strains from which viral polypeptides were obtained are: lane A, type 3 Dearing; lane B, type 1 Lang; lane C, type 1 clone 28; lane D, type 1 clone 50; lane E, type 1 Lang; lane F, type 3 Dearing; lane G, type 3 clone 8; lane H, type 3 clone 9; lane I, type 3 clone 18; lane J, type 3 clone 31; lane K, type 3 Dearing; lane L, type 1 Lang.

polypeptides were pulse-labeled with [^{35}S]methionine as described above. Viral polypeptides were then immunoprecipitated from infected cell extracts and resolved on discontinuous slab gels, and the gels were dried and autoradiographed (Fig. 1). As shown previously for type 1 Lang, type 2 Jones, and type 3 Dearing strains (16), the polypeptide species from different isolates within the same serotype show reproducible differences in electrophoretic migration. For example, compare the outer capsid polypeptides σ3 or σ1 of type 1 Lang and type 1 clones 28 and 50 (lanes B to D) or the σ3 or σ1 polypeptides of type 3 Dearing and type 3 clones 8, 9, 18, and 31 (lanes F to K). The σ1 polypeptide was the slowest-migrating σ polypeptide of each strain, whereas σ3 was the fastest-migrating σ polypeptide species. Note that the type 1 σ1 polypeptides (lanes B to D) all migrated slower than did the type 3 σ1 polypeptides (lanes F to K) (16). In contrast to this consistent serotype-specific difference, the σ3 and μ1C polypeptides varied in their migrations independent of the serotype.

Tryptic peptide structure of the μ1C polypeptide. We previously reported that the methionine-containing tryptic peptides of the μ1C polypeptides of our prototype strains type 1 Lang, type 2 Jones, and type 3 Dearing (human isolates) are highly conserved (8). To analyze further the degree of conservation of the μ1C polypeptide, we carried out tryptic peptide mapping of μ1C polypeptides from reoviruses isolated from diverse sources. Each strain (Table 1) was grown in L-cells in the presence of [^{35}S]methionine to label viral polypeptide species. After purification of virions

from infected cells, viral polypeptides were resolved on preparative slab gels and recovered, and their tryptic peptides were analyzed as described previously (8).

The peptide maps of the μ1C polypeptides from three type 1 and five type 3 reovirus strains are shown in Fig. 2. The origin of each strain is shown in Table 1. Note that two type 1 isolates (28 and 50) and two type 3 isolates (18 and 31) were obtained from a single bovine population between the years 1959 and 1961. The major numbered tryptic peptides of each strain were nearly identical, except that there was one peptide found only in the type 3 Dearing μ1C polypeptide (peptide 10) (Fig. 2A and B). The position where peptide 10 would be in the peptide map of clone 9 is indicated by an arrow. To confirm that the numbered peptides are conserved among strains, we analyzed the following mixtures of μ1C tryptic peptides: type 3 Dearing plus clone 8, type 3 Dearing plus clone 9, type 3 Dearing plus clone 18, and type 3 Dearing plus clone 31. As an example of these analyses, the peptide map of the mixture of tryptic peptides between clone 18 and type 3 Dearing μ1C polypeptides is shown (Fig. 2A, 3 + 18). The analysis of these mixtures confirms our observation that most of the peptides derived from the μ1C polypeptide are conserved. The peptide maps of the μ1C polypeptides from the type 1 strains are shown in Fig. 2B. Except for the absence of peptide 10, nearly all of the peptides were identical to each other as well as to those of the type 3 clones 8, 9, 18, and 31. (This result was also confirmed by analysis of the appropriate mixtures; e.g., Fig. 2B, 1 + 28 and 28 + 31.)

During the tryptic peptide analysis of the μ1C polypeptides from type 1, 2, and 3 prototype strains, we observed several faint peptides (which were not assigned numbers) in addition to the 8 to 10 numbered methionine-containing tryptic peptides (8). During the present analysis, we observed that several of these faint peptides were more prominent in the six natural isolates (Fig. 2A and B). To assist in determining the degree of conservation of these peptides, we have assigned them letters (A to D). A summary of the shared and unique μ1C-derived tryptic peptides is presented schematically in Fig. 2B. Of the 13 lettered or numbered methionine-containing tryptic peptides present in μ1C polypeptides, 10 were present in all eight type 1 and 3 strains (open circles), and two other peptides (B and D) were present in most of the strains examined. Peptide 10 is present only in the type 3 Dearing strain (8). The 13 methionine-containing tryptic peptides we have observed in μ1C polypeptides are compatible with the 16 methionines present in type 3 Dearing μ1C polypeptides (15).

Although the methionine-containing tryptic peptides of all the μ1C polypeptides we have examined are highly conserved (8) (Fig. 2), it is possible that another area of the μ1C molecule which does not contain any methionines might show more variability in peptide structure. To analyze this possibility, we carried out tryptic peptide analyses of type 1 Lang, type 2 Jones, and type 3 Dearing μ1C polypeptides labeled with a different amino acid (L-[^{3}H]lysine). Our results indicated that 24 of 26 lysine-containing tryptic peptides are shared among the μ1C polypeptides of the three serotypes (data not shown). These results confirm our observations that the μ1C polypeptide of reoviruses contains extensive regions of conserved amino acid sequence not only in our laboratory strains but in all the different natural isolates we have examined.

Tryptic peptide structure of the σ3 polypeptides of type 1 and 3 reovirus isolates. We reported previously that 40 to 60% of the tryptic peptides from σ3 polypeptides of the
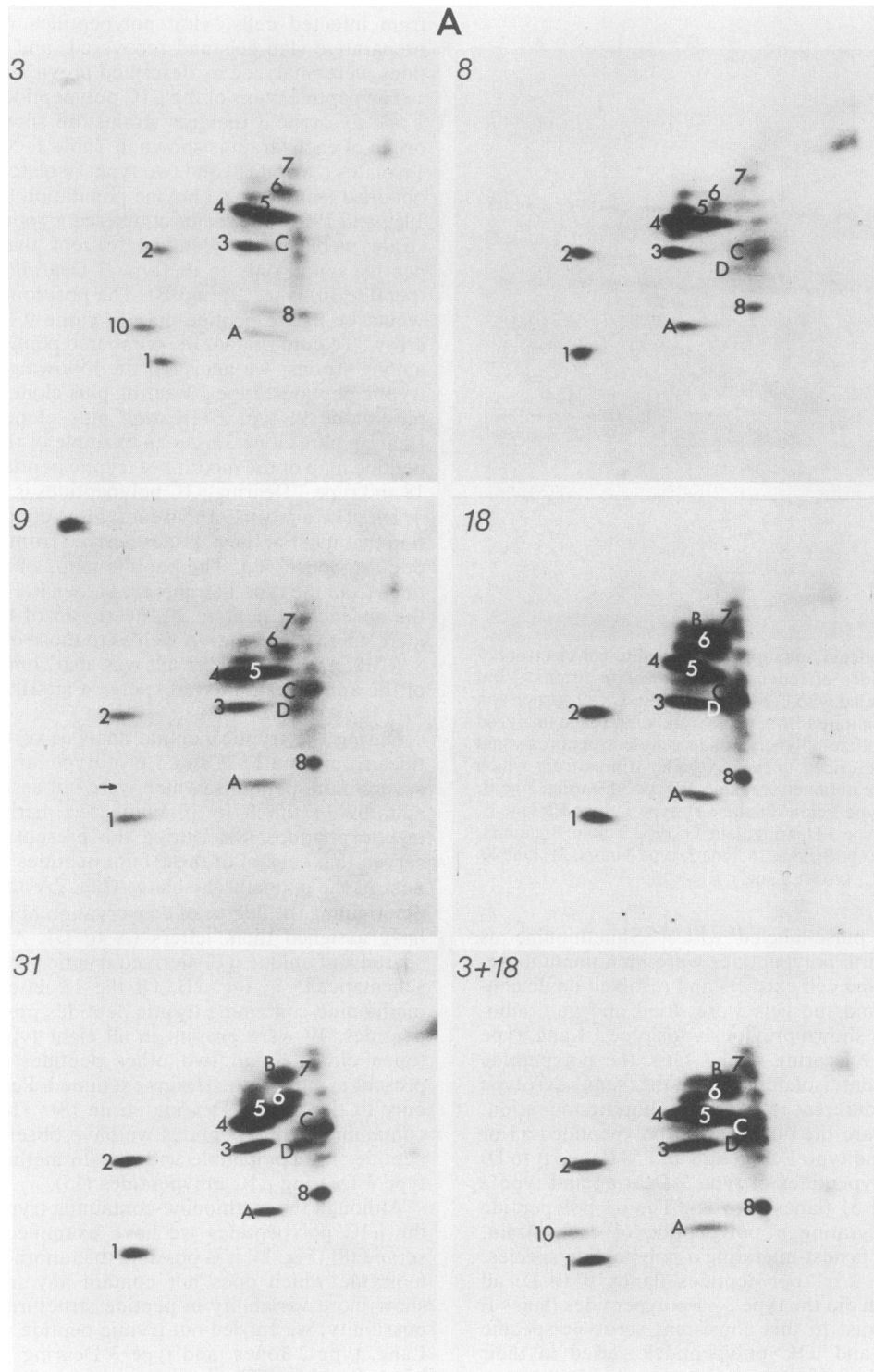
FIG. 2

human isolates type 1 Lang, type 2 Jones, and type 3 Dearing are conserved (8). This suggested that the σ3 polypeptide is less highly conserved than the μ1C polypeptide.

To study the σ3 polypeptides present in natural populations of reoviruses, we analyzed two type 1 and two type 3 strains isolated from a single bovine population (Table 1).

Tryptic digests of [³⁵S]methionine-labeled σ3 polypeptides from two type 1 bovine isolates and two type 3 bovine isolates were compared with each other and with our prototype human laboratory strains by two-dimensional thin-layer chromatography. The results are shown in Fig. 3 and Table 2. The numbering system adopted is the same as that
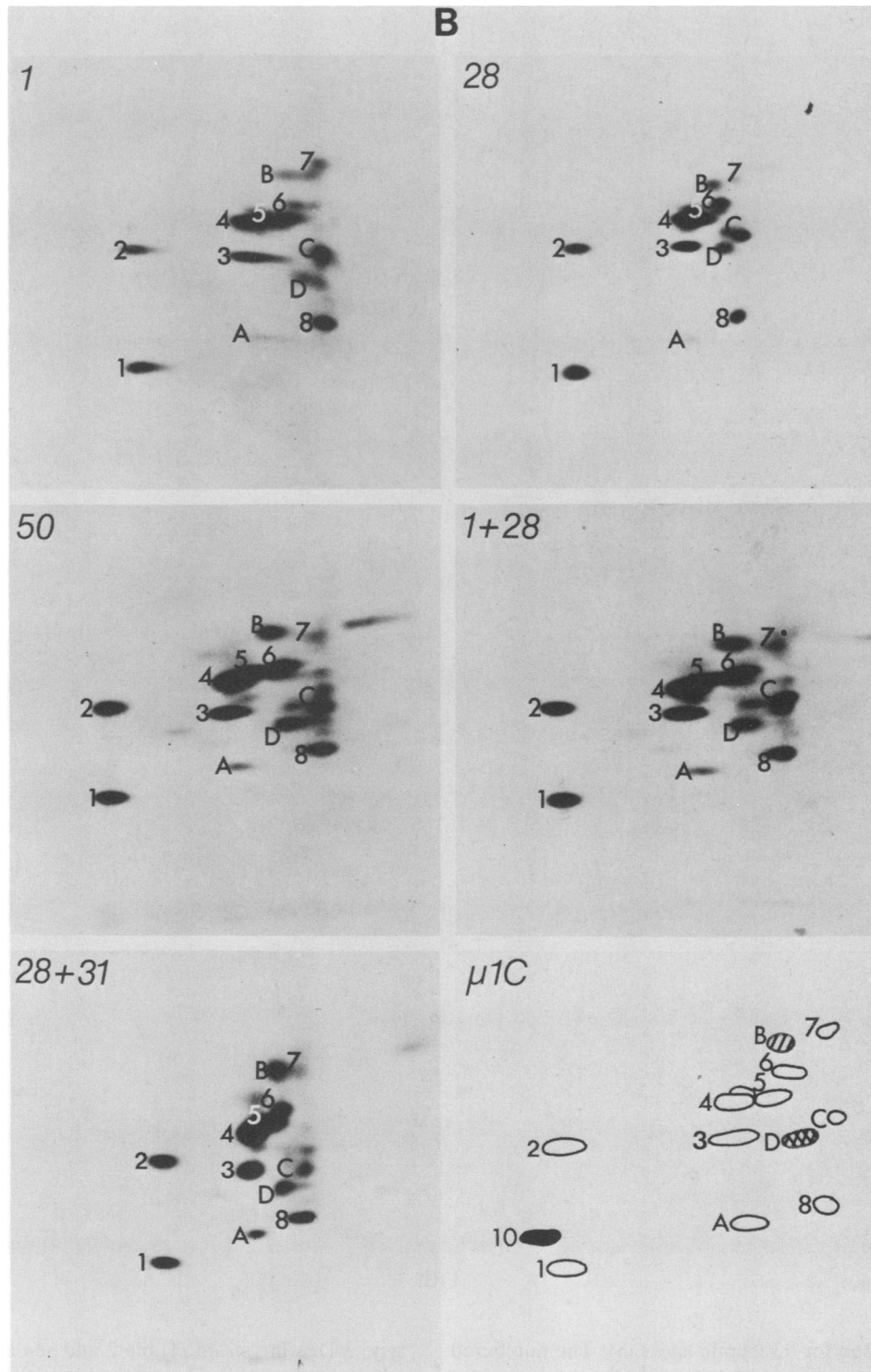
FIG. 2. Tryptic peptide maps of the μ1C polypeptides of type 1 and 3 natural-isolate strains. [^{35}S]methionine-labeled μ1C polypeptides were isolated, digested with trypsin, and analyzed by two-dimensional thin-layer chromatography and fluorography as described in the text. In these and subsequent peptide maps, peptides were spotted on the lower right corner. Peptides were then electrophoresed in the first dimensions from right to left and chromatographed in the second dimension from bottom to top. (A) Tryptic peptide maps of μ1C polypeptides from the following type 3 strains: type 3 Dearing (3), type 3 clone 8 (8), type 3 clone 9 (9), type 3 clone 18 (18), type 3 clone 31 (31), and a mixture of peptides from type 3 Dearing and clone 18 μ1C polypeptides (3 + 18). (B) Tryptic peptide maps of μ1C polypeptides from the following type 1 strains: type 1 Lang (1), type 1 clone 28 (28), type 1 clone 50 (50), a mixture of peptides from type 1 clone 28 and type 3 clone 31 (28 + 31), and a mixture of peptides from type 1 Lang and type 1 clone 50 (1 + 50). A schematic drawing of all of the methionine-containing tryptic peptides present in type 1 or 3 μ1C polypeptides is also shown. Tryptic spots (designated with open circles) were present in all strains examined. The spot present in only type 1 Lang and clones 18, 28, 31, and 50 (B) is indicated with a cross-hatched circle. Spot D (missing from type 3 Dearing) is indicated by a double-cross-hatched circle. The filled spot (10) was present only in type 3 Dearing.
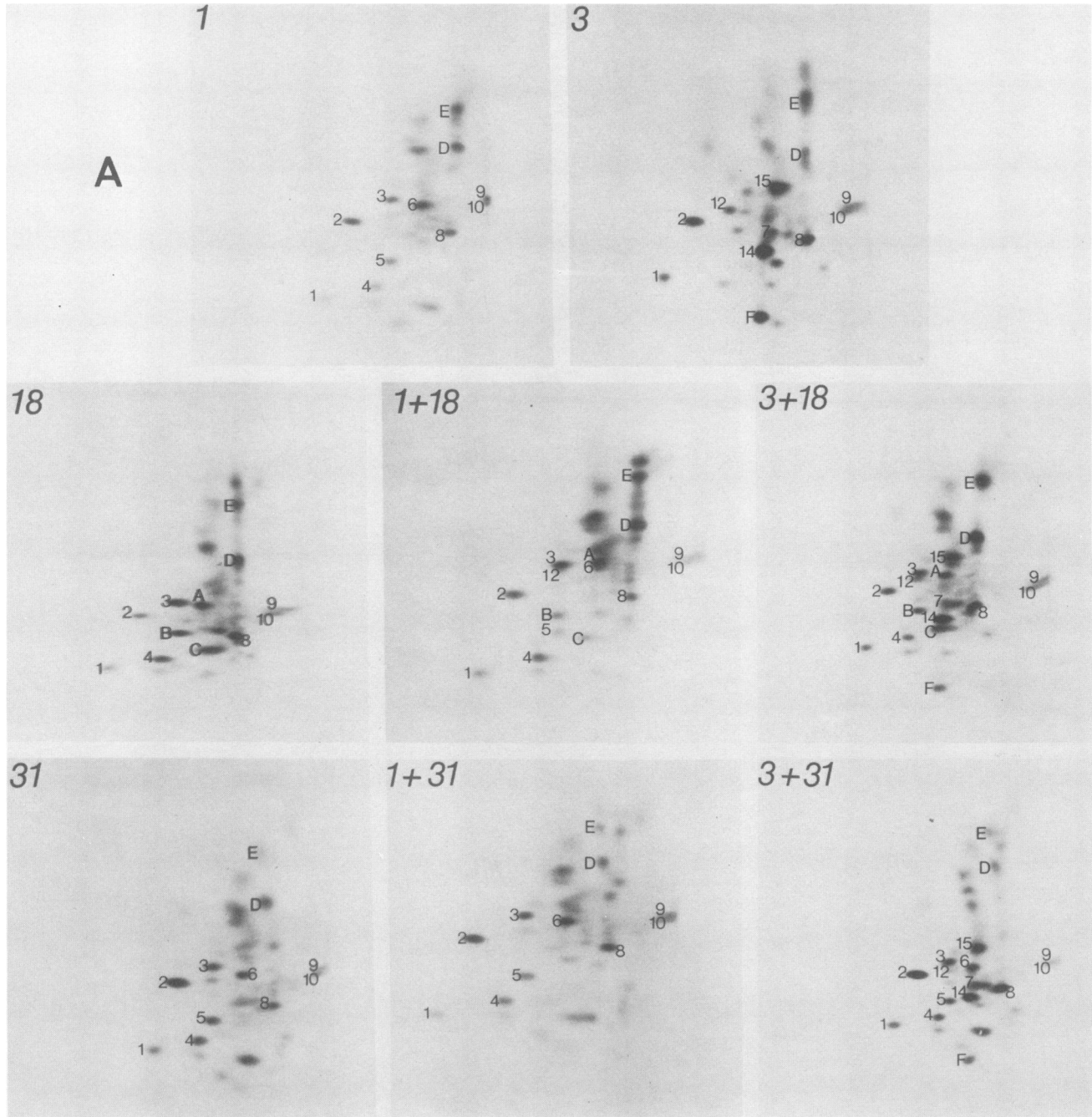
FIG. 3

previously described for σ3 tryptic spots (8). The numbered spots present in these maps represent only those which were clearly resolved in two or more independent tryptic digests. Faint spots or those running near the solvent front during chromatography were not numbered.

The tryptic maps of the σ3 polypeptides from the bovine type 3 isolates 18 and 31 were compared with the maps of σ3 polypeptides from our type 3 and type 1 laboratory strains (Fig. 3A). There were several notable differences between the peptide maps of clones 18 and 31 and that of the type 3 Dearing strain. Clones 18 and 31 and the type 1 Lang strain lacked peptides 12, 14, and 15, which were present in the

type 3 Dearing strain (Table 2 and see also reference 8). In addition, clones 18, 31, and the type 1 Lang strain contained two peptides (3 and 4) which were absent in the type 3 Dearing strain. Clone 18 also contained three new tryptic peptides (designated A, B, and C) which were absent from both type 1 Lang and type 3 Dearing strains (Table 2 and see also reference 8). Although peptides A and 6 were not easily resolved here, mixing experiments between clone 18 and 31 σ3 tryptic peptides (data not shown) and between type 1 and clone 31 σ3 tryptic peptides (Fig. 3A) showed that peptides A and 6 (and B and 5) are quite distinct. The σ3 polypeptide of type 3 clone 31 contained two peptides (5 and 6) which
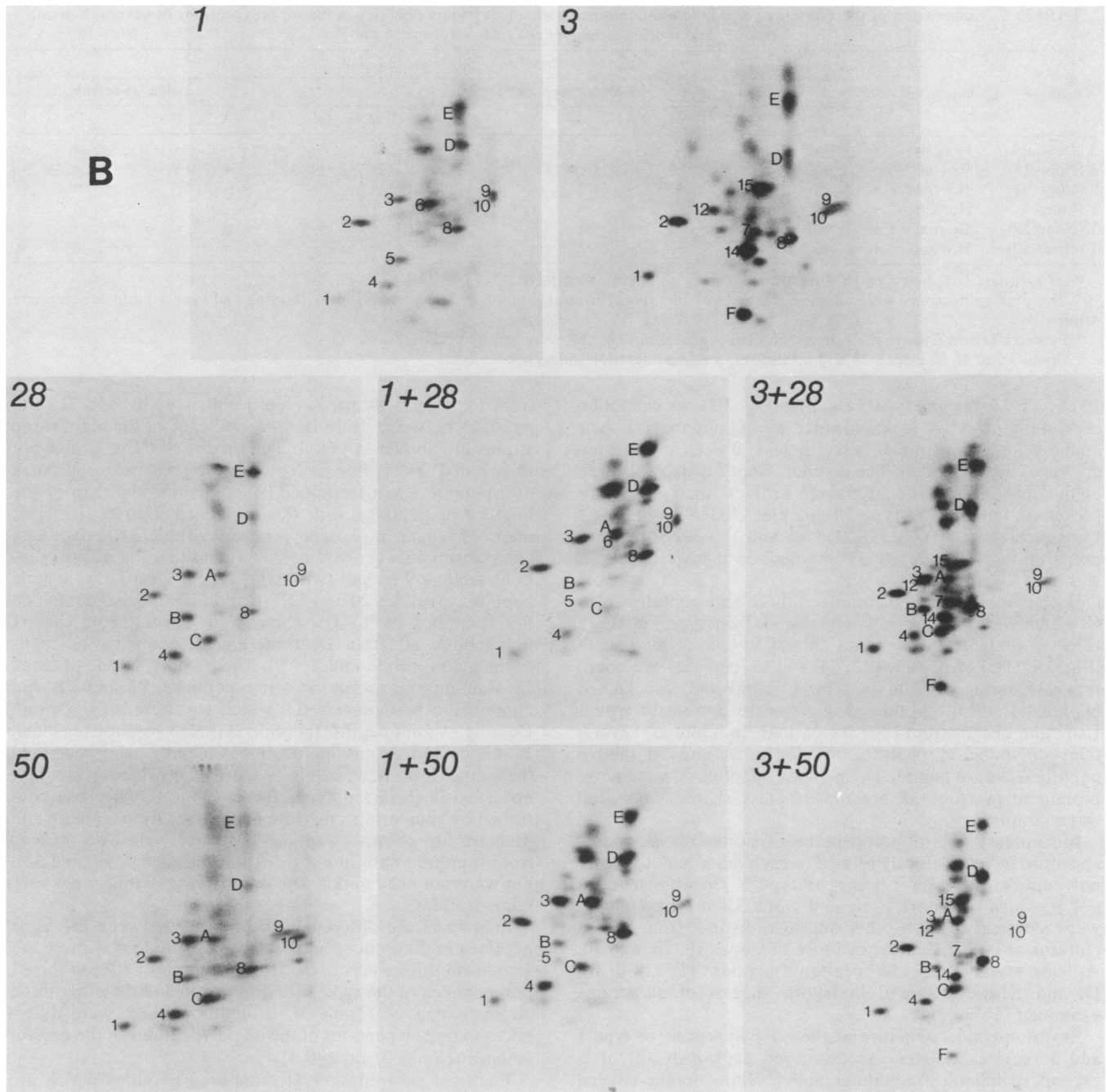
FIG. 3. Tryptic peptide maps of σ3 polypeptides from type 1 and type 3 natural isolate strains. [^{35}S]methionine-containing tryptic peptides from different σ3 polypeptides were analyzed as described in the legend to Fig. 1. (A) Type 1 Lang (1), type 3 Dearing (3), type 3 clone 18 (18), type 1 Lang plus type 3 clone 18 (1 + 18), type 3 Dearing plus type 3 clone 18 (3 + 18), type 3 clone 31 (31), type 1 Lang plus type 3 clone 31 (1 + 31), and type 3 Dearing plus type 3 clone 31 (3 + 31). (B) Type 1 Lang (1), type 3 Dearing (3), type 1 clone 28 (28), type 1 Lang plus type 1 clone 29 (1 + 28), type 3 Dearing plus 1 clone 29 (3 + 28), type 1 clone 50 (50), type 1 Lang plus type 1 clone 50 (1 + 50), and type 3 Dearing plus type 1 clone 50 (3 + 50).

were absent from type 3 Dearing but present in type 1 Lang σ3 polypeptides. In fact, when we analyzed a mixture of tryptic peptides from type 1 Lang and type 3 clone 31 (Fig. 3A), we found that they were essentially identical. This was a surprising result since type 1 Lang was isolated from humans, whereas clone 31 was a type 3 bovine isolate (Table 1).

A comparison of the peptide maps of σ3 polypeptides from the type 1 bovine strains 28 and 50 with type 1 Lang and type 3 Dearing strains is shown in Fig. 3B. We observed that the tryptic peptides of σ3 of clones 28 and 50 were closely related to each other (they were identical, except for a small difference in mobility of peptides 9 and 10). We confirmed this result by analyzing a mixture of σ3 tryptic peptides from clones 28 and 50 (data not shown). Although peptides 9 and 10 of both clones 28 and 50 comigrated with peptides 9 and

TABLE 2. Comparison of the conserved and unique methionine-containing tryptic peptides in the σ3 polypeptides of several human, bovine, and murine strains of reovirus serotypes 1 and 3

Serotype	Origin	Presence[a] (+) or absence (−) of:																	
		Prototype peptide[b]													New σ3 peptide				
		1	2	3	4	5	6	7	8	9	10	12	14	15	A	B	C	D[c]	E
3 (Dearing)	Human	+	+	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+
3 (clone 18)	Bovine	+	+	+	+	−	−	?	+	+	+	−	−	−	+	+	+	+	+
3 (clone 31)	Bovine	+	+	+	+	+	+	?	+	+	+	−	−	−	−	−	−	+	+
1 (Lang)	Human	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+
1 (clone 28)	Bovine	+	+	+	+	−	−	?	+	+[d]	+	−	−	−	+	+	+	+	+
1 (clone 50)	Bovine	+	+	+	+	−	−	?	+	+	+	−	−	−	+	+	+	+	+

[a] σ3 peptides 1, 2, and 8 to 10 were shared among all strains examined.

[b] The peptide numbers were adapted from a previous report from this laboratory (8), using type 3 Dearing and type 1 Lang as reference strains.

[c] Peptides D and E were faint in our original preparations (8) and were not numbered.

[d] Spots 9 and 10 of clones 28 and 50 showed a slight migration difference.

10 of type 1 Lang and type 3 Dearing (Fig. 3B), we cannot be certain that they are identical since we did not analyze their amino acid compositions. Nevertheless, it is clear that they are very closely related. The distinct "spot" which appeared below spot 10 in clone 50 was an artifact since it was not present in the maps of type 1 Lang plus clone 28 and type 1 Lang plus clone 50 (Fig. 3B, 1 + 28 and 1 + 50) or in other preparations of clone 50 σ3 polypeptide digests (data not shown).

Both clones 28 and 50 contained three new peptides (A to C) which were also present in σ3 digests from type 3 clone 18 (Fig. 3A and Table 2). Clones 28 and 50 (and also clone 18 [Fig. 3A]) lacked peptides 12, 14, and 15 (peptides present in type 3 Dearing but not in type 1 Lang) and also lacked peptides 5 and 6 (peptides 5 and 6 were present in type 1 Lang and absent from type 3 Dearing). In addition, several faint unnumbered peptides of clones 28 and 50 (above peptide A, left of peptide D, and above peptide E) were more prominent in clone 18 but nevertheless shared among all three strains.

In summary, the σ3 polypeptides of clones 18, 28, and 50 appear to be more closely related to each other than to the σ3 polypeptides of type 1 Lang or type 3 Dearing. The σ3 polypeptides of type 1 Lang and clone 31 (a type 3 clone) were identical to each other and also distinct from the σ3 patterns of type 3 Dearing or those of clones 18, 28, and 50. At least seven numbered or lettered peptides (1, 2, 8 to 10, D, and E) were shared in tryptic digests of all strains examined (Table 2).

Tryptic peptide structure of the σ1 polypeptide of type 1 and 3 reovirus isolates. As described previously (8), it is extremely difficult to obtain enough [³⁵S]methionine-labeled σ1 polypeptides to perform tryptic peptide analysis. As an alternate approach, we have utilized radioiodination of σ1 polypeptides in acrylamide gel slices (4). σ1 polypeptides labeled with ¹²⁵I were digested out of gel slices with trypsin, recovered, and subjected to two-dimensional thin-layer chromatography as previously described (4, 8). We have found that this method gives a reproducible number and distribution of tyrosine-containing tryptic spots. However, there are some differences in spot intensity from one preparation to the next. Although we have included some faint spots in our analysis to conform to the numbering system we described previously (8), we have based our conclusions only on tryptic spots which have been reproducibly intense and clearly resolved in two or more independent tryptic digests of the same σ1 polypeptide.

The tryptic maps of individual ¹²⁵I-labeled σ1 polypeptides

from the type 3 Dearing, type 3 clones 9, 18, and 31, and mixtures between strain Dearing and each of the other three strains are shown in Fig. 4. The origins of these strains are shown in Table 1. For each strain, the presence or absence of a peptide was determined by comparing the map of the mixture of peptides with those of each individual tryptic map. The data have been arranged (Table 3) to facilitate comparison between strains. We found that the σ1 polypeptide of clone 9 lacked two major type 3 Dearing σ1 tryptic peptides (peptides 20 and 22). In addition, clone 9 contained four peptides (A to D) which were absent from the σ1 polypeptide of strain Dearing (Fig. 4; compare the maps designated for 3, 9, and 3 + 9). The σ1 polypeptide of clone 18 was missing strain Dearing peptides 19 and 22 and contained a new peptide (D) which was absent from strain Dearing. A faint peptide (peptide 2) is also absent from clone 18. Clone 31 was missing peptides 20 and 22 and contained the same four new peptides (A to D) which were first observed in the σ1 polypeptide of clone 9. This was confirmed by comparing a mixture of peptides from clones 9 and 31 (data not shown). Peptides 8 and 17 were also missing from the three natural-isolate strains (clones 9, 18, and 31), but were not used in this discussion because they were very faint peptides.

In spite of the differences in intensity between the same peptides in different strains, we can conclude that there are significant differences in the tryptic peptides between the σ1 polypeptides of the type 3 Dearing strain and the other three strains examined (Table 3). In addition, there were differences in tryptic peptides of the σ1 polypeptides of the natural isolates (clones 9, 18, and 31).

Each gel showed several numbered peptides which are faint in these reproductions. All of them, however, were present in darker exposures of these same autoradiographs; we have used lighter exposures for Fig. 4 to optimize the presentation of the more intense σ1 tryptic peptides, upon which we have based our conclusions. (Similarly, we have confirmed the absence of a given peptide by exposing an autoradiograph five times longer than for the ones shown.) The faint peptides were included to conform to our previous numbering system (8). The peptide marked with an asterisk (Fig. 4, 3, 3 + 9, 3 + 18, and 3 + 31) has not been assigned a peptide number because it is usually very faint in tryptic peptide preparations of type 3 σ1 (8).

DISCUSSION

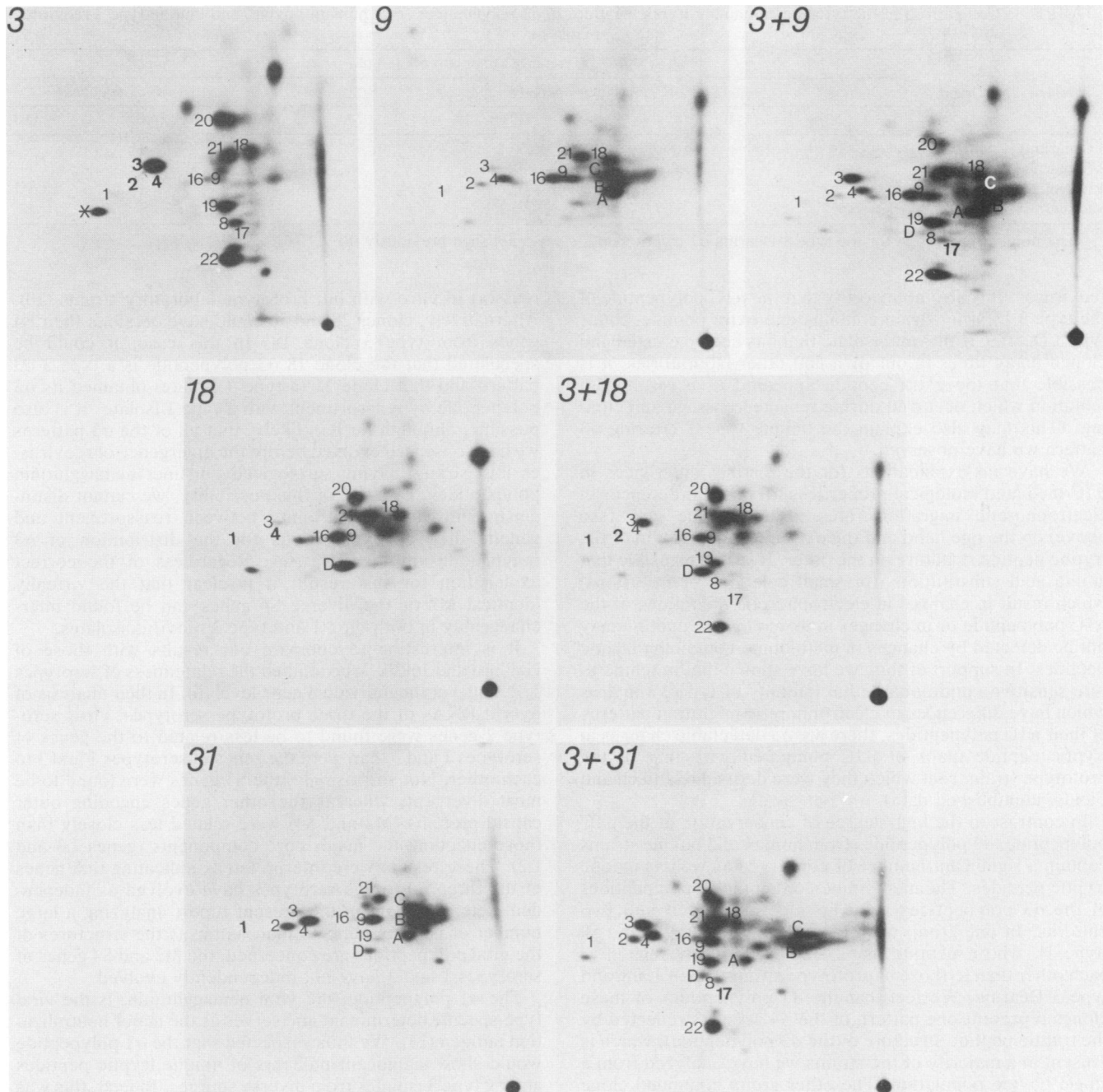The studies described in this report have confirmed and extended our previous data on the structural relationship of

FIG. 4. Tryptic peptide maps of σ1 polypeptides from type 3 natural isolate strains. σ1 polypeptides were iodinated in situ with [125]I (4), digested out of gel slices with trypsin, and then analyzed by two-dimensional thin-layer chromatography as described in the legend to Fig. 1 and in the text. Peptide maps of: type 3 Dearing σ1 polypeptide (3), type 3 clone 9 (9), type 3 Dearing plus type 3 clone 9 (3 + 9), type 3 clone 18 (18), type 3 Dearing plus type 3 clone 18 (3 + 18), type 3 clone 31 (31), and type 3 Dearing plus type 3 clone 31 (3 + 31).

the three outer capsid polypeptides of the prototype human reovirus strains type 1 Lang, type 2 Jones, and type 3 Dearing.

The tryptic peptide structure of μ1C polypeptides is highly conserved (8). Since we examined only human isolates and tryptic peptides containing methionine, we could not be certain whether there was significant variability in other regions of the μ1C molecule or among isolates from nonhuman sources. We have now shown that lysine-containing tryptic peptides present in μ1C polypeptides obtained from

type 1 Lang, type 2 Jones, and type 3 Dearing are also highly conserved (data not shown). Thus, the conservation of methionine-containing peptides is a true indication of the high degree of structural conservation of the μ1C molecule. The μ1C polypeptides of two bovine and one human type 1 isolate and two bovine, one murine, and two human type 3 isolates all showed the same high degree of conservation as that of the prototype virus. On the basis of these results, it seems likely that only one tryptic peptide pattern of the M2 gene product is present in natural populations of type 1 and 3

TABLE 3. Comparison of the tyrosine-containing tryptic peptides in σ1 polypeptides from human, bovine, and murine type 3 reovirus isolates

Serotype	Origin	Presence (+) or absence (−) of:																
		Prototype peptide[a]													New peptides			
		1	2	3	4	8	9	16	17	18	19	20	21	22	A	B	C	D
3 (Dearing)	Human	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
3 (clone 9)	Murine	+	+	+	+	−	+	+	−	+	+	−	+	−	+	+	+	+
3 (clone 18)	Bovine	+	−	+	+	−	+	+	−	+	−	+	+	−	−	−	−	+
3 (clone 31)	Bovine	+	+	+	+	−	+	+	−	−	+	−	+	−	+	+	+	+

[a] The numbering system for the type 3 Dearing σ1 tryptic peptide was published previously (8).

reoviruses. It is also noteworthy that the μ1C polypeptide of the type 3 Dearing strain contains one extra peptide. Since type 3 Dearing is the only isolate that has been passaged and cloned many times in our own and other laboratories, it is possible that the extra peptide appeared as a result of a mutation which occurred during repeated passage and cloning. (This may also explain the unique type 3 Dearing σ3 pattern we have observed.)

We have no explanations for the distinct differences in μ1C-mediated biological properties (10) or the differences in electrophoretic migration rates in acrylamide gels (see above) on the one hand and the extreme conservation of the tryptic peptide structure on the other. It seems plausible that amino acid substitutions (or small deletions or insertions) which result in changes in electrophoretic migrations of the μ1C polypeptide or in changes in its biological function may not be detected by changes in methionine-containing tryptic peptides. In support of this, we have shown that in temperature-sensitive conditional lethal mutants of type 3 reovirus which have differences in electrophoretic migration patterns of their μ1C polypeptides, there are no detectable changes in tryptic peptide maps of μ1C compared with that of the prototype strain from which they were derived (Gentsch and Fields, unpublished data).

In contrast to the high degree of conservation of the μ1C polypeptide, σ3 polypeptides from human and bovine strains contain a significant number of conserved as well as unique tryptic peptides. The methionine-containing tryptic peptides of the σ3 polypeptides from bovine isolates fell into two patterns. In one group were clones 18 (type 3), 28, and 50 (type 1), whose peptide maps are more closely related to each other than to those of prototype strains type 1 Lang and type 3 Dearing. We feel that the σ3 polypeptides of these clones represent one pattern of the S4 gene (as reflected by the tryptic peptide structure of the σ3 polypeptide), which is present in a majority of the strains we have analyzed from a single bovine population. The other group contained clone 31 (a type 3 isolate) and the laboratory strain type 1 Lang. This pattern may represent a second form of the S4 gene that is present in type 1 and type 3 isolates from different geographical areas. The σ3 polypeptide of type 2 Jones is distinguishable from both of these σ3 tryptic peptide patterns (8). Since we have only analyzed one type 2 isolate, we are not sure whether type 2 σ3 represents a third S4 gene pattern.

The different tryptic peptide patterns we have observed for σ3 polypeptides can be explained in two ways. The most likely explanation is that type 3 clones 18 and 31 have obtained their S4 genes (encoding the σ3 polypeptide) by reassortment with type 1 strains that were cocirculating in the population and whose σ3 polypeptides are closely related to those of clones 28 or 50 and type 1 Lang, respectively. It has recently been demonstrated that bovine strains can

reassort in vitro with our prototype laboratory strains (10). Alternatively, clones 28 and 50 could have obtained their S4 genes from type 3 clone 18. In this case, it could be speculated that the clone 18 σ3 polypeptide is a type 3 σ3 pattern and that clone 31 (a type 3 isolate) obtained its σ3 polypeptide by reassortment with a type 1 isolate. It is also possible, although far less likely, that all of the σ3 patterns we have observed evolved before the divergence of reoviruses into strains having serologically distinct hemagglutinin polypeptides. Because of this possibility, we cannot distinguish with absolute certainty between reassortment and genetic drift as explanations for the distribution of σ3 polypeptides in these viruses. Regardless of the correct explanation for this result, it is clear that the virtually identical M2 or the diverse S4 genes can be found interchangeably in both type 1 and type 3 reovirus isolates.

It is interesting to compare our results with those of Gaillard and Joklik, who studied the relatedness of serotypes 1, 2, and 3 at the individual gene level (6). In their analysis of hybrid RNAs of the three prototype serotypes, virus serotype 2 genes were found to be less related to the genes of serotypes 1 and 3 than were the genes of serotypes 1 and 3 to each other. Not surprisingly, the S1 genes were found to be most divergent, whereas the other genes encoding outer capsid proteins (M2 and S4) were related less closely than those encoding the major core components (genes L3 and L2). These results were interpreted as indicating that genes of the three prototype serotypes have evolved as independent sets. The data in the present report analyzing a large number of isolates indicate that, as far as the structures of the viral polypeptides are concerned, the M2 and S4 genes of serotypes 1 and 3 have not independently evolved.

The σ1 polypeptide, the viral hemagglutinin, is the viral type-specific determinant and serves as the major neutralization antigen (23). We thus suspected that the σ1 polypeptide would show significant numbers of unique tryptic peptides among type 3 isolates from diverse sources. Indeed, this was the case; about 1/3 of the tryptic peptides were conserved among all four isolates examined. A surprising result was the fact that a murine isolate was almost identical to one of the bovine isolates. This was particularly interesting since the σ1 polypeptides of two isolates from the same herd isolated 1 year apart differed in several peptides. Thus, either the two bovine isolates have undergone genetic drift or, alternatively, two distinct type 3 S1 gene patterns were present in the bovine herd during the 2-year period. We need to examine a larger number of bovine and murine isolates to distinguish between these possibilities.

An interesting question prompted from these data is whether antigenic changes in the type 3 σ1 take place. Studies by Burstin et al. (2) have shown that certain monoclonal antibodies directed against the type 3 Dearing HA polypeptide inhibited hemagglutination by type 3 Dearing

clones 9 and 31, and other type 3 natural isolates to a similar extent (clone 18 was not tested). Of two efficient neutralizing α-type 3 σ1 monoclonal antibodies studied, one had the same neutralization titer against strain Dearing as it did against clones 9 and 31. The other, however, neutralizes type 3 Dearing 25 times as efficiently as it does clones 9 and 31 (2). These data are compatible with the hypothesis that some antigenic differences exist in the HA molecules of clones 9 and 31 when compared with those of type 3 Dearing. In addition, antigenic variants of type 3 Dearing σ1 polypeptide have been selected in vitro in the presence of monoclonal antibodies against the neutralizing antigen (22). However, we have not yet been able to correlate differences in tryptic peptides with changes in the antigenicity of the HA polypeptide.

Future studies correlating genetic analyses of reovirus isolates with more detailed structural data should allow us to more clearly understand the precise contribution of genome segment reassortment versus mutation in generating the diversity seen among reoviruses in natural populations.

ACKNOWLEDGMENTS

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