JOURNAL OF VIROLOGY, Apr. 1980, p. 73-84
0022-538X/80/04-0073/12$02.00/0

Vol. 34, No. 1

Comparative Biochemical Studies of Type 3 Poliovirus

PHILIP D. MINOR

Division of Viral Products, National Institute for Biological Standards and Control, Hampstead, London NW3 6RB, United Kingdom

A study of the biochemistry of type 3 poliovirus strains which involves the examination of the virus-coded polypeptides in infected cells and the preparation of oligonucleotide maps is reported. The polypeptide patterns were shown to be a relatively stable property of virus strains and distinguished Sabin vaccine strains from wild strains of poliovirus type 3. This approach may be of value in deciding the origin (vaccine or nonvaccine) of field isolates of poliovirus. Oligonucleotide maps were found to be sensitive indicators of differences among strains and appear to form a basis for determining genetic relationships among strains. The nucleotide maps of two viruses isolated from human cases of paralytic poliomyelitis temporally associated with the administration of attenuated vaccine suggested a vaccine origin for the strain. In one case the nucleotide map was indistinguishable from that of the vaccine strain.

Although the molecular biology and genetics of poliovirus have been studied extensively (6), with few exceptions (15) these methods have not been applied to comparisons of poliovirus strains. The biochemical basis of variations among strains is of considerable interest in itself and is potentially of practical value on several grounds. Biochemical methods may be able to demonstrate unambiguous relationships among strains or to detect subtle differences among related strains. They may, therefore, provide valuable epidemiological tools or information on the evolution of virus strains under various conditions either in the wild or under controlled culture conditions as in live virus vaccine manufacture.

The poliovirus vaccines currently in use originate from strains derived by Sabin (19), and certain differences have been shown to exist between the original virulent virus strains and the attenuated strains derived from them (1, 8, 9). It has not been clearly established that these are the only differences or whether the Sabin vaccine strains possess multiple mutations, any or all of which are required for the attenuated state. The biochemical basis for the loss of virulence is of interest and of obvious practical significance. It is possible that a biochemical marker for the virulence of polioviruses might be identified to supplement the information obtained from the currently used neurovirulence test in monkeys.

In populations with a high degree of immunity conferred by Sabin live polio vaccine, the few cases of poliomyelitis occurring tend to be attributed to a type 3 poliovirus (5), and the study reported here was therefore concerned with the comparative biochemistry of type 3 viruses. Two methods were employed: an examination of the polypeptides found in cells as a result of infection and the preparation of oligonucleotide maps from RNA of purified virions.

MATERIALS AND METHODS

Cell growth. HEp-2 cells (Cincinnati strain) were routinely grown at 37°C in 16-ounce (ca. 0.473-liter) glass bottles in Eagle basal medium containing 5% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.) and 0.88 g of sodium bicarbonate per liter (BME).

Virus stocks. Virus stocks were grown in HEp-2 cell cultures at 35°C and stored at −70°C in small samples. Stocks were at most two passes from the material received from outside sources. Strains were isolated in this laboratory or as shown in the tables. Strains isolated from paralytic cases prior to 1960 before oral polio vaccines had been developed (pre-OPV strains) are listed in Table 1; strains isolated by the World Health Organization from cases temporally associated with the administration of oral polio vaccine are listed in Table 2. Leon $12a_1b$ (19) Sabin vaccine batches used were 12B, 3J, 7E, 7I, 6AG, and 4a (by the code of Boulger et al. [2]) and five batches designated a, b, c, d, and e. Vaccines other than Leon $12a_1b$ used were USOL-D-BAC and USOL-D-BAC-VONKA, obtained from M. Böttiger and V. Vonka, respectively, and WM315 and WM326, obtained from H. Koprowski and J. Gear, respectively. Type 3 virus isolates from paralytic cases in England between 1973 and 1975 were obtained from Colindale Public Health Laboratory, London, United Kingdom, and are listed in Table 3. Isolates from an agammaglobulinemic patient over a period of 16 months are listed in Table 4.

Virus titers were assayed by infectivity in HEp-2 cells by the microtiter method (7) and expressed as $\log_{10}$ 50% tissue culture infectious doses per milliliter.

Infection of cells and preparation of samples. HEp-2 cells were seeded at 5×10^5 cells per 60-mm

dish the day before use and incubated at 34°C in an atmosphere of 3% CO_2. They were then infected with 0.1 ml of infected tissue culture fluid virus stock (>7.5 $\log_{10}$ 50% tissue culture infectious doses per ml), and virus adsorption was allowed to occur for 1 h at room temperature. Five milliliters of warmed BME was then added to each culture, and incubation was continued at 34°C. Under these conditions, a cytopathic effect was observed at 9 h postinfection. At 6 h after the virus addition, the medium was removed and the cultures were rinsed with Gey buffer. Ten microcuries of [^{35}S]methionine (700 Ci/mmol, 26.3 TBq/mmol; Radiochemical Centre, Amersham, England) was then added in 0.5 ml of Gey buffer, and incubation was continued at 34°C for another hour, after which time the cell sheets were washed with phosphate-buffered saline and the cells were harvested in 100 μl of phosphate-buffered saline with a rubber policeman. Twenty microliters of denaturing mix (8% sodium dodecyl sulfate [SDS], 8% β-mercaptoethanol, 8 M urea) was added, and the mixture was heated in a boiling water bath for 1 min before loading 20 μl (50 μg of protein) per sample onto the gel.

SDS-polyacrylamide gel electrophoresis. Electrophoresis of proteins was carried out on 10 to 30% linear gradient polyacrylamide slab gels. Gels were poured from the bottom with a Perspex constant-pressure gradient maker, initially with 30% acrylamide (BDH Chemicals Ltd., Poole, England), 0.141% methylenebisacrylamide (BDH Chemicals Ltd.), 0.1% SDS, 8% glycerol, and 0.372 M Tris-hydrochloride (pH 8.6) in the reservoir and 10% acrylamide, 0.047% methylenebisacrylamide, 0.1% SDS, and 0.372 M Tris-hydrochloride (pH 8.6) in the mixing chamber. A stacking gel of 5% acrylamide, 0.133% methylenebisacrylamide, 0.125 M Tris (pH 6.8), and 0.1% SDS was cast on the resolving gel. Electrophoresis buffer was 0.18 M glycine, 0.025 M Tris (pH 8.8), and 0.1% SDS (12). Samples were electrophoresed overnight at a constant 80 V, and the gels were fixed in 7% acetic acid–40% methanol–53% water for at least 2 h before drying on a sheet of Whatman 3MM paper between silicone rubber sheets under vacuum in a water bath at 90 to 100°C. Dried gels were exposed to Fuji Rx or Kodirex X-ray film for 3 days.

Preparation of ^{32}P-labeled virus RNA. For each virus, 20 glass test tubes (15 cm by 1.3-cm internal diameter) were each seeded with 10^7 HEp-2 cells, placed in a roller drum adjusted to hold the tubes horizontally, and rolled for 16 to 24 h at 34°C for the cells to attach to the glass. The medium was then decanted, and each tube was infected with 0.3 ml of virus stock diluted 1:10 in Hanks citrate buffer (140 mM NaCl; 5 mM KCl; 1 mM each $MgSO_4$, $CaCl_2$, and

TABLE 1. *Type 3 poliovirus pre-OPV strains isolated from paralytic cases*

Virus strain	Yr of isolation	Place of isolation	Source[a]
III-1	1951	New Mexico	CDC
III-2	1958	Indiana	CDC
III-5	1958	Washington, D.C.	CDC
III-7	1957	New Mexico	CDC
III-8	1957	Arkansas	CDC
III-9	1957	Georgia	CDC
III-10	1957	Georgia	CDC
III-12	1958	Vermont	CDC
III-19	1957	Missouri	CDC
III-23	1959	Maine	CDC
III-41	1956	Louisiana	CDC
III-374	1957	Japan	CDC
III-694	1957	Bombay	CDC
III-699	1957	Bombay	CDC
III-710	1958	Bombay	CDC
III-715	1958	Bombay	CDC
77689	1953	California	E.H.L.
77692	1956	California	E.H.L.
77693	1956	California	E.H.L.
77729	1953	California	E.H.L.
77730	1952	California	E.H.L.
77731	1953	California	E.H.L.
77732	1954	California	E.H.L.
77750	1954	California	E.H.L.
Leon	1937	California	ATCC
190	1952	Montana	NIH
30	1952	Canada	NIH

[a] CDC, Center for Disease Control, Atlanta, Ga.; E.H.L., E. H. Lennette, State of California Department of Public Health, Berkeley; ATCC, American Type Culture Collection, Rockville, Md.; NIH, National Institutes of Health, Bethesda, Md.

TABLE 2. *World Health Organization isolates of type 3 poliovirus from paralytic cases temporally associated with the use of oral vaccine*

Strain[a]	Source of material
101/0/8	Stool
102/0/8	Stool
103/0/8	Stool
104/0/8	Stool
106/0/8[b]	Medulla
109/0/8	Stool
110/0/8	Stool
111/0/8	Stool
114/0/8[b]	Spinal cord
115/0/8[b]	Medulla
116/0/3	Stool
118/0/5	Stool
119/0/8[b]	Spinal cord
122/1/4	Stool
131/1/7	Stool
132/1/7	Stool
146/1/8	Stool
152/1/8	Stool
153/1/8	Stool
156/1/10	Stool
161/1/10	Throat swab
158/1/10	Stool
177/2/7	Stool

[a] Strains within braces were isolated from the same individual. Full epidemiological and serological details of strains are to be published in the *World Health Organization Bulletin* (I. Dömök, manuscript in preparation).

[b] Fatal case.

TABLE 3. *Type 3 poliovirus isolates obtained from paralytic cases occurring in England between 1973 and 1975*[a]

Strain	Yr of isolation	City
20278	1973	Norwich
21746	1973	Gloucester
285	1974	Chester
5229	1974	Reading
9052	1974	Newcastle upon Tyne
11340	1974	Leeds
13118	1974	Sheffield
15282	1974	Newcastle upon Tyne
16057	1974	Leicester
10196	1975	Liverpool
13533	1975	Manchester
15521	1975	Taunton

[a] Strains were provided by Colindale Public Health Laboratory. There was no known administration of oral polio vaccine or specific contact with a recent vaccine e.

20 mM sodium citrate) supplemented with Eagle minimum essential medium, amino acids, vitamins, glucose, antibiotics, 25 mM HEPES buffer (N-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and sodium bicarbonate to 0.88 g/liter (P-free medium). After rolling for an additional hour at 34°C, 1 ml of P-free medium containing 160 μCi of carrier-free ^{32}P (Radiochemical Centre) and actinomycin D (Sigma Chemical Co., St. Louis, Mo.) to 0.6 μg/ml was added to each tube, and rolling was continued for another day. Cells were then lysed by freezing and thawing at −20°C, cell debris was removed by centrifugation (2,000 × *g*, 5 min), and solid ammonium sulfate was added at 40 g/100 ml of supernatant. Virus was then pelleted by centrifugation at 3,000 × *g* for 2 h at 4°C, and the pellet was dissolved in 3 to 6 ml of phosphate-buffered saline. Nonidet P-40 detergent (BDH Chemicals Ltd.) was added to 1%, and the whole harvest was layered on a 32-ml gradient of 15 to 45% RNase-free sucrose (Cambrian Chemicals, Croyden, United Kingdom), 10 mM Tris-hydrochloride (pH 7.4), and 50 mM NaCl. The gradient was spun on a Beckman L5-50 ultracentrifuge (SW27 head) at 24,000 rpm at 4°C for 4 h, and fractions of approximately 2 ml were collected by bottom puncture. Fractions containing labeled virus were pooled and extracted three times with phenol-chloroform-isoamyl alcohol (50:48:2) containing 0.1% dihydroxyquinoline. The RNA in the aqueous phase was precipitated twice with ethanol at −20 or −70°C. This process yielded RNA with an activity of 5×10^6 to 15×10^6 cpm.

Preparation of oligonucleotide fingerprints. The method for the preparation of oligonucleotide fingerprints, provided by J. F. Obijeski, was essentially as described by Lee and co-workers (13, 14). RNA extracted from the virus was digested without further purification with 20 U of T_1 RNase (Calbiochem, La Jolla, Calif.) in 10 mM Tris-hydrochloride, pH 7.4 (1 U/μl). In early experiments, RNA was purified on sucrose gradients before digestion, but this had no visible effect on the quality of the fingerprints obtained and was therefore subsequently omitted. Incubation was for 30 min at 37°C and terminated by the addition of 40 μl of 9 M urea and 40 μl of 50% glycerol saturated with bromophenol blue and xylene cyanol FF. The samples were electrophoresed in the first dimension on a gel (20 by 40 cm) of 8% acrylamide–0.1% methylenebisacrylamide–6 M urea brought to pH 3.25 to 3.3 with saturated citric acid. Electrophoresis buffer was 6 M urea brought to pH 3.25 to 3.3 with saturated citric acid, and electrophoresis was at 300 V at 4°C overnight until the bromophenol blue had migrated 20 cm. The second-dimension electrophoresis was performed on a gel (40 by 40 cm) of 23% acrylamide– 1.5% methylenebisacrylamide–0.05 M Tris-borate (pH 8.2). The electrophoresis buffer was 0.05 M Tris-borate (pH 8.2), and electrophoresis was at 500 V at 4°C until the bromophenol blue had migrated 19 cm from the bottom of the first-dimension gel. Gels were exposed to Cronex 4 X-ray film with an intensifying screen at 4°C.

RESULTS

Comparison of vaccine and paralytic type 3 polioviruses. Cells were infected with poliovirus strains as indicated, and the polypeptides were synthesized in the presence of [^{35}S]-methionine resolved by electrophoresis on linear gradient gels. Although all data reported here were obtained with HEp-2 cells, all virus strains have also been tested in HeLa cells. The strain of HeLa cells used was less susceptible to infection than were HEp-2 cells, and the course of infection was more prolonged. Although HEp-2 is the cell of choice, the same polypeptide pattern was observed in our HeLa strain if satisfactory infection was achieved. The same results were also obtained in Vero cells and secondary monkey kidney cultures, although these have been studied far less extensively. Figure 1 shows an autoradiogram of a gel of HEp-2 cell extracts. The uninfected track on the extreme left indicates the normal pattern of protein synthesis in these cells. The adjacent five tracks were from cells infected with Leon $12a_1b$ vaccines from various manufacturers and with varying degrees of neurovirulence (2), and the remaining five tracks were from cells infected with pre-OPV strains isolated from paralytic cases (Table 1). Virion proteins (VP0, VP1, and VP3) were iden-

TABLE 4. *Viruses isolated from a hypogammaglobulinemic patient fed monovalent type 3 oral poliovirus vaccine on 21 January 1962*

Strain	Date of isolation
H7405	5 March 1962
H18095	13 June 1962
HA	3 December 1962
HC	25 February 1963
HD	17 April 1963
HF	25 May 1963

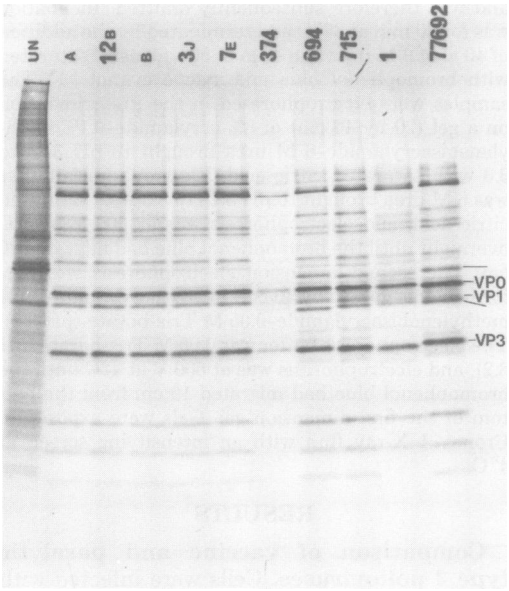

FIG. 1. *Autoradiogram of a 10 to 30% linear gradient gel of [^{35}S]methionine-labeled HEp-2 cell extracts. UN; Uninfected cells; A, 12B, B 3J, and 7E; cells infected with different batches of type 3 Sabin oral polio vaccine; 374, 694, 715, 1, and 77692, cells infected with type 3 pre-OPV strains isolated from paralytic cases.*

tified by co-electrophoresis of purified [^{35}S]methionine-labeled full and empty capsids. Seven different viruses and cells infected with them were examined in this way (data not shown). Host cell protein synthesis was almost completely shut off as a result of infection.

A striking feature of the pattern of polypeptide synthesis was the presence of two additional polypeptides in the cells infected with the nonvaccine virus which were completely absent from the cells infected with Leon 12a$_1$b strains (lines in Fig. 1). The migration rates of these polypeptides varied slightly but consistently from strain to strain. Figure 2 shows an autoradiogram of a gel of extracts from cells infected with 14 pre-OPV strains isolated in various countries from paralytic cases. The extra bands were present in all extracts except 12 and possibly 9. (Strain 77689 gave a poor infection.) Virus 9 gave a unique pattern in which two polypeptides were resolved in the VP0 region. Virus III-12 has been reported to be serologically vaccine-like (17). Figure 3 shows infected cell extracts for four vaccine batches (4I, 6AG, c, and d), one of which (6AG) was of a low titer, resulting in poor infection. The extra polypeptides were clearly absent from the tracks when satisfactory infection was achieved. The remaining five tracks were of extracts from cells infected with

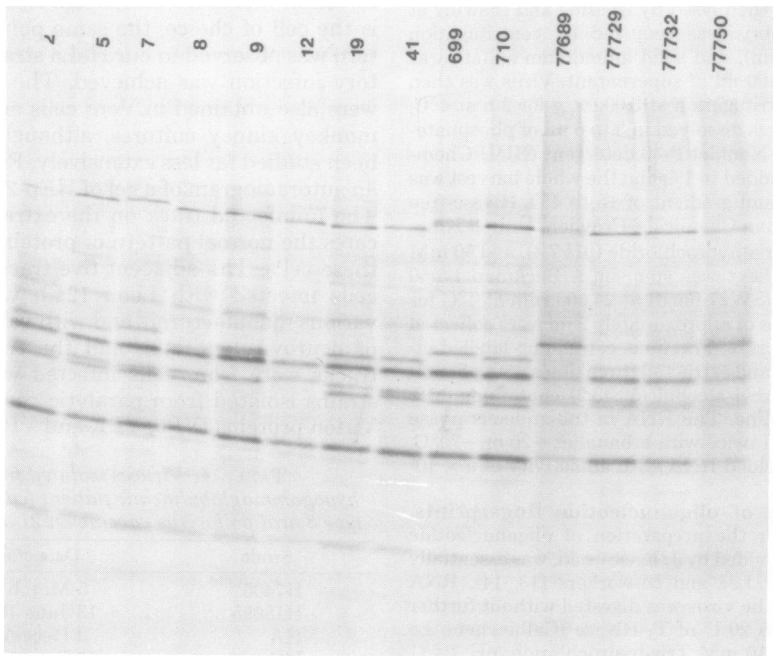

FIG. 2. *Autoradiogram of a 10 to 30% linear gradient gel of [^{35}S]methionine-labeled HEp-2 cell extracts. Cells were infected with the type 3 preOPV strains isolated from paralytic cases shown.*

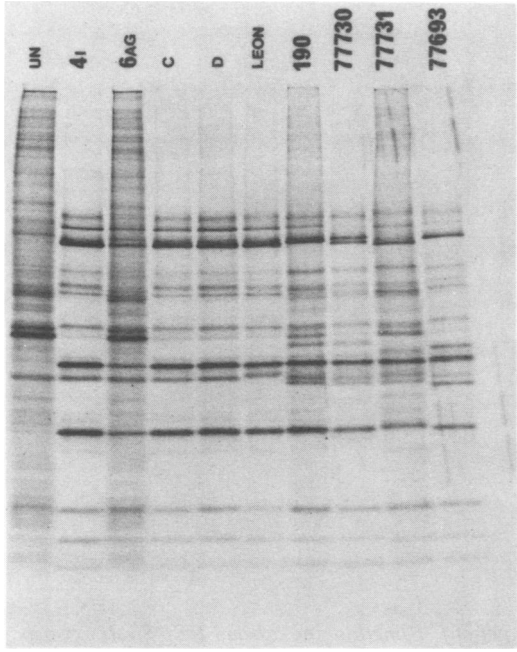

FIG. 3. *Autoradiogram of a 10 to 30% linear gradient gel of [^{35}S]methionine-labeled HEp-2 cell extracts. UN, uninfected cells; 4I, 6AG, C, and D, cells infected with different batches of type 3 oral polio vaccine; Leon, cells infected with Sabin vaccine precursor; 190, 77730, 77731, and 77693, cells infected with type 3 pre-OPV strains isolated from paralytic cases.*

pre-OPV strains isolated from paralytic cases, including Leon, the strain from which the Sabin vaccine strain was derived. The Leon pattern lacks the two polypeptides found for other pre-OPV strains. Figure 4 includes infected-cell extracts for two vaccine batches (e and 4a) and two pre-OPV strains (III-10 and III-30). Both vaccine batches gave patterns which lacked the two bands found in the pre-OPV strain-infected cells.

Of the 27 strains of pre-OPV poliovirus examined, only 3 (III-9, III-12, and Leon) gave infected cell patterns lacking the two extra bands. It was noticeable that the mobility of VP1 was slightly lower for Leon than for the Sabin vaccine strains which were derived from it (19). This has been observed consistently under our conditions of electrophoresis. Other slight, consistent variations in the migration rates of proteins from strain to strain have also been observed.

Two attenuated but non-Sabin-derived strains, WM3 and USOL-D-BAC (which was given a field trial as a vaccine strain), gave the patterns shown in Fig. 4. Two independent batches of each strain were tested. WM315 and WM326 gave very similar and characteristic patterns, as did USOL-D-BAC and USOL-D-BAC-VONKA, indicating that the pattern is a relatively stable feature of the virus strain used, and both showed the extra polypeptide bands when compared with a Sabin vaccine strain (strain 4a).

Stability of the peptide pattern with virus passage. Six serial passages of viruses 4a and III-10 in HEp-2 cells did not affect the patterns obtained (data not shown). Strains isolated from paralytic cases temporally associated with the administration of oral polio vaccine were examined. Patterns from the 23 strains tested (Table 2) are shown in Fig. 5 and 6, and 9 of the strains (106, 114, 116, 118, 152, 153, 115, 156, and 161) gave a pattern unlike the Sabin type 3 vaccine. The migration of the extra bands varied from strain to strain (compare 156 and 115, and 152 and 116), but some viruses gave identical patterns (156 and 161, 114 and 115, and 152 and 153). These pairs of strains were isolated from the same individuals (Table 3). The pattern produced was the same when the virus was isolated from different batches of material or different types of starting material (strains 156 and 161) and was therefore stable in the process of isolation.

These results suggested that the presence of the extra polypeptides in infected-cell extracts could provide an indication of the origin of a strain and specifically whether it derived from the Sabin vaccine strain. Figure 7 shows the patterns of polypeptide synthesis of cells infected with isolates from 12 recent paralytic cases of poliomyelitis from the United Kingdom listed in Table 3. Four (21746, 16057, 13532, and 15521) gave the vaccine-type pattern, whereas the remaining eight gave patterns having the extra polypeptide bands.

However, the strains derived from cases temporally associated with the administration of polio vaccine listed in Table 2 have been studied extensively by serological methods, and the results suggest that all were vaccine derived.

Data are shown in Fig. 4 for a series of isolates made over a period of 16 months from a hypogammaglobulinemic patient fed a type 3 Sabin oral vaccine (Table 3). The polypeptide patterns indicate that the excreted virus was initially Sabin vaccine-like (H7405 and H18095) but then became non-Sabin vaccine-like, acquiring the two extra bands. Strain HA differs from all other isolates in the intensity and position of the extra bands, and it is therefore possible that the patient was reinfected with another virus during the period of study. However, the data can also

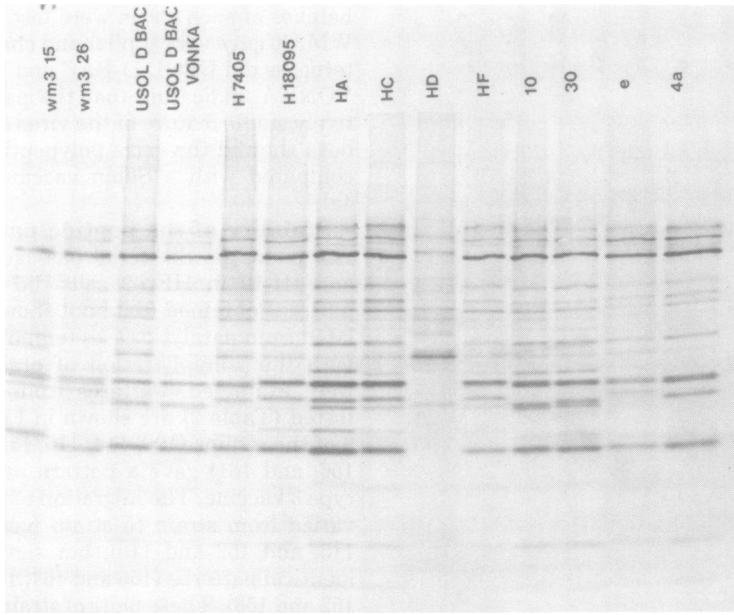

FIG. 4. *Autoradiogram of a 10 to 30% linear gradient gel of [^{35}S]methionine-labeled HEp-2 cell extracts. WM315, WM326, USOL-D-BAC, and USOL-D-BAC-VONKA, cells infected with attenuated non-Sabin type 3 strains; H7405, H18095, HA, HC, HD, and HF, cells infected with sequential isolates from hypogammaglobulinemic patient; III-10 III-30, cells infected with type 3 pre-OPV strains isolated from paralytic cases; e and 4a, cells infected with different batches of type 3 Sabin oral polio vaccine.*

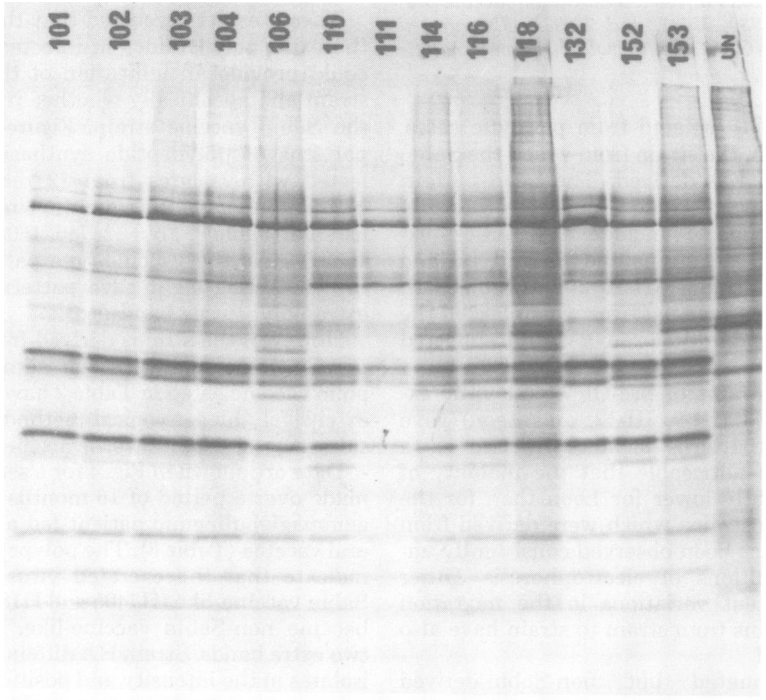

FIG. 5. *Autoradiogram of a 10 to 30% linear gradient gel of [^{35}S]methionine-labeled HEp-2 cell extracts. 101, 102, 103, 104, 106, 110, 111, 114, 116, 118, 132, 152, and 153, Cells infected with type 3 strains isolated from paralytic cases temporally associated with the use of oral polio vaccine; UN, uninfected cells.*

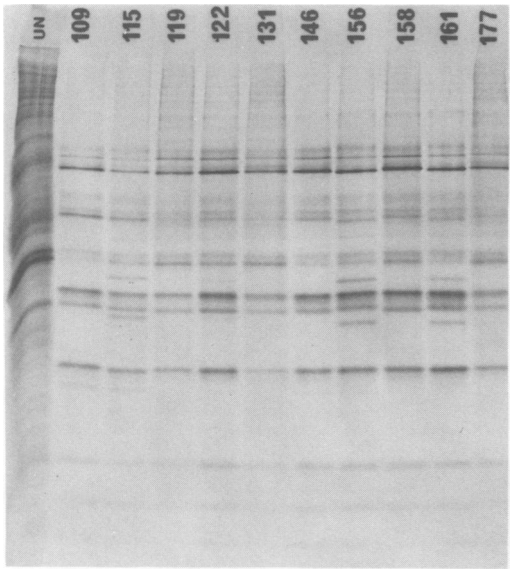

FIG. 6. *Autoradiogram of a 10 to 30% linear gradient gel of [^{35}S]methionine-labeled HEp-2 cell extracts. UN, Uninfected cells; 109, 115, 119, 122, 131, 146, 156, 158, 161, and 177, cells infected with type 3 strains isolated from paralytic cases temporally associated with the use of oral polio vaccine.*

be interpreted to mean that the polypeptide pattern apparently characteristic of Sabin strain virus is not stable indefinitely on prolonged passage in humans.

Oligonucleotide maps. Oligonucleotide maps of the T_1 RNase digest of the RNA of certain key strains were prepared. Figures 8a, b, and c show the maps prepared from three pre-OPV strains: 30, III-10, and III-694 (the polypeptide patterns are given in Fig. 1, 2, and 4, respectively). Spots corresponding to large, characteristic oligonucleotides are found in the lower part of the pattern. More than 50 such spots are readily distinguishable. All three maps are distinct and completely different from the vaccine pattern (strain 4a) given in Fig. 9a. Figure 9b is a map prepared from strain 119 (Table 2 and Fig. 6). The polypeptide pattern is vaccine-like, and the oligonucleotide map is indistinguishable from that of the vaccine strain. This strain was isolated from the central nervous system of a fatal case (Table 2) and has been shown to be intensely neurovirulent in monkeys (F. Taffs, personal communication). Figure 9c is a map prepared from strain 106 (Table 2 and Fig. 5). This strain is non-vaccine-like in the peptide pattern, but the map is clearly very

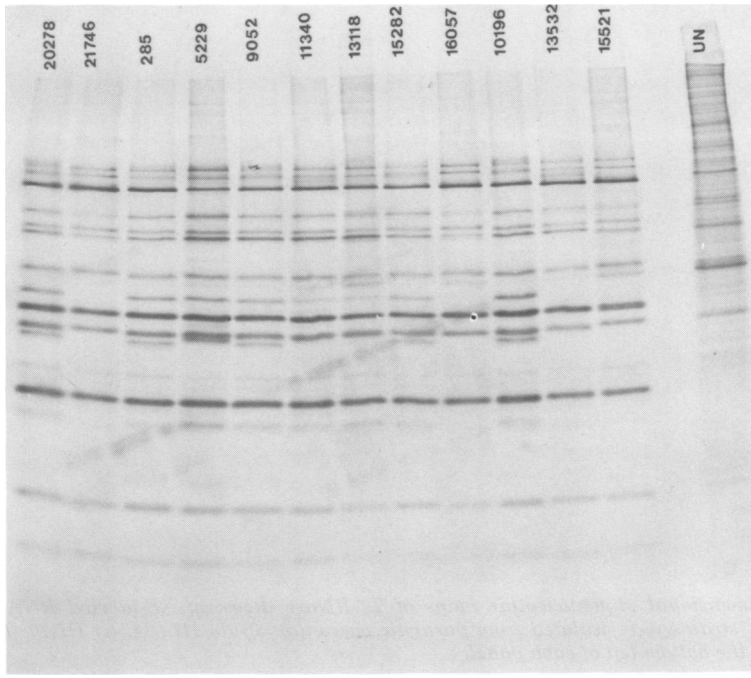

FIG. 7. *Autoradiogram of a 10 to 30% linear gradient gel of [^{35}S]methionine-labeled HEp-2 cell extracts. Cells were infected with type 3 strains isolated from paralytic cases occurring in England between 1973 and 1975. UN, Uninfected cells.*

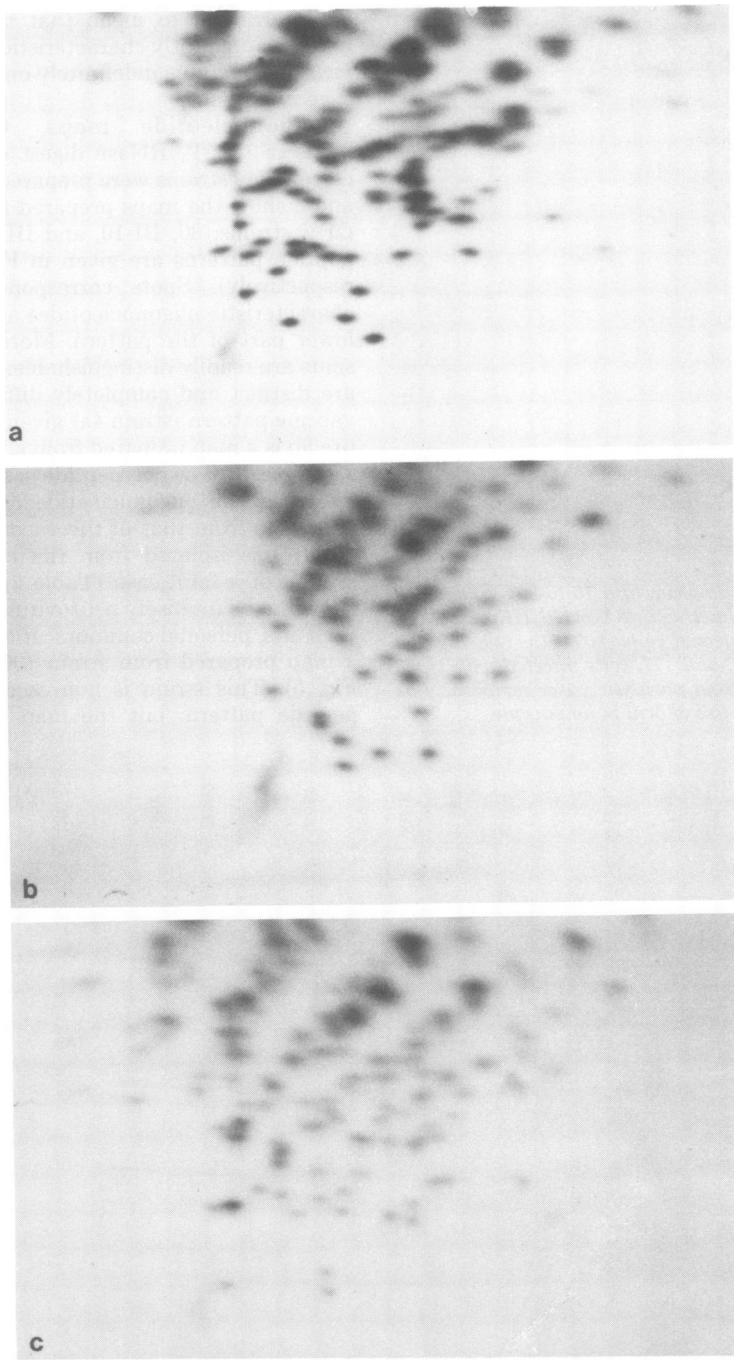

FIG. 8. *Two-dimensional oligonucleotide maps of T_1 RNase digest of ^{32}P-labeled RNA from purified viruses. Pre-OPV strains were isolated from paralytic cases. (a) 30; (b) III-694; (c) III-10. The origin (not shown) is toward the bottom left of each panel.*

however, at least seven spots present in the 106 map which are absent from the vaccine pattern and at least four which are present in the vaccine pattern but absent from the 106 map. Strain 106 was also isolated from the central nervous system of a fatal case and has been shown to be of a similar high neurovirulence for monkeys as was strain 119 (Taffs, personal communication).

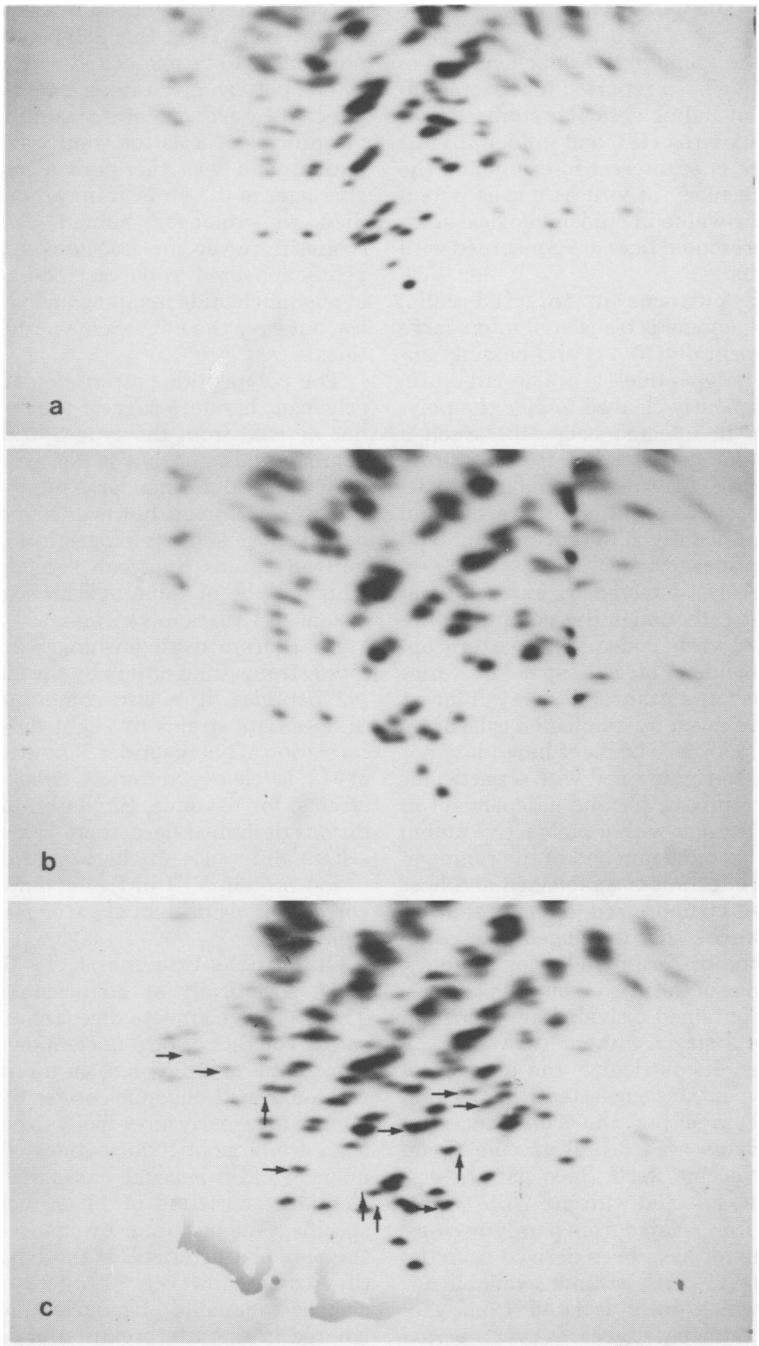

FIG. 9. *Two-dimensional oligonucleotide maps of T_1 RNase digest of ^{32}P-labeled RNA from purified viruses. (a) Sabin strain 4a; (b) strain 119, isolated from a case of paralytic polio temporally associated with the administration of oral polio vaccine; (c) strain 106, isolated from a case of paralytic polio temporally associated with the administration of oral polio vaccine. (Horizontal arrows indicate spots not found in [a]. Vertical arrows indicate spots found in [a] but absent here.) The origin (not shown) is toward the bottom left of each panel.*

DISCUSSION

Studies of the comparative biochemistry of virus strains have been reported for several different viruses, including vesicular stomatitis virus (4), smallpox virus (18), and influenza virus (16). Such work is of interest in examining the evolution and ecology of viruses and is potentially of practical value in epidemiological studies. The data presented here are concerned with type 3 poliovirus.

Polypeptide patterns in infected cells. The poliovirus genome is translated into a large precursor polypeptide (10, 21) and possibly another, smaller polypeptide (11). The large precursor is subsequently cleaved to give the polypeptides found in infected cells (10). Such a processing scheme can give rise to other products by alternative cleavages (3), and minor variations in the proteins of different strains could result in a significantly different amount and type of polypeptides formed.

The polypeptides detected by autoradiography in infected cells under the conditions described here are virus coded. The pattern obtained is independent of cell type, but varies slightly with the virus strain and is very different from the pattern given by uninfected cells or by staining with Coomassie brilliant blue (data not shown). The pattern obtained with a particular virus is highly reproducible and independent of the length of the pulse with a radioactive amino acid over a period of 60 min and of the timing of the pulse once infection is established and host protein synthesis is suppressed (data not shown). It is likely, however, that prolonged incubation would modify the pattern by allowing more extensive processing of the precursor polypeptides.

The pattern obtained provided an indication of the origin of a strain, although it was not a rigorous marker. In particular, the absence of two minor polypeptides suggested that a strain was derived from either the same ancestral strain as the Sabin type 3 oral vaccine (Leon $12a_1b$) or the vaccine itself. This pattern was very rare in cells infected with pre-OPV strains of type 3 poliovirus isolated from paralytic cases; such viruses cannot have been derived from the vaccine. Of the 27 such strains available for examination, which were isolated from geographically widely separated cases over a period of time, only 2 gave a polypeptide pattern lacking the two minor bands. One of these strains is Leon, the precursor of type 3 Sabin vaccine. In contrast, this pattern was more common in strains isolated from recent paralytic cases. Two attenuated non-Sabin strains gave the two extra polypeptides in infected cells, Leon is virulent and did not give them, and vaccine batches of varying neurovirulence in monkeys gave identical patterns. Thus, this polypeptide pattern did not appear to be associated with virulence.

The pattern given in an infected cell was unaffected by passage of a strain in culture or by the process of isolation from a specimen from a patient and was therefore a stably inherited character of the virus. It may, however, be modified on prolonged human passage. Certain strains from vaccine-associated cases of poliomyelitis appeared to be vaccine-like on the basis of oligonucleotide mapping and serological studies, but gave the nonvaccine pattern of polypeptides.

The polypeptide pattern detected in infected cells can therefore suggest that a strain is or is not derived from the type 3 oral vaccine or a common ancestor, but is not a rigorous test. It may be of value when large numbers of samples are to be examined, however, and can sometimes detect more subtle changes in known vaccine-derived strains than can serological methods. This may be of value in searches for subpopulations of viruses in vaccines.

Apart from distinguishing Sabin vaccine-related strains from others by the clear absence of polypeptides, it is also sometimes possible to differentiate strains by slight differences in the migration of polypeptides. The attenuated strain WM3 is clearly different from other type 3 strains, for example. Similarly, under the conditions described here, there is a slight but consistent difference in the migration rate of the virion protein VP1 of Leon compared with the corresponding protein of vaccine strains derived from it.

Oligonucleotide maps. T_1 RNase cleaves RNA specifically at guanosine residues (20). Therefore, a complete digest of an RNA preparation consists of oligonucleotides of a range of sizes, none of which has an internal guanosine residue. Small oligonucleotides of a specific sequence are clearly more likely to occur by chance than are large oligonucleotides of a specific sequence, and it is usually assumed that oligonucleotides consisting of 10 or more bases in a specific sequence occur by chance so rarely that they are characteristic of the RNA from which they come. However, 90 to 95% of the RNA is cleaved to smaller oligonucleotides and is thus ignored in the interpretation of oligonucleotide maps. Nonetheless, such maps appear to be a very sensitive method for distinguishing strains of vesicular stomatitis virus (4) or influenza virus (16), and the data presented here suggest that this is also true for poliovirus. Three presumably unrelated pre-OPV strains isolated from paralytic cases gave maps totally different from each other and from the Sabin vaccine strain 4a.

The oligonucleotide map prepared from the vaccine strain is essentially identical to that described by Lee et al. (13) and maps prepared by other workers (J. F. Obijeski, personal communication). The broad similarity of the oligonucleotide maps of strain 106 and the vaccine strain suggests that the two are related. Strain 106 is, however, not vaccine related by the criterion of the polypeptide pattern that it gives in infected cells, and the map from strain 106 has at least seven spots not found in that of the vaccine strain and lacks at least four which are found in that of the vaccine strain. As every large characteristic spot present in the vaccine map could in theory yield two spots in the 106 map by the introduction of a suitably sited guanosine residue, the differences between the two maps could be generated by an absolute minimum of four mutations. As the characteristic spots make up only 5 to 10% of the genome, this figure is likely to be a gross underestimate (possibly by a factor of 10) of the number of mutations required to derive strain 106 from the vaccine strain.

This suggests a number of possible origins of strain 106. First, it may not be derived from the Sabin vaccine strain or a strain resembling its "wild" progenitor (e.g., Leon). This is unlikely in view of the many similarities between the oligonucleotide maps of strain 106 and the vaccine strain, the unique nature of maps derived from strains known to be unrelated to the vaccine, the serological similarity of strain 106 and the vaccine strain, and the epidemiological circumstances of its isolation (I. Dömök, personal communication).

Second, it could have been derived from the vaccine by mutation and selection after administration of the vaccine to the patient. Virus was isolated from this patient within 30 days of vaccination; therefore, this explanation would suggest a high mutation rate and strong selection pressures on the virus in the course of human passage. Little is known of the biochemical consequences of human passage of Sabin oral polio vaccines, although the progeny virus may be more neurovirulent (D. I. Magrath, personal communication), and data presented here indicate that the pattern of polypeptides found upon infection of cells by a virus may alter with human passage of the virus.

Third, the vaccine administered may have contained a subpopulation of the strain in question.

Strain 119 was indistinguishable from the vaccine virus in both the polypeptide pattern and the oligonucleotide fingerprint. It was isolated from human central nervous system material of a fatal case and has been shown to be as highly neurovirulent in monkeys as is strain 106. It therefore appears that changes in the genome as a result of reacquiring neurovirulence are not necessarily reflected in changes in the oligonucleotide map.

Straightforward biochemical methods are therefore of possible value in studying the relatedness of different type 3 poliovirus strains. This is of considerable interest in the field evaluation of vaccines and the laboratory study of virus populations. However, neither of the techniques applied here provided information on the virulence of a type 3 poliovirus. Moreover, both methods have been applied initially to uncloned virus populations on the grounds that these are of most clinical interest. Variation within populations is under investigation at the moment.

ACKNOWLEDGMENTS

I thank J. F. Obijeski for assistance in setting up the oligonucleotide mapping technique, M. Roebuck for providing recent paralytic virus strains, and A. John and J. Bootman for locating other virus strains. I also thank S. Jefferies for technical assistance and D. I. Magrath and G. C. Schild for helpful discussions.

LITERATURE CITED

1. **Adler, R., and D. R. Tershak.** 1974. Temperature sensitive defect of type 2 poliovirus. Virology **58**:209–218.
2. **Boulger, L. R., S. A. Marsden, D. I. Magrath, L. F. Taffs, and G. C. Schild.** 1979. Comparative monkey neurovirulence of Sabin type III poliovirus vaccines. J Biol. Stand. **7**:97–111.
3. **Butterworth, B. E., and B. D. Korant.** 1974. Characterization of the large picornaviral polypeptides produced in the presence of zinc ion. J. Virol. **14**:282–291.
4. **Clewley, J. P., D. H. L. Bishop, C.-Y. Kang, J. Coffin, W. M. Schnitzlein, M. E. Reichmann, and R. E. Shope.** 1977. Oligonucleotide fingerprints of RNA species obtained from rhabdoviruses belonging to the vesicular stomatitis virus subgroup. J. Virol. **23**:152–166.
5. **Collingham, K. E., T. M. Pollock, and M. O. Roebuck.** 1978. Paralytic poliomyelitis in England and Wales. Lancet **i**:976–977.
6. **Cooper, P. D.** 1977. Genetics of picornaviruses, p. 133–207. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 9. Plenum Publishing Corp., New York.
7. **Dömök, I, and D. I. Magrath.** 1979. Guide to poliovirus isolation and serological techniques. World Health Organization offset publication no. 46. World Health Organization, Geneva.
8. **Fiszman, M., M. Reynier, D. Bucchini, and M. Girard.** 1972. Thermosensitive block of the Sabin strain of poliovirus type I. J. Virol. **10**:1143–1151.
9. **Garfinkle, B. D., and D. R. Tershak.** 1972. Degradation of poliovirus polypeptides *in vivo*. Nature (London) New Biol. **238**:206–208.
10. **Jacobson, M. F., J. Asso, and D. Baltimore.** 1970. Further evidence on the formation of poliovirus proteins. J. Mol. Biol. **49**:657–669.
11. **Jense, H., F. Knauert, and E. Ehrenfeld.** 1978. Two initiation sites for translation of poliovirus RNA in vitro: comparison of LSc and Mahoney strains. J. Virol. **28**:387–410.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.

13. **Lee, Y. F., N. Kitamura, A. Nomoto, and E. Wimmer.** 1979. Sequence studies of poliovirus RNA. IV. Nucleotide sequence complexities of poliovirus type 1, type 2 and two type 1 defective interfering particle RNAs and fingerprint of the poliovirus type 3 genome. J. Gen. Virol. **44**:311-312.
14. **Lee, Y. F., and E. Wimmer.** 1976. "Fingerprinting" high molecular weight RNA by two dimensional gel electrophoresis: application to poliovirus RNA. Nucleic Acids Res. **3**:1647-1658.
15. **Milstien, J. B., J. R. Walker, and L. J. Ebron.** 1977. Correlation of virus polypeptide structure with attenuation of poliovirus type 1. J. Virol. **23**:811-815.
16. **Nahajima, K., V. Desselberger, and P. Palese.** 1978. Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. Nature (London) **274**:334-339.
17. **Nakano, J. H., H. M. Gelfond, and J. T. Cole.** 1966. Antigenic segregation of type 3 poliovirus isolates related and unrelated to Sabin's vaccine strain with the use of modified Wecker and McBride techniques. Am. J. Epidemiol. **83**:130-145.
18. **Obijeski, J. F., E. L. Palmer, L. G. Gafford, and C. C. Randall.** 1973. Polyacrylamide gel electrophoresis of fowl pox and vaccinia virus proteins. Virology **51**:512-516.
19. **Sabin, A. B., and L. R. Boulger.** 1973. History of Sabin attenuated poliovirus oral live vaccine strains. J. Biol. Stand. **1**:115-118.
20. **Takashi, K.** 1960. The structure and function of ribonuclease T1. Part 1. Chromatographic purification and properties of ribonuclease T1. J. Biochem. (Tokyo) **49**: 1-8.
21. **Villa-Komaroff, L., N. Guttman, D. Baltimore, and H. F. Lodish.** 1975. Complete translation of poliovirus RNA in a eukaryotic cell-free system. Proc. Natl. Acad. Sci. U.S.A. **72**:4157-4161.