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Polypeptide Composition of Poliovirions, Naturally Occurring Empty Capsids, and 14S Precursor Particles

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The three serotypes of poliovirus were compared with respect to their polypeptide composition. Type 1, 2, and 3 strains were clearly different from each other in the electrophoretic mobilities of their larger structural polypeptides. Some of the viral polypeptides formerly identified as single peaks (e.g., VP 2) were shown to contain multiple components, indicating that purified virions contain at least six polypeptides. Three type 1 strains were indistinguishable in their viral polypeptides. A quantitative estimate was made of the polypeptide composition of the type 1 Mahoney poliovirion, as well as of naturally occurring empty capsids and 14S precursor particles. The data are discussed in light of the antigenic differences among polioviruses and the possible modes of virion morphogenesis.

Previous studies have shown that picornaviruses generally are composed of multiple polypeptides (2, 14, 15, 16). Quantitative estimates of the molar ratios of the structural polypeptides have been made for ME virus (22) and a bovine enterovirus (14). The molar ratios of the four major components were found to be 1:1:1:1 for ME virus and 1:1:1:0.5 for the bovine enterovirus. In both cases the possibility remained that other minor components existed in the infectious particle. Recently, Vanden Berghe and Boeyé (25) reported that purified type 1 Mahoney poliovirions contained at least seven distinct structural polypeptides. The molar ratios were calculated to be 3 VP 1:4 VP 2a + 2b + 2c:4.5 VP 3a + 3b:5 VP 4.

The possibility that poliovirus particles contained more than four polypeptides was first recognized during experiments aimed at comparing the structural polypeptides of the different poliovirus serotypes. The experiments reported here are an extension of those preliminary findings and include an attempt to determine the stoichiometry of the structural polypeptides of the virion and of two poliovirus-related particles which may be precursors in the morphogenesis of the poliovirion.

MATERIALS AND METHODS

Polioviruses. The type 1 Mahoney (Ma) and type 1 Brunhilde (Br) strains were obtained from J. V. Maizel in 1965. The type 2 MEF$_1$ and type 3 Leon (Le) strains were purchased from the American Type Culture Collection and passaged directly in HeLa S3 monolayers. Type 1 LSc poliovirus was obtained from J. S. Youngner. After growth in HeLa cell suspension cultures, all viruses were initially purified by the detergent method previously described (20). The final virus preparation had a A_{260}/A_{280} of 1.68 to 1.70. Each serotype was tested for specific neutralization by homologous antiserum by using nonhomologous antisera as controls. Viral titers were determined by plaque formation by using the agar cell-suspension method of Cooper (3).

Cells. HeLa S3 cells were propagated in Eagle minimum essential medium containing 2 to 4 mM glutamine and 5% calf serum. No antibiotics were used in the routine cultivation of cells, but penicillin (100 U/ml) and streptomycin (100 μg/ml) were included in the medium just prior to virus infection.

Infection of cells and preparation of radioactive particles. The infection of cells with purified poliovirus at high multiplicities of infection (100 to 300 PFU/cell) in the presence of guanidine and actinomycin D (24), the preparation of cytoplasmic extracts by Dounce homogenization, the monitoring of viral RNA synthesis, and the isolation of labeled virions, empty capsids, and 14S precursor particles from sucrose gradients were described previously (18; B. A. Phillips and J. V. Maizel, Jr., Fed. Proc., p. 482, 1967).

Polyacrylamide gel electrophoresis of viral proteins. The procedures of Summers et al. (24) for the solubilization (dissociation and reduction) of viral polypeptides and their electrophoretic separation on

polyacrylamide gel columns were used routinely. In most experiments, 10% polyacrylamide gels (20 by 0.6 cm) were subjected to pre-electrophoresis at 50 V, 5 mA per gel for at least 5 h before use. However, no significant differences were noted when gels not subjected to pre-electrophoresis were employed. The precise conditions of electrophoresis are given in the figure legends. Gel fractionation was accomplished by using a Savant auto-gel divider and fraction collector.

Determination of radioactivity. Either Bray or Aquasol (NEN Corp.) scintillation fluid was employed. The results obtained for a given labeled preparation were not affected by the scintillation fluid used, although the recovery of radioactivity from the gel did depend on how the gel fractions were treated. Recoveries ranged from 50 to 100%. However, the results obtained (i.e., the relative distribution of radioactivity in viral peaks) were essentially the same for a given preparation regardless of the recovery attained. In double labeling experiments, discrimination settings were such that there was a 10% spillover of ^{14}C radioactivity into the ^{3}H channel and a 1% spillover of ^{3}H radioactivity into the ^{14}C channel.

Source of materials. The following radioisotopes were obtained from New England Nuclear Corp.: ^{14}C- and ^{3}H-labeled amino acid mixtures (0.1 mCi/ml and 1.0 mCi/ml, respectively), ^{14}C- and ^{3}H-labeled valine, leucine, and isoleucine (approximately 250 mCi/mmol for the ^{14}C isotopes and >2 Ci/mmol for the ^{3}H isotopes). The same qualitative and quantitative results were obtained when a reconstituted amino acid mixture or a mixture made from valine, leucine, and isoleucine was used to label viral products. Guanidine HCl (A grade) was purchased from Calbiochem (Calif.). NCS reagent (a quaternary ammonium base in toluene), used to extract polypeptides from acrylamide gel fragments, was obtained from Amersham/Searle (Arlington Heights, Ill.). Actinomycin D was a gift from Merck, Sharp and Dohme, Rahway, N. J.

Statistical analyses. A Student's *t* test for nonpaired data was applied to the data where indicated in the tables.

RESULTS

Comparison of polypeptides from poliovirus serotypes. To assess the reliability and precision of the methodology, two different preparations of type 1 Ma poliovirus, labeled with ^{14}C- and ^{3}H-amino acids, respectively, were subjected to co-electrophoresis on the same gel column. The results (Fig. 1A) showed that all ^{14}C and ^{3}H radioactive peaks were coincident with one another. The dissociation and electrophoresis of type 1 Br and type 1 LSc strains resulted in profiles which were similar to those obtained for type 1 Ma poliovirus. No differences in the mobilities of the four viral polypeptides (VP 1–VP 4) were discernible among the type 1 strains tested. A small amount of NCVP 6 (noncapsid viral polypeptide 6) was always detected in labeled virus partially purified in sucrose gradients, but when this virus was subsequently banded in CsCl gradients, no NCVP 6 was detected.

To obtain greater resolution with the polyacrylamide gels, electrophoresis was carried out over longer periods of time, namely, 24 to 25 h. Figure 1B shows the results obtained when two differently labeled type 1 Ma preparations were subjected to co-electrophoresis as described above. Under these conditions, two distinguishable VP 2 components (VP 2a, VP 2b) were detected. By comparing their migration relative to the VP 1 and VP 3 polypeptides, the molecular weights of VP 2a and 2b were calculated to be 30,000 and 27,500, respectively. Prolonged electrophoresis also usually resulted in the detection of a distinct shoulder to the right of the peak fraction of the VP 3 polypeptide; occasionally two distinct peaks were resolved (see Fig. 4).

Because of the antigenic relatedness of the type 1 strains, other serotypes were also examined using these techniques. Type 2 MEF_1 poliovirus, labeled with ^{3}H-amino acids and partially purified in sucrose gradients, was dissociated, reduced, and subjected to electrophoresis with a similarly treated ^{14}C-labeled type 1 Ma preparation (Fig. 2). A distinct and reproducible difference in the VP 1 polypeptides of the two viruses was detected; the VP 1 of the type 2 virus had a molecular weight of about 38,000 versus 35,000 for the VP 1 polypeptide of the type 1 strain. In contrast, no clear differences were seen in the NCVP 6 and VP 2 polypeptides. There sometimes was a rapidly migrating shoulder to the VP 3 polypeptide of the type 1 virus that was not apparent in the type 2 strain (Fig. 2).

To verify the differences noted between these two virus types, empty capsids, obtained from cells infected with one or the other virus and labeled with either ^{14}C- or ^{3}H-amino acids, were isolated by sucrose gradient fractionation and subjected to dissociation, reduction, and co-electrophoresis as above (Fig. 3). Again, a clear difference in the VP 1 polypeptides of types 1 and 2 poliovirus was detected. In addition, the VP 3 polypeptide(s) of the type 1 Ma empty capsids appeared to migrate slightly faster than that of the type 2 empty capsids. No differences in the NCVP 6 or VP 2 polypeptides were noted. The radioactivity found in fractions 15 to 25 was identified as NCVP 1 and NCVP 2 by co-electrophoresis with differentially labeled, infected cytoplasmic extract (data not shown). However, when empty capsids were further purified in

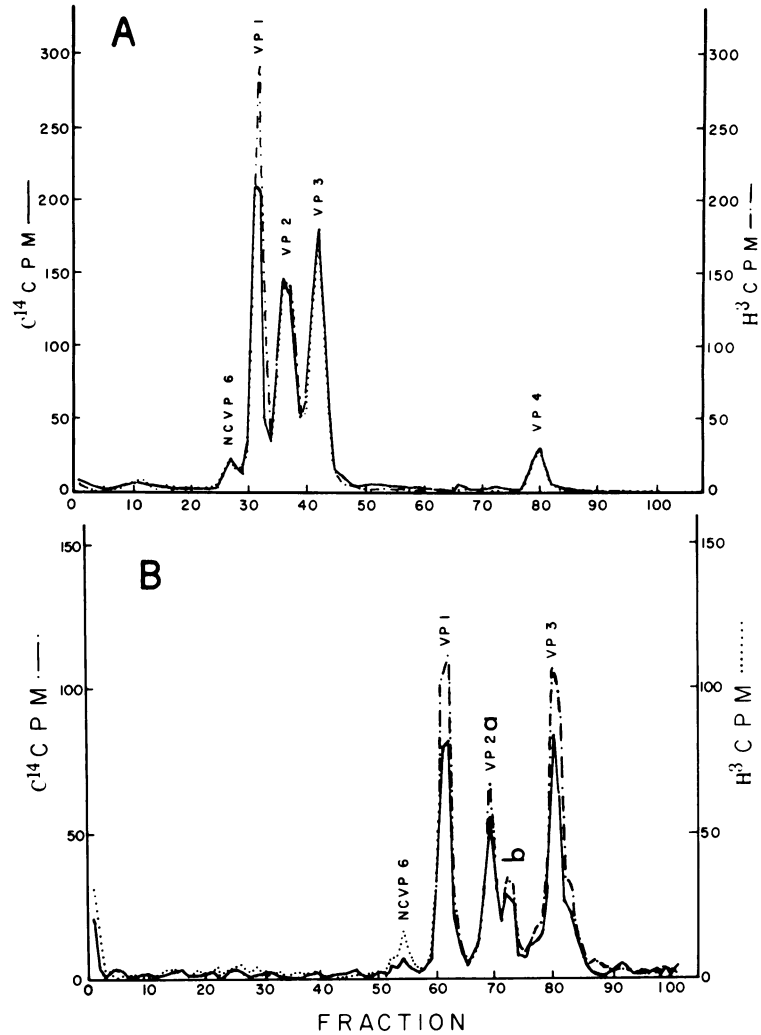

FIG. 1. *Comparison of the capsid polypeptides of ^{14}C- and ^{3}H-labeled type 1 Ma poliovirions. Radioactive poliovirus was isolated from infected HeLa cells which were incubated with either ^{14}C- or ^{3}H-amino acid mixtures from 2 to 6 h postinfection. A cytoplasmic extract in reticulocyte standard buffer (RSB) was prepared and fractionated in a 15 to 30% (wt/wt) sucrose-RSB gradient. After collecting the ^{14}C- and ^{3}H-labeled virion bands, the two preparations were mixed, dissociated, and reduced, and subjected to electrophoresis in 10% polyacrylamide gels at 80 V, 10 to 11 mA per gel, for 15 h (A) or 25 h (B). The anode is to the right.*

CsCl, no NCVP 1 or NCVP 2 was detected.

Figure 4 shows the electrophoretic comparison of type 3 Le poliovirus with type 1 Ma virions. Like the type 2 strain, the VP 1 of type 3 virions was significantly larger (migrated more slowly) than that of the type 1 virus. Type 3 virions contained two VP 2 components. One migrated like the VP 2a of the type 1 virion, whereas the other was significantly larger.

Differences in the structural polypeptides of types 2 and 3 virions were also detected (Fig. 5). As expected from the comparison with type 1 virus, the VP 1 polypeptides of these two viruses migrated almost at the same rate. It appeared that there were at least two VP 3 components in the type 3 virions of which the slower migrating one was coincident with a VP 3 component present in type 2 virions. A similar difference was noted for the VP 2 polypeptide(s). The slower moving VP 2 polypeptide of the type 3 strain was the predominant component, whereas the faster migrating one was the predominant component in the type 2 strain. In every instance, co-electrophoresis of ^{3}H- and

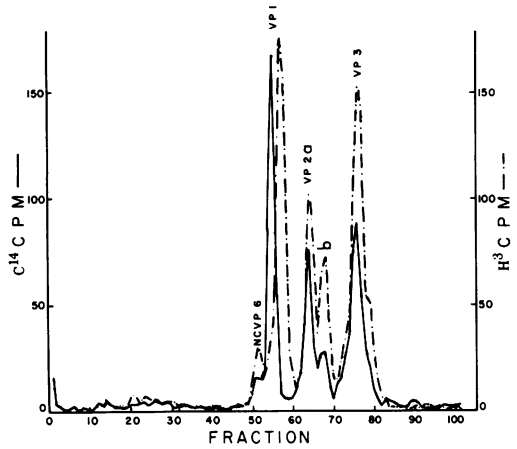

FIG. 2. *Comparison of the capsid polypeptides of ^{14}C-labeled type 2 MEF_1 and ^3H-labeled type 1 Ma poliovirions. The experimental conditions were identical to those described in Fig. 1. Electrophoresis was carried out for 25 h. The anode is to the right.*

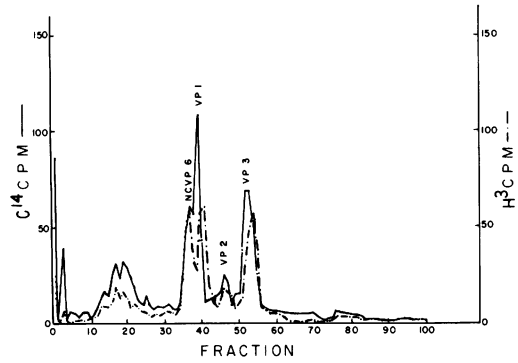

FIG. 3. *Comparison of the structural polypeptides of ^{14}C-labeled type 2 MEF_1 empty capsids and ^3H-labeled type 1 Ma empty capsids. Naturally occurring empty capsids were labeled and prepared using the same methodology described in Fig. 1. Electrophoresis was carried out for 15 h. The anode is to the right.*

^{14}C-labeled polypeptides of the same virus strain showed no differences between the respective viral polypeptides, as illustrated for the type 1 Ma strain (Fig. 1).

Percent distribution of viral polypeptides. Table 1 contains data compiled over a 5-year period for several type 1 strains as well as type 2, MEF_1, and type 3 Le strains. All of the strains contained nearly the same relative amounts of the VP 2 and VP 4 polypeptides. Although only limited data are available for the type 1 Br strain, it seems likely that, within the experimental error of these techniques, the relative amount of the VP 3 polypeptide(s) of this virus is about the same as that of the other viruses tested. In contrast, there were significant differences in the relative amounts of the VP 1 polypeptides among the type 1 strains. No differences were detected in the relative amounts of the VP 1 polypeptides in types 2 and 3 viruses.

Percent distribution of viral polypeptides in virions, empty capsids, and 14S precursor particles. Table 2 summarizes data compiled over several years on the relative distribution of radioactivity in the polypeptides of poliovirions and poliovirus-related particles obtained from HeLa cells infected with type 1 Ma poliovirus. These results indicated that, in the assembly of 14*S* particles (about 420,000 daltons) into empty capsids (about 5×10^6 daltons), there

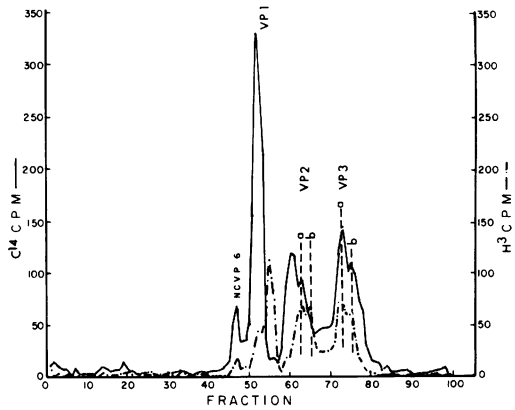

FIG. 4. *Comparison of the capsid polypeptides of ^{14}C-labeled type 3 Le and ^3H-labeled type 1 Ma poliovirions. Experimental conditions were the same as described in Fig. 1. Only the components of the type 1 Ma virus are identified. Electrophoresis was carried out for 25 h. The anode is to the right.*

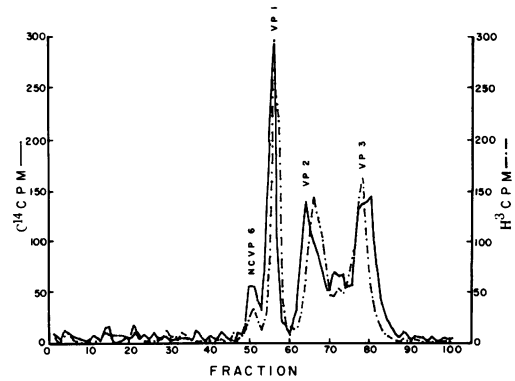

FIG. 5. *Comparison of the capsid polypeptides of ^{14}C-labeled type 3 Le and ^3H-labeled type 2 MEF_1 poliovirions. Experimental conditions were the same as described in Fig. 1. Electrophoresis was carried out for 25 h. The anode is to the right.*

TABLE 1. *Percent distribution of radioactivity in the virion polypeptides of poliovirus serotypes*

Virus	No. of preparations	No. of determinations	Viral polypeptide			
			VP 1	VP 2[a]	VP 3	VP 4
Type 1 Ma	6	18	23.9 ± 5.4	30.1 ± 6.2	33.0 ± 3.6	5.0 ± 1.5
Type 1 Br	2	2	32.4	31.5	27.7	5.8
Type 1 LSc	3	7	17.3 ± 2.4	29.2 ± 3.8	30.8 ± 4.7	4.7 ± 0.6
Type 2 MEF_1	2	6	28.5 ± 2.8	27.3 ± 2.6	35.2 ± 4.3	4.9 ± 1.0
Type 3 Le	2	4	27.3 ± 2.6	25.4 ± 4.0	32.4 ± 3.7	4.2

[a] For type 1 Ma virus: VP 2a = 16.8 ± 2.5; VP 2b = 9.7 ± 1.0.

TABLE 2. *Fraction of total radioactivity present in the polypeptides of type 1 Mahoney virions, empty capsids, and 14S particles*

Particle	Viral polypeptides					
	NCVP 1-2	NCVP 6	VP 1	VP 2	VP 3	VP 4
Virion (6)[a]	0	0.02	0.239 ± .054	0.301 ± .062	0.33 ± .036	0.05 ± 0.15
No. of polypeptides[b]		2.4 (0)	34 (36)	53 (60)[c]	68 (60 or 72)	41 (36)
Empty capsid (5)	0.113 ± 5.0	0.216 ± .040	0.218 ± .039	0.107 ± .027	0.274 ± .018	0
No. of polypeptides	5-6 (?)	26 (36)	30 (36)	18 (24)	55 (60)	0
14S particles (7)	0.05 ± .027	0.231 ± 5.6	0.211 ± 3.0	0.105 ± 0.8	0.235 ± 3.2	0
No. of polypeptides	0.2 (?)	2.3 (3)	2.4 (3)	1.5 (2)	4.0 (5)	0

[a] Number of preparations.

[b] Estimates of the number of each of the polypeptides were calculated by multiplying the particle weight by the fraction of radioactivity in a given polypeptide and dividing by the molecular weight of that polypeptide. The molecular weight of the 14S particle was assumed to be 403,000; empty capsids were assumed to be composed of twelve 14S particles (12 × 403,000) or 4.84 × 10^6 daltons; the virion capsid was assumed to arise from empty capsids through the loss of 36 NCVP 6 polypeptides and a gain in 36 VP 2a, 36 VP 4, and 12 VP 3 polypeptides with a resulting molecular weight of 4.93 × 10^6. The number in parentheses represents the hypothesized number of copies.

[c] VP 2a = 36 copies; VP 2b = 24 copies.

TABLE 3. *Net changes in the relative distribution (in percent) of radioactivity in the viral polypeptides of virions, empty capsids, and 14S particles*

Morphogenetic reaction	Viral polypeptide				
	NCVP 6	VP 1	VP 2	VP 3	VP 4
14S → Empty capsid	−1.5	+0.7	+0.2	+3.9[a]	–
Empty capsid → Virion	−21.6	+2.1	+19.4	+5.6[b]	+5.0
14S → Virion	−21.1	+2.8	+19.6	+9.5[b]	+5.0

[a] $P \sim 0.05$.

[b] $P < 0.01$.

was no gain or loss of radioactivity in the NCVP 6, VP 1, or VP 2 polypeptides (Table 3). However, there was an increase ($P \sim 0.05$) in the radioactivity associated with the VP 3 polypeptide(s). The amount of radioactivity found in the VP 3 polypeptides of virions (Table 2) was significantly greater than that found in empty capsids or 14S particles ($P < 0.01$; Table 3). These data also showed that 14S particles, as well as 73S empty capsids, contained a VP 2 polypeptide (probably VP 2b) as described earlier (B. A. Phillips and J. V. Maizel, Jr., Fed. Proc., p. 482, 1967). These experiments did not permit an assessment as to the origin of the VP 4 polypeptide; the data were not incompatible with the previous evidence that it arises from the cleavage of NCVP 6 (12).

The data were used to calculate the quantity of each polypeptide component in each of these particles. Because the radioactivity in the VP 3 peak was not routinely resolved clearly into distinct components, the data were dealt with

as if VP 3 was a single entity. In the case of the VP 2 complex, several electrophoretic analyses of virion preparations clearly resolved two components, thus permitting an estimation of the relative distributions and the molar ratios of VP 2a and VP 2b.

The results (Table 2) suggested that the 14S particles contained 13 polypeptides (4.03×10^5 daltons [18]); the empty capsids contained 156 polypeptides (4.84×10^6 daltons); and the poliovirions contained 204 polypeptides (assuming 72 VP 3 polypeptides: 4.93×10^6 daltons + 2.5×10^6 daltons of RNA). The major components detected in the 14S preparations only accounted for about 83% of the recoverable radioactivity. The remainder was distributed in several minor components, present in variable amounts in different preparations, and thus were not considered as part of the 14S particle. The stoichiometric composition of virions (VP 1:VP 2:VP 3:VP 4) was determined to be 3:4.5:6:3.

Evidence for changes with time in the polypeptide composition of type 1 Ma poliovirions. Over the years in the course of these and other experiments, type 1 Ma virus was passaged and, at irregular intervals, radioactive virus was prepared and subjected to electrophoresis as described above. Beginning in 1970, it became apparent that the relative amounts of the structural polypeptides appeared to have changed (Table 4). The most striking change was that the type 1 Ma virus contained only half the VP 1 polypeptide concentration it had contained originally. A smaller but apparently not significant reduction in the VP 3 polypeptide(s) also was detected. No such changes in the relative concentrations of the other viral polypeptides were noted.

Several experiments were performed to further investigate the apparent change in our virus preparation. Neutralization tests clearly confirmed that the virus was a type 1 poliovirus; antisera directed against types 2 and 3 viruses did not inactivate the virus, whereas anti-type 1 serum did inactivate it (4 $\log_{10}$ drop in titer after 15 min at 37 C). Another possible explanation was that the type 1 Ma virus stocks had become contaminated with another type 1 virus, such as the LSc strain; the latter virus strain was introduced into this laboratory in 1970. The LSc strain, in contrast to all of the other viruses handled in this laboratory, was known to be temperature sensitive (39 C). When the replication cycles of type 1 Ma and LSc stocks were tested for their sensitivity to 39 C, the temperature sensitivity of the LSc virus was confirmed (Table 5). This strain was apparently leaky in that a significant amount of progeny virus was produced (23-40 PFU/cell) at 39 C. In contrast, later stocks of type 1 Ma virus (1971) produced about the same amount of virus as the original stock virus at 37 C, but showed a twofold drop in yield at 39 C. This finding would be expected if the later stocks of T1 Ma in fact were composed of equal amounts of the T1, Ma, and LSc strains. However, such a virus population should exhibit a significantly higher concentration (about 21%) of the VP 1 polypeptide than that found (14.7%).

Finally, the plaquing efficiency of the type 1, Ma (1971) stocks was similar to the earlier stocks of virus and was clearly different from the type 1, LSc strain (Table 5).

DISCUSSION

A careful analysis by polyacrylamide gel electrophoresis of the polypeptides of prototype strains of three poliovirus serotypes revealed clear and reproducible differences in the major components of the virion. These differences also were detected in the naturally occurring empty capsids obtained from infected cells (Fig. 3). The results indicate that the different serotypes contain differences in all three of the major components. In contrast, no qualitative differences were detected in any of the polypeptides of the three strains of type 1 polioviruses tested. A quantitative difference was measured for the VP 1 polypeptide of the type 1 strains (Table 1), whereas the polypeptides of the type 2 and type 3 strains tested were different qualitatively but not quantitatively (Fig. 2, 4, and 5, Table 1). (In spite of the fact that the LSc strain contained

TABLE 4. *Changes in the relative amounts of structural polypeptides of type 1 Mahoney poliovirions with prolonged passage*

Time period	No. of preparations	No. of determinations	Viral polypeptide[a]			
			VP 1	VP 2	VP 3	VP 4
1966-1967	2	8	27.9 ± 3.4	30.2 ± 5.4	34.7 ± 1.9	4.8 ± 1.2
1970-1972	4	6	14.7 ± 2.4	29.1 ± 5.8	29.1 ± 2.4	5.3 ± 1.3

[a] The significance of VP 1 was $P < 0.01$. The other viral polypeptides were not significant ($P > 0.05$).

significantly less VP 1 polypeptide, ^{14}C-LSc virions and ^{3}H-type 1 Ma virions were found to band coincidently after isopycnic centrifugation in CsCl [data not shown].) No differences were detected in the VP 4 polypeptides of any of the viruses tested. However, in 10% polyacrylamide gels, the VP 4 polypeptide migrated almost as rapidly as the dye marker (bromophenol blue); therefore, the resolving power of these gels would be expected to be very limited with regard to polypeptides the size of VP 4 (about 6,000 daltons).

These results are of particular interest in that Breindl (1) recently showed that the VP 4 polypeptide was the antigenic determinant of the "D" specificity manifested by intact virions. Anti-D antibody reacted with the virion in such a way that subsequent exposure of the virion-antibody complex to pH 12 resulted in the release of the antibody-VP 4 complex (which was unable to react with virions) and a particle exhibiting the "C" antigenicity. That VP 4 resides on the surface of the virion is further supported by its specific release following virus attachment to susceptible cells (5).

The results obtained here suggest that if the VP 4 component is the sole determinant of the D antigenic state, then it must mask the other structural components. Alternatively, the D specificity may be determined by components in conjunction with the VP 4 polypeptide, but only the latter is released with the antibody after alkali treatment. The finding that each of the virus types contained different VP 1 and VP 3 polypeptides indicates that the empty capsids of each serotype should exhibit unique antigenicities, for which there is some indirect evidence (8), although it does not rule out the existence of common determinants as well. For example, the NCVP 6 polypeptides in the empty capsids of each of the virus serotypes (Fig. 3 and unpublished experiments) always exhibited the same relative mobility. This finding is particularly interesting in light of the proposal that the VP 4 polypeptide, which must be unique for each serotype if it is the D determinant, arises from the cleavage of the NCVP 6 polypeptide. It is possible that all of the different poliovirus serotypes produce the same NCVP 6 polypeptide but that the cleavage reaction that gives rise to VP 2 and VP 4 occurs at a site unique for each serotype. It must be emphasized that the methodology employed in these studies is one that discriminates on the basis of molecular weight (6) and very likely would not resolve polypeptides of similar molecular weight which have unique amino acid sequences.

One of the aims of this study was to attempt to determine the stoichiometry of the structural polypeptides of virions as well as poliovirus-related particles suspected of playing a precursor role. There is evidence that 14S particles are precursors to viral empty capsids (18, 19; B. A. Phillips and J. V. Maizel, Jr., Fed. Proc., p. 482, 1967) as well as to virions (10). In addition, Jacobson and Baltimore (12) presented evidence that the empty capsid may be a direct precursor in the formation of virions.

The results shown in Tables 2 and 3 indicate that the assembly of 14S particles into empty capsids probably occurs without the addition or loss of polypeptides. The increase measured for VP 3 is of marginal significance and probably is attributable to experimental variations in the determinations. Certainly the simplest explanation would be one assuming no qualitative or quantitative changes. This is supported by the capability of 14S particles to self-assemble into

TABLE 5. *Temperature sensitivity of the replicative cycles of type 1 poliovirus strains*

Virus	Yield (PFU/ml × 10^{8})[a]			Plaquing efficiency (PFU/ml × 10^{10})		
	37 C	39 C	39/37	37 C	39.5	39.5/37
Type 1 Mahoney, 1966	11.2 ± 2.8 (280)[b]	8.8 ± 4.8 (220)	0.8 ± 0.2	710 ± 50 (8-9)[c]	420 ± 20 (5-6)	0.6
Type 1 Mahoney, 1971	10.3 ± 4.6 (257)	4.7 ± 0.5 (120)	0.5 ± 0.2	630 ± 130 (7-8)	500 ± 50 (4-5)	0.8
Type 1 LSc	6.5 ± 2.1 (162)	1.3 ± 0.3 (32)	0.2 ± 0.2	3.8 ± 0.9 (5-7)	0.55 ± 0.22 (1-3)	0.14

[a] A cell suspension (4 × 10^{6} cells/ml) in medium missing serum was divided into three 20-ml portions and infected with each strain at an MOI of about 5.0. After 1 h at 37 C, each cell culture was centrifuged, the cells were resuspended in medium containing serum, and one-half of each culture incubated at 37 C and 39.2 C (± 0.1), respectively. At 6.5 h postinfection, the cells were pelleted, resuspended in 0.02 M PO_4 buffer (pH 7.0), and freeze-thawed three times. After spinning down the cell debris, the supernatant fluid was assayed for viral infectivity at 37 C. The results are the average of two experiments.

[b] Plaque-forming unit per cell.

[c] Plaque diameter in millimeters.

a 73S empty capsid (19). However, a significant difference in the amount of radioactivity migrating as the VP 3 polypeptide(s) was found when virions were compared to either the empty capsids or the 14S particles. If the above assumption concerning no change in the structural components during the conversion of 14S into empty capsids is true, then a component, migrating in the same region of VP 3, becomes part of the virion presumably when the RNA becomes encapsidated. Vanden Berghe and Boeyé (25) were able to detect three VP 2 components in type 1 Ma poliovirions, of which one (VP 2c) was present in small amounts (5%) and migrated between VP 2b and VP 3a. It is possible that this component, which we failed to detect, migrated in the VP 3 region of our gels.

The results (Table 2) also indicate that 14S particles and 73S empty capsids contain a small amount of a VP 2 component (VP 2b). Therefore, it is proposed that the NCVP 6 polypeptide is cleaved into the VP 2a component at a later stage of virion formation and this accounts for the presence of two VP 2 components in the virion.

In spite of repeated attempts using a variety of negative-staining techniques, we have failed to visualize capsomeres in highly purified poliovirion preparations. It has come to our attention that other conscientious attempts to demonstrate capsomeres or any meaningful surface detail also have been unsuccessful (J. V. Maizel, personal communication). To our knowledge there is only one report (17) claiming to have demonstrated capsomeres in poliovirions and, even in this study, techniques for contrast enhancement had to be employed because direct micrographs failed to resolve capsomeres. On the other hand, crystallographic experiments indicate that poliovirus particles possess icosahedral (5:3:2) symmetry (7). We believe that empty capsids are formed by the self-assembly of twelve 14S particles, each positioned at the 12 vertices of an icosahedron, and that, upon encapsidation of the viral RNA, reactions involving the cleavage of NCVP 6 to form VP 2a + VP 4 further alter the capsid structure. These ideas are in accord with the model proposed by Rueckert et al. (22) in which polio and ME viruses were best represented by an icosahedron of the T = 1 class. It is even conceivable that the 14S particle is produced directly by the folding and cleavage of precursor polypeptide(s) (NCVP 1?) and thus is the formal equivalent of a structure unit. This implies that, for any given viral strain, NCVP 1 (about 110,000 daltons) is cleaved to form a VP 1, a VP 2b, and at least two VP 3 components; the latter polypeptides total about 118,000 daltons. Alternatively, there may be ambiguity in the cleavage reactions so that, for example, VP 3a and VP 3b exist in different particles (4).

Finally, the polypeptide composition of the type 1 Ma virus used in these experiments changed over a 3- or 4-year period (Table 5). This change may be the result of a selection process exerted by the host cells. Another possible explanation is that the later stocks of type 1 Ma virus contain an increased amount of a defective particle which contains less (or no) VP 1. The possibility that the Mahoney strain was contaminated with a temperature-sensitive virus (LSc strain) seems unlikely. The LSc strain always produced about ⅓ to ½ the virus yield at 37 C as the type 1 Ma virus. One would expect this virus, therefore, to be at a disadvantage over a period of years and thus should comprise only a very small proportion of a virus population containing wild-type virus. If such were the case, its presence would not influence the quantitative measurement of the structural proteins of the virus population. In addition, the 1971 stocks of type 1 Ma virus did not show the significant reduction in plaquing efficiency at 39.5 C characteristic of LSc virus. Also, LSc virus always produced a smaller plaque, especially at the elevated temperature whereas the 1971 type 1 Ma virus produced larger plaques characteristic of the original type 1 Ma stock virus. Another possibility may be the action of a cytoplasmic proteinase recently demonstrated in infected cells (9) and found to be associated with another picornavirus, Mengo virus (11). However, we have no evidence at this time for such a viral-associated enzyme activity.

If virus populations can undergo shifts which result in quantitative changes in their structural composition, this may account for the different estimates reported by different workers for the stoichiometric composition of type 1 Ma poliovirions.

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