Antigenic and Structural Relatedness among Non-capsid and Capsid Polypeptides of Polioviruses belonging to Different Serotypes

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SUMMARY

Antibodies were raised by immunization of Macaca fascicularis monkeys with extracts of M. fascicularis kidney cells separately infected with one of the three poliovirus serotypes. Preparations of antibodies were shown to be strictly type-specific in the neutralization test but formed immune complexes adsorbable on Staphylococcus aureus, Cowan I strain cells, with heterotypic as well as homotypic virus-specific polypeptides present in extracts from the virus-infected cells. The presence of intertypic antigenic determinants was demonstrated by this technique on both the non-capsid and capsid poliovirus polypeptides.

Structural variations of poliovirus polypeptides were studied by analysing products of their partial proteolysis. Non-capsid polypeptides encoded in the central portion of the virus genome (polypeptides 5b and X) as well as in the 3'-terminal region (NCVP2 and NCVP4) were found to be highly conserved, whereas capsid polypeptides VP1, VP2, and VP3, which are encoded in the 5'-terminal region of the virus RNA, displayed a much greater variability.

INTRODUCTION

Virions of all the known strains of poliovirus belong to one of the three distinct serotypes. No cross-reactions between the serotypes have as yet been revealed. Nevertheless, a significant intertypic genome relatedness has been found by RNA molecular hybridization experiments (Young et al., 1968; Young, 1973a, b) and electron microscopy of RNA heteroduplexes (Cumakov et al., 1979).

We have extended these studies by investigating the antigenic and structural relatedness between analogous capsid and non-capsid polypeptides of different polioviruses. We report here that all of the virus-specified polypeptides show intertypic antigenic relatedness and this is supported by structural analysis of the non-capsid polypeptides. However, capsid polypeptides of the three poliovirus types are structurally dissimilar despite their antigenic cross-reactivity.

METHODS

Viruses and cells. The following cloned poliovirus strains were used for preparation of antigens and labelled virus-specific polypeptides: Mahoney (type 1), P40, which is a temperature-resistant (tr) revertant isolated from the strain P712 ch 2ab (type 2), and 452/62 IID isolated from a human polio case (type 3). For immunization, (see below), Sabin's vaccine strains LSc 2ab (type 1), P712 ch 2ab (type 2) and Leon 12 ab (type 3) were also utilized. Viruses were grown either in primary cultures of Macaca fascicularis kidney cells or in HeLa cells.
Partially purified antibody preparations. Monolayer cultures of *M. fascicularis* kidney cells were infected with the appropriate virus strain and at the end of the growth cycle the cells were suspended in physiological saline (2 × 10⁷ cells/ml) and stored frozen until use. Prior to immunization with such antigens, each *M. fascicularis* monkey received two injections of the appropriate vaccine virus (10⁸ p.f.u. intramuscularly with a 2-week interval) to prevent illness. Each monkey then received from five to seven weekly intramuscular injections of frozen and thawed suspension (1 ml) of cells infected with the virulent virus of the same type. Partially purified antibodies were prepared from sera taken 1 week after the last immunization. The purification procedure consisted of a cycle of adsorption on to and elution from a solid-phase immunosorbent prepared from the virus-infected HeLa cells.

For preparation of the immunosorbent, cells were harvested 4 h post-infection (p.i.), suspended (2 × 10⁷ cells/ml) in a buffer (NET) containing 0.15 M-NaCl, 5 mM-EDTA, 50 mM-tris-HCl, pH 7.8, and 0.02% sodium azide. Nonidet P40 (NP40) was added to 1% final concentration and after 5 min incubation at 4 °C the nuclei and cell debris were removed by low-speed centrifugation. Cross-linking of the extracts with 0.5% glutaraldehyde was carried out essentially as described by Avrameas & Ternynck (1969). The immunosorbents were repeatedly washed with 0.01 M-phosphate, pH 7.2, and 0.1 M-glycine buffer, pH 2.9.

For antibody isolation, a portion of the immunosorbent equivalent to 3 ml of infected cell homogenate was mixed with 2 ml of homotypic antisera, and after 1 h incubation at room temperature, the unadsorbed material was repeatedly washed away with 0.01 M-phosphate, pH 7.2. The adsorbed virus-specific antibodies were eluted in a small vol. of 0.1 M-glycine buffer, pH 2.9, dialysed against NET buffer and stored at -20 °C.

Labelled virus-specific polypeptides. Monolayer roller cultures of HeLa cells were infected with the appropriate virus at an m.o.i. of about 20 p.f.u./cell. The labelled amino acids, either ³⁵S-methionine (790 to 1240 Ci/mmol, 50 or 100 μCi/ml) or ¹⁴C-labelled protein hydrolysate (1000 mCi/g, 5 μCi/ml), were added at 2 h p.i. Two h later the infected cells were harvested, washed and lysed in NET buffer containing 1% NP40 as described in the preceding section. Capsid polypeptides were prepared from poliovirus particles purified according to a modified procedure of Medappa *et al.* (1971). Naturally occurring procapsids were isolated from the crude extracts of infected cells by centrifugation (Beckman rotor SW25.1; 24000 rev/min, 4 h) through a sucrose concentration gradient (15 to 30%, w/w).

Formation and analysis of immune complexes. The procedure used was a modification of that described by Kessler (1975). Suspensions of inactivated *Staphylococcus aureus*, Cowan I, cells were kindly donated by Dr G. M. Mashilova (Mechnikov Institute for Vaccines and Sera, Moscow). The cells were repeatedly washed and suspended in NET buffer with 0.05% NP40 to give a 10% (v/v) suspension. To reduce non-specific adsorption, which was relatively high in some preparations of *S. aureus* cells, these cells were incubated for 1 h at 4 °C with a fivefold diluted and clarified (12000 g, 20 min) extract of uninfected and unlabelled HeLa cells.

Prior to assay, labelled nuclei-free extracts from virus-infected HeLa cells (20 μl) were usually incubated with 0.5 ml 8 M-urea in 0.1 M-NaCl, 0.01 M-tris-HCl, pH 8, for 15 min at 37 °C. The samples were dialysed against NET buffer supplemented with NP40 to a final concentration of 0.05% and clarified by centrifugation at 12000 g at 4 °C for 20 min. The sample (0.5 ml) was mixed with a preparation of homotypic or heterotypic antibodies (5 μl), and immune complexes were allowed to form at 4 °C for 15 min. Then, 0.2 ml of a 10% suspension of *S. aureus* cells was added and after a 15 min incubation at 4 °C bacterial cells were pelleted by centrifugation at 7000 g for 5 min at 4 °C. The cells were thoroughly washed with NET buffer containing 0.05% NP40 until no radioactivity could be detected in the supernatants. It should be noted that under the conditions described, a great excess of
antigen was present and, therefore, only 5 to 10% of the total radioactive polypeptides were adsorbed on *S. aureus* cells in a homotypic reaction.

To elute immune complexes, 0.1 ml of a dissociation buffer solution [2% sodium dodecyl sulphate (SDS), 50 mM-tris-HCl, pH 6.8, 5% mercaptoethanol, 10% glycerol, 0.003% bromophenol blue] was added to the pelleted cells. The suspension was agitated for 15 min at room temperature and centrifuged at 12000 g for 10 min. The supernatant was used for determination of radioactivity and for electrophoretic analysis in SDS-containing 15% polyacrylamide gel slabs essentially according to the procedure of Laemmli (1970).

**Fractionation of virus-specific polypeptides on Sepharose-6B.** One ml of the 8 M-urea-treated (non-dialysed) homogenate of the virus-infected cells containing dextran blue was loaded on to a column of Sepharose-6B (50 × 2 cm) equilibrated with a solution of 8 M-urea in 0.1 M-NaCl, 0.01 M-tris-HCl, pH 8. Gel filtration was carried out at a rate of about 2 ml/h at room temperature. Fractions (2 ml) were dialysed against NET buffer after withdrawal of small samples for radioactivity determinations.

**Analysis of partial proteolysis products.** A modification of previously described procedures (Cleveland *et al.*, 1977; Svitkin *et al.*, 1979) was used. First-dimension electrophoresis was run in 15% polyacrylamide slabs using the discontinuous system of Laemmli (1970) and 2.7 cm-wide wells. After electrophoresis, each sample lane was cut into strips, about 4 mm wide, one of which was processed for autoradiography while others were frozen at −20 °C until the second-dimension electrophoresis. This was carried out in the following way. Strips with the separated labelled proteins were soaked for 30 min at 4 °C in a solution containing 0.1% SDS, 125 mM-tris-HCl, pH 6.8, and then overlaid on a concentrating gel (5% polyacrylamide). A small volume of not yet polymerized 5% polyacrylamide was added and after polymerization, a solution of chymotrypsin (250 μg/ml) in 0.05 M-tris-HCl, pH 6.8, 0.1% SDS, 10% glycerol, 0.003% bromophenol blue was loaded on to the gel. Eighteen% polyacrylamide slabs were used for separation of partial proteolysis products.

**RESULTS**

**Formation of homotypic and heterotypic immune complexes by poliovirus-specific polypeptides**

Preliminary experiments showed that labelled virus-specific polypeptides present in extracts from infected cells could form complexes adsorbable on *S. aureus* cells with heterotypic as well as homotypic antibody preparations (Table 1, expt. 1). The data show that the heterotypic immune complexes contain usually only 1.5- to 2-fold less radioactive material than the homotypic ones, whereas the non-specific binding of labelled polypeptides to microbial cells in the absence of antibody preparations was very low. The following controls attested to the specificity of the technique used: (i) non-immune sera failed to exert any significant effect on the binding of label to *S. aureus* cells (Table 1, expt. 2), and (ii) antibodies against polio 1 polypeptides did not form detectable immune complexes with labelled polypeptides contained in extracts from either uninfected HeLa or L cells, or encephalomyocarditis (EMC) virus-infected Krebs-2 cells (Table 1, expt. 3).

High levels of adsorption of label on *S. aureus* cells in heterotypic reactions might be due to formation of intermolecular aggregates containing virus-specific polypeptides with antigenic determinants able to form complexes with heterotypic antibodies as well as polypeptides lacking such a determinant. To disrupt such aggregates, the extracts from virus-infected cells were treated with 8 M-urea, dialysed and assayed for capacity to form immune complexes. Expt. 4 of Table 1 shows that this treatment did not appreciably change the ability of virus-specific polypeptides to form heterotypic immune complexes.
Table 1. *Formation of homotypic and heterotypic immune complexes*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Source of labelled polypeptides</th>
<th>Antibody</th>
<th>Amount of label eluted from <em>S. aureus</em> cells (ct/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polio 1-infected monkey cells</td>
<td>None</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td>Polio 1-infected monkey cells</td>
<td>Anti-polio 2</td>
<td>4921</td>
</tr>
<tr>
<td></td>
<td>Polio 1-infected monkey cells</td>
<td>Anti-polio 3</td>
<td>4162</td>
</tr>
<tr>
<td></td>
<td>Polio 2-infected monkey cells</td>
<td>None</td>
<td>235</td>
</tr>
<tr>
<td></td>
<td>Polio 2-infected monkey cells</td>
<td>Anti-polio 2</td>
<td>10839</td>
</tr>
<tr>
<td></td>
<td>Polio 2-infected monkey cells</td>
<td>Anti-polio 3</td>
<td>5860</td>
</tr>
<tr>
<td></td>
<td>Polio 3-infected monkey cells</td>
<td>None</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>Polio 3-infected monkey cells</td>
<td>Anti-polio 2</td>
<td>2538</td>
</tr>
<tr>
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<td>Anti-polio 3</td>
<td>3700</td>
</tr>
<tr>
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<td>None</td>
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<td>Polio 2-infected monkey cells</td>
<td>Non-immune serum</td>
<td>191</td>
</tr>
<tr>
<td>3</td>
<td>EMC-infected Krebs-2 cells</td>
<td>None</td>
<td>318</td>
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<tr>
<td></td>
<td>EMC-infected Krebs-2 cells</td>
<td>Anti-polio 1</td>
<td>237</td>
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<tr>
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<td>Uninfected HeLa cells</td>
<td>None</td>
<td>330</td>
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<td>Anti-polio 1</td>
<td>251</td>
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<tr>
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<td>Anti-polio 2</td>
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<tr>
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<td>Uninfected L cells</td>
<td>None</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Uninfected L cells</td>
<td>Anti-polio 1</td>
<td>80</td>
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<td>285</td>
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<td>2012</td>
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<td>1520</td>
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<td></td>
<td>Polio 3-infected monkey cells</td>
<td>Anti-polio 3</td>
<td>2700</td>
</tr>
</tbody>
</table>

* For expts. 1 to 3, cellular extracts not treated with urea were utilized, whereas expt. 4 was carried out with extracts treated with 8 M-urea as described in Methods.

Table 2. *Neutralization tests with original antisera and purified antibody preparations*

<table>
<thead>
<tr>
<th>Antibody preparation</th>
<th>Type 1 (p.f.u./ml)</th>
<th>Type 2 (p.f.u./ml)</th>
<th>Type 3 (p.f.u./ml)</th>
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<tbody>
<tr>
<td>None</td>
<td>$14 \times 10^7$</td>
<td>$85 \times 10^7$</td>
<td>$78 \times 10^6$</td>
</tr>
<tr>
<td>Anti-polio 1 serum</td>
<td>$&lt;6 \times 10^4$</td>
<td>$77 \times 10^7$</td>
<td>$38 \times 10^6$</td>
</tr>
<tr>
<td>Anti-polio 2 serum</td>
<td>$12 \times 10^7$</td>
<td>$&lt;3 \times 10^6$</td>
<td>$32 \times 10^6$</td>
</tr>
<tr>
<td>Anti-polio 3 serum</td>
<td>$16 \times 10^7$</td>
<td>$91 \times 10^7$</td>
<td>$&lt;6 \times 10^4$</td>
</tr>
<tr>
<td>Anti-polio 1 antibodies</td>
<td>$&lt;6 \times 10^4$</td>
<td>$99 \times 10^7$</td>
<td>$90 \times 10^6$</td>
</tr>
<tr>
<td>Anti-polio 2 antibodies</td>
<td>$20 \times 10^7$</td>
<td>$&lt;3 \times 10^6$</td>
<td>$54 \times 10^6$</td>
</tr>
<tr>
<td>Anti-polio 3 antibodies</td>
<td>$22 \times 10^7$</td>
<td>$111 \times 10^7$</td>
<td>$&lt;6 \times 10^4$</td>
</tr>
</tbody>
</table>

* Infectious virus titres (number of plaques on *M. fascicularis* kidney cell cultures) after incubation of undiluted virus for 60 min at room temperature with 1:100 dilution of either original immune sera or purified antibody preparations. Two replicate cultures were used for each dilution of the viruses.

The type specificities of the antisera, as judged by infectivity neutralization, were repeatedly checked, and the results of one such experiment carried out with both the original immune sera and purified antibody preparations derived from it are shown in Table 2. It can be seen that the sera and antibody preparations proved to be type-specific in the neutralization reaction. When assayed in the agar diffusion precipitation test with purified virions, the sera were again demonstrated to be type-specific (data not shown).
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Fig. 1. Electrophoretic analysis of homotypic and heterotypic immune complexes formed by poliovirus-specific polypeptides. 1, Non-specific adsorption on S. aureus cells in the absence of antibodies; 2, complexes with antibodies against poliovirus type 1; 3, complexes with antibodies against poliovirus type 2; 4, complexes with antibodies against poliovirus type 3; 5, polypeptides contained in the extracts from virus-infected cells. The positions of the capsid proteins in the slab were determined using preparations of purified virions and procapsids as a source of marker polypeptides.

Identification of poliovirus-specific polypeptides having intertypic antigenic determinants

To identify virus-specific polypeptides contained in the homotypic and heterotypic immune complexes, the material eluted from S. aureus cells was analysed by polyacrylamide gel electrophoresis. In all the experiments described in this paragraph, the extracts used in the assay had been pretreated with 8 M-urea. The electrophoretic pattern of samples taken from expt. 4 of Table 1 are presented in Fig. 1. It can be seen that nearly all detectable virus-specific polypeptides of each of the three polioviruses form immune complexes with heterotypic as well as homotypic antibodies. Although the technique used is non-quantitative, the capsid polypeptides VP1, VP2 and VP3 appear to be more abundant in homotypic compared to heterotypic complexes. The non-capsid polypeptides do not seem to demonstrate such a preference for homotypic antibodies.

Fig. 1 also shows that non-specific (without antibodies) adsorption on S. aureus cells is somewhat selective, with protein X being most prominent. It has been suggested that this polypeptide is hydrophobic in nature (Butterworth et al., 1976). However, even polypeptide X is clearly specifically precipitated from immune complexes. The majority of virus specific
Fig. 2. Gel filtration profile of a labelled extract from poliovirus type 3-infected cells. Fractionation was carried out on a Sepharose-6B column in the presence of 8 M-urea. Letters denote fractions used for the subsequent analysis.

Fig. 3. Electrophoretic analysis of homotypic and heterotypic immune complexes formed by fractionated poliovirus type 3-specific polypeptides. Fractions A, B, C, D and E were taken from the profile shown in Fig. 2. 1, Non-specific adsorption on S. aureus cells in the absence of antibodies; 2, complexes with antibodies against poliovirus type 1; 3, complexes with antibodies against poliovirus type 2; 4, complexes with antibodies against poliovirus type 3; 5, polypeptides contained in the respective fractions of the gel filtration profile.

The most straightforward interpretation of the results presented in Fig. 1 was that all the virus-specific polypeptides adsorbed on S. aureus cells possessed intertypic antigenic determinants. A possibility remained, however, that at least some of these polypeptides actually lacked intertypic determinants but merely formed urea-resistant aggregates with polypeptides, including X, can also be seen in immune complexes by inspection of less-exposed films showing practically no non-specifically adsorbed polypeptides.
Fig. 4. Partial proteolysis maps of non-capsid polypeptides of polioviruses belonging to different serotypes. The upper row of autoradiographs was obtained from a gel in which products of partial proteolysis of intracellular polypeptides of poliovirus type 1 (T1) and poliovirus type 3 (T3) were investigated. The lower row of autoradiographs was obtained from another gel in which products of partial proteolysis of intracellular polypeptides of poliovirus type 2 (T2) and poliovirus type 3 (T3) were compared. Spots derived from the main polypeptides indicated can easily be distinguished from additional spots originating from the neighbouring virus-specific proteins.
Fig. 5. Partial proteolysis maps of capsid polypeptides of polioviruses belonging to different serotypes. The autoradiograph was obtained from a gel in which products of partial proteolysis of virion proteins of the three poliovirus serotypes were investigated simultaneously.

other proteins that did possess such determinations; in other words, it might be suggested that some polypeptides were non-specifically co-adsorbed on *S. aureus* cells in aggregated form. To exclude such aggregates from the reaction, an extract containing labelled virus-specific polypeptides of poliovirus type 3 was subjected to gel filtration on a Sepharose-6B column in the presence of 8 M-urea. Fig. 2 shows the profile of radioactivity obtained upon gel filtration, and Fig. 3 presents the electrophoretic patterns of polypeptides contained in separate fractions of this profile. It can be seen that fractions A and B contained polypeptides of considerably lower molecular masses than should be expected on the basis of positions of these fractions in the gel filtration profile. It may be concluded that the corresponding polypeptides are indeed components of large urea-resistant aggregates (some of these aggregates perhaps include dextran blue which was used in the eluent as an additional hydrophobic fractionation tool). Therefore, the ability of virus-specific polypeptides present in fractions A and B to form immune complexes with heterotypic antibodies cannot be interpreted unambiguously.

By contrast, fractions C, D and E contain labelled polypeptides within an expected range of molecular masses, which indicates that the corresponding polypeptides in these fractions are largely in a non-aggregated form. These polypeptides again form immune complexes not only with homotypic but also heterotypic antibody preparations (Fig. 3), which implies that they do contain intertypic antigenic determinants. This is also true of capsid polypeptides VP1, VP2 and VP3 (the presence of the latter in immune complexes formed by fraction E
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proteins is clearly seen on over-exposed films), although they appear more readily to form
grouped than heterotypic immune complexes.

Partial proteolysis maps of virus-specific polypeptides of different polioviruses

The above results suggested the existence of an antigenic relatedness among homologous
proteins of poliovirus belonging to different serotypes. To obtain information concerning
chemical relatedness among these proteins, a modified technique of partial proteolysis
described by Cleveland et al. (1977) was used as described in Methods. This technique
allowed us to obtain partial proteolysis maps of the polypeptides which were well resolved
upon first-dimension electrophoresis. These included polypeptides NCVP5b and NCVPX,
representing the central portion of the poliovirus genome, as well as NCVP2 and NCVP4
translated from the 3'-terminal region of the virus RNA (Rueckert et al., 1979).

Fig. 4 shows that all these non-structural polypeptides displayed a markedly high level of
homology among polioviruses belonging to three different serotypes. At the same time, some
minor differences between homologous polypeptides could also be revealed, e.g. NCVP2 of
poliovirus type 3 yielded reproducible spots apparently absent from NCVP2 of poliovirus
types 1 and 2.

It should be noted that various polypeptides in a given extract were labelled to a different
degree. Therefore, photographs presented in Fig. 4 were printed using films exposed for
different time intervals; an attempt was made to present bands of homologous peptides at
approximately the same intensity. Prolonged exposures of films usually revealed more minor
bands than are seen in Fig. 4, but overall resolution was relatively poor in these cases.

Capsid polypeptides were analysed using purified preparations of polio virions to avoid
overlapping with non-capsid polypeptides. The results are presented in Fig. 5. It can be seen
that the three larger capsid proteins of different polioviruses yielded dissimilar patterns of
partial proteolysis products. This latter result agrees well with preliminary data of Kew et al.
(1978).

DISCUSSION

Antibodies against intracellular poliovirus-specific proteins were elicited by immunization
of monkeys with homogenates of virus-infected monkey cells. These antibodies, when tested
in the infectivity neutralization reaction, were shown to react in a strictly type-specific
manner, revealing no cross-reactions. Nevertheless, the antibody preparations were shown to
efficiently form immune complexes with both homotypic and heterotypic intracellular
virus-specific polypeptides; the amounts of S. aureus cell-bound homotypic and heterotypic
complexes differed from each other by a factor of 2 at the most. This result demonstrated the
existence of intertypic antigenic determinants on poliovirus-specific polypeptides. Such
determinants were shown by electrophoretic analysis to be present on both non-capsid and
capsid polypeptides. It should be emphasized that these results were obtained in experiments
where special care was taken to avoid artefacts due to intermolecular aggregation of
virus-specific polypeptides.

We are aware of no previous publications concerning the type specificity of non-capsid
poliovirus polypeptides. The presence of common antigenic determinants on homologous
non-capsid polypeptides of different poliovirus serotypes is in good accord with a striking
similarity of partial proteolysis maps of these polypeptides revealed in the present study.

More unexpected was the discovery of intertypic antigenic determinants on poliovirus
capsid proteins. This finding appears to conflict with the known type specificity of the
determinants on polio virions demonstrable not only in the neutralization reaction but also in
such tests as agar immunodiffusion (Le Bouvier et al., 1957; Le Bouvier, 1959) and immune
electron microscopy (Hummeler et al., 1962). Moreover, the partial proteolysis maps of
homologous capsid polypeptides of different poliovirus serotypes were also shown to be clearly distinct. The most likely explanation for these seemingly contradictory data is that the intertypic antigenic determinants present on intracellular poliovirus capsid polypeptides are either lost or masked upon the maturation of virions. This interpretation is supported by recent findings of Meloen et al. (1979) that antisera raised against any of the four individual poliovirus capsid proteins failed to neutralize the infectivity of virions or react with them in a radioimmune assay.

It may be noted that during an early stage of investigating poliovirus antigens some evidence was obtained suggesting the existence of determinants shared by heated preparations of virions belonging to all the three serotypes (cf. Hummeler & Hamparian, 1958).

The results reported here suggest that the genes coding for poliovirus capsid proteins diverged more widely, upon evolutionary separation of serotypes, than the genes encoding non-structural polypeptides. This notion is compatible with the results of an electron microscopic analysis of poliovirus 1/poliovirus 3 RNA heteroduplexes (Cumakov et al., 1979), and also agrees with observations made upon comparison of polypeptides of different strains of foot-and-mouth disease virus (Brown, 1979; Newman et al., 1979; Robson et al., 1980).

It is obvious that techniques used in the present study are adequate for a search for some common structural features and insensitive to relatively minor alterations in the virus genomes. If one needs to find genetic changes of the latter sort, other approaches, such as the analysis of products of complete rather than partial proteolysis of virus-specific polypeptides (cf. Kew et al., 1980) or fingerprinting of RNase T1 digests of the virus genomes (Frisby et al., 1976; Lee & Wimmer, 1976; Lee et al., 1979; Nomoto et al., 1979) or ultimately the RNA genome sequencing (cf. Porter et al., 1978) should be used.

This study was reported in part at the Symposium 'Strategy of the Genomes of RNA-containing Animal Viruses' (Moscow, March 19–21, 1980).

REFERENCES


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