Topographical Studies on Poliovirus Capsid Proteins by Chemical Modification and Cross-linking with Bifunctional Reagents

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SUMMARY
Poliovirus capsid proteins comprise 15.1 lysines in VP1, 5.6 lysines in VP2, 11.7 lysines in VP3 and 5.5 lysines in VP4. Treatment with the monofunctional reagent N-succinimidyl 2,3-3H-propionate leads to the modification of 3.4 lysines in VP1, 0.6 lysines in VP2, 2.0 lysines in VP3 and 0.03 lysines in VP4. Chemical modification with the monofunctional reagent N-succinimidyl 3-(4-hydroxy,5-125I-iodophenyl)propionate results in a predominant labelling of VP1 and VP3, whereas VP2 is less accessible and VP4 is not modified. Cross-linking of poliovirus with bifunctional imidoesters, dimethyl suberimidate (DMS, 1.1 nm) and dimethyl adipimidate (DMA, 0.8 nm) leads to a new protein complex of mol. wt. which corresponds to the sum of VP1 and VP3. By cleavage with ammonia and electrophoresis on polyacrylamide gels in SDS, the proteins are identified as VP1 and VP3. This result gives evidence for a direct neighbourhood of VP1 and VP3 in the virus capsid. Treatment of the virus with the monofunctional reagents has no influence on the stability of the particle. The infectivity is reduced only by the bifunctional reagent.

INTRODUCTION
The picornaviruses appear to be physically and chemically similar. The capsid of poliovirus consists of 60 copies of each of the four different proteins known as VP1, VP2, VP3 and VP4. The proteins and the genomic RNA are assembled into particles with icosahedral symmetry (Finch & Klug, 1959). From the morphogenesis of poliovirus it seems likely that the 5S structural protein precursor NCVP1 represents a fundamental repeating structural unit of the virus (Rueckert, 1976). There is support for the idea that the capsid of the mature virion is generated from this precursor after proteolytic cleavage and assembly via 14S pentamers and 80S procapsids (12 pentamers; Jacobson & Baltimore, 1968; Phillips, 1971; Phillips & Wiemert, 1978). This process, in turn, follows similar pathways which have been shown by dissociation experiments on several cardioviruses (Dunker & Rueckert, 1971; Rueckert, 1976). However, the stepwise degradation of Coxsackie virus (Philipson et al. 1973), of bovine enterovirus (Martin & Johnston, 1972) and of poliovirus (Katagiri et al. 1971) supports the idea that at least these viruses consist of a stable matrix of VP1 and VP3.

Different models of the capsid structure have been proposed (Finch & Klug, 1959; Rueckert et al. 1969; Philipson et al. 1973; Rueckert, 1976). Although general information
exists concerning the architecture of the capsid of the mature virion, there is minimal information concerning the individual arrangement or specific functions of each polypeptide.

Topographical investigations on the capsid proteins of the picornaviruses have been made using several methods. Antibody-blocking experiments demonstrate that in the case of Mengo virus (Lund et al. 1977), VP1 is involved in cell attachment. Therefore, it seems likely that VP1 is a surface protein. Proteolytic cleavage in situ of VP1 of foot-and-mouth disease virus (Cavanagh et al. 1977), which results in reduced infectivity, supports this result. Chemical modification by the lactoperoxidase technique of bovine enterovirus (Carthew & Martin, 1974), of Mengo virus (Lund et al. 1977) and of poliovirus (Beneke et al. 1977; Lonberg, Holm & Butterworth, 1976) leads to a predominant labelling of VP1. Acetylation of the amino groups of poliovirus provides similar results (Lonberg-Holm & Butterworth, 1976). In all the studies so far reported, VP2 and VP3 are less accessible for chemical modification and in no case has VP4 been modified.

In the present communication, the accessibility of the capsid proteins of poliovirus to different monofunctional reagents was investigated. The reagents used bind covalently to cysteine residues or lysine residues. Protein neighbourhoods were investigated by cross-linking of the virus with bifunctional reagents, binding also to lysine groups. The results are discussed with respect to the topography of the proteins.

**METHODS**

*Virus.* Poliovirus type I (Mahoney) was used throughout these experiments.

*Cells.* HEp-2 cells were grown as monolayers in plastic bottles (Nunc, Roskilde, Denmark). Growth medium consisted of Eagle's minimal essential medium (MEM, Gibco, Paisley, Scotland), supplemented with 1·7 g/l bicarbonate, 5% new-born calf serum (Flow, Bonn, Germany), heat-inactivated at 56 °C for 30 min, and antibiotics: penicillin, 100 international units (i.u.)/ml; streptomycin sulphate, 50 µg/ml; and Nystatin, 25 i.u./ml.

*Propagation, labelling and purification of virus.* Cells were inoculated with virus at an m.o.i. of 30 to 50 and incubated at 36 °C. The inoculum was replaced by growth medium at 0·5 h.p.i. ³H or ¹⁴C-labelled virus was prepared in a similar way, except that growth medium lacking amino acids and a mixture of ³H or ¹⁴C-amino acids was added at 0·5 h.p.i. ³H-lysine- or ³⁵S-cysteine-labelled virus was prepared in growth medium by replacement of either lysine or cysteine by the appropriate radioactive amino acids. Virus was harvested at 18 h.p.i. Cells and supernatant were frozen and thawed. After centrifugation in an SW 27 rotor (Beckman Spinco) at 8000 rev/min for 45 min at 4 °C, to remove the cell debris, the crude virus was collected by centrifuging the supernatant in an SW 27 rotor at 27000 rev/min for 3 h at 4 °C. The pellet was homogenized in 1 ml phosphate-buffered saline (PBS) in a tight-fitting Dounce homogenizer and layered on a pre-formed linear sucrose gradient (15 to 45%, w/w, in PBS). After centrifugation in an SW 40 rotor at 40000 rev/min for 5 h at 4 °C, the gradient was monitored at 260 nm. The virus peak was collected and spun down in an SW 65 rotor at 50000 rev/min for 1·5 h at 4 °C. The pellet was re-suspended in PBS and CsCl was added to a final density of 1·33 g/ml. The virus was banded by isopycnic centrifugation in an SW 65 rotor at 40000 rev/min for 20 h at 4 °C and the gradient was fractionated at 260 nm. The virus peak was collected and dialysed against PBS if not otherwise stated. In several cases purification was followed by a second equilibrium run in CsCl.

*Plaque titration.* The infectivity of the virus was titrated by the plaque method of Cooper (1967), using HEp-2 cells.

*Radioisotopes and chemicals.* N-succinimidyl-2,3-³H-propionate (66 Ci/mmol), N-succinimidyl 3-(4-hydroxy,5-¹²⁵I-iodophenyl)propionate (1700 Ci/mmol), N-ethyl-2,3-¹⁴C-maleimide (3·7 mCi/mmol), iodo-2-³H-acetic acid (152 mCi/mmol), ¹⁴C-amino acid mix-
ture, $^3$H-amino acid mixture, L-cysteine-$^{35}$S-hydrochloride (65 mCi/mm), L-4,5n-$^3$H-lysine monohydrochloride (25 Ci/mm), were purchased from the Radiochemical Centre, Amersham, England. Dimethyl suberimidate and dimethyl adipimidate were from Pierce Chemical Company, Rockford, Illinois, U.S.A.

**Modification with monofunctional reagents.** For modification of amino groups $^1$4C-labelled virus ($5 \times 10^9$ p.f.u./ml) was dialysed against 0·1 M-triethanolamine-HCl (pH 8·2) and allowed to react with 2 mCi N-succinimidyl-2,3-$^3$H-propionate (SIP) for 0·5 h at room temperature. The modified virus was extensively dialysed against 10 mM-tris-HCl (pH 7·5) or layered on a linear sucrose gradient (15 to 30%, w/w, in PBS) and run in an SW 40 rotor (40000 rev/min, 3 h, 4 °C). After fractionation, samples were taken and 1 ml of 3% formaldehyde was added to each vial. The extent of modification was estimated by counting in a Packard Tricarb liquid scintillation counter after dispersion in 10 ml of Instagel (Packard). The virus fractions were collected and centrifuged in an SW 65 rotor (45000 rev/min, 1 h, 4 °C). The pellet was resuspended in 0·2 ml PBS.

The method of Bolton & Hunter (1973) was used for the modification of the lysine groups with $N$-succinimidyl 3-(4-hydroxy, 5-$^{13}$I-iodophenyl)propionate. The reaction is similar to that described above with the exception that the time of reaction was 15 min at 0 °C and that unlabelled virus was used for modification. After separation of excess reagent by either dialysis against PBS or by centrifugation through a sucrose gradient, the radioactivity was determined in an Auto-Gamma scintillation spectrometer (Packard).

Modification of the sulphydryl groups was carried out either with $^3$H-iodoacetic acid (IAA) or $^{14}$C-N-ethylmaleimide (NEM). Virus ($5 \times 10^9$ p.f.u./ml) was dialysed in 50 mM-tris-HCl (pH 7·2) and added to 0·5 mCi of NEM at room temperature. After 1 h, the reaction was quenched by addition of excess of 2-mercaptoethanol (2-ME). The reaction with IAA was carried out in the same way except that the time of reaction was for 2 h. The treated virus preparation was dialysed against 20 mM-tris-HCl (pH 7·5) or fractionated through a sucrose gradient as described above.

**Amino acid analysis.** Determination of the amino acid composition of the capsid proteins was made after hydrolysis of poliovirus for 18 h with 6 N-HCl in vacuo at 11 °C using a Durrum amino acid analyser (Durrum, Model D 500, Palo Alto, California, U.S.A.).

**Cross-linking with bifunctional imidoesters.** The reactions were carried out according to the method of Clegg & Hayes (1974). $^3$H-labelled virus ($5 \times 10^9$ p.f.u./ml) was dialysed against 0·2 M-triethanolamine-HCl (pH 8·2) and allowed to react with dimethyl suberimidate (DMS) or dimethyl adipimidate (DMA) at a final concentration of 5 mg/ml for 0·5 h at 37 °C. The reaction was stopped by addition of 1 M-tris-HCl (pH 7·5) to a final concentration of 10 mM-tris-HCl. Purification of the treated virus was carried out by centrifugation through a sucrose gradient as described above.

**SDS polyacrylamide gel electrophoresis.** For analysis of the modified proteins, the virus was dialysed against 10 mM-tris-HCl (pH 7·5), 1 M-urea and 6 mM-2-ME containing 4% SDS, and incubated for 2 min at 100 °C. After addition of 10% glycerol and 0·001% bromophenol blue (B), the samples were run on cylindrical 12·5% polyacrylamide gels ($5 \times 120$ mm), containing 0·33% methylene-bis-acrylamide, 0·1% SDS and 3% urea, or on 15% polyacrylamide gels containing 0·38% methylene-bis-acrylamide, 0·1% SDS and 3% urea. Electrophoresis was carried out in 80 mM-tris-glycine (pH 8·6) containing 0·1% SDS, 3% urea and 5 mM-thioglycollic acid for 4 h at a constant current of 3 mA per gel.

To visualize the proteins, the gels were stained with 0·05% Coomassie brilliant blue in 25% methanol and 10% acetic acid. For preparative purposes, radioactively labelled proteins were used. Such gels were frozen at −20 °C after electrophoresis and sliced longitudinally. One half was kept for ammonolysis of the cross-linked proteins, while the other half was cut into 1 mm slices for accurate determination of the position of the cross-linked proteins.
Fig. 1. SDS polyacrylamide gel electrophoresis of poliovirus polypeptides in vivo labelled with $^{35}$S-cysteine. Electrophoresis was performed on 12.5% polyacrylamide gels as described in Methods. The direction of migration is from left to right. B, bromophenol blue.

Fig. 2. SDS polyacrylamide gel electrophoresis of poliovirus polypeptides in vivo labelled with $^3$H-lysine. Electrophoresis was performed on 12.5% polyacrylamide gels as described in Methods. The direction of migration is from left to right.

The slices were placed in sealed vials and solubilized by incubation with 0.3 ml of 30% $\text{H}_2\text{O}_2$ for 5 h at 60 °C and then counted as described above.

Ammonolysis of the cross-link. Reversal of the cross-linking reaction was by the method of Bickle et al. (1972) which was slightly modified. Slices of the polyacrylamide gel containing the $^3$H-labelled cross-link were dialysed for 10 h at room temperature against a mixture of 15 vol. of concentrated ammonia (0.88 g/ml) and 1 vol. of glacial acetic acid containing 1% SDS. The slices were then dialysed several times against 10 mM-tris-$\text{HCl}$ (pH 7.5) containing 0.1% SDS.

RESULTS

Labelling of poliovirus proteins and chemical modification with monofunctional reagents

$^{35}$S-cysteine-labelled poliovirus proteins were investigated on SDS polyacrylamide gels. VP1, VP2 and VP3 contain cysteines, while VP4 is devoid of this amino acid (Fig. 1). If the proteins were stained after electrophoresis, the four polypeptides were detectable at their expected positions including VP4 (not shown). The extent of radioactivity bound to each of the polypeptides is shown in Table 1. The amount of label reflects the ratio of cysteine in the polypeptides; VP1: VP2: VP3: VP4 was 1.4:5.0:3.6:0. There is also a small amount of cysteine in VPo. For chemical modification, virus was treated with either $^3$H-iodoacetic acid or with $^{14}$C-N-ethyl maleimide. In neither case was radioactivity bound to the proteins (Table 1), indicating that cysteine residues are not available for modification in any capsid protein.

When $^3$H-lysine-labelled virus was subjected to SDS polyacrylamide gel electrophoresis radioactivity was found in each of the four structural proteins and even a small amount in VPo (Fig. 2, Table 1). The total amount of radioactivity in the four proteins is equivalent to 38 lysine residues, which was calculated from the amino acid analysis. Assuming that the lysines are uniformly labelled, the number in each virus protein can be calculated from the percentage of the radioactivity bound to each polypeptide (Table 2).
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Table 1. Distribution of radioactivity bound to virus proteins

<table>
<thead>
<tr>
<th>Sample of poliovirus I (Mahoney)</th>
<th>Virus proteins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled with $^{35}$S-cysteine</td>
<td>VP1</td>
</tr>
<tr>
<td>Modified with $^{3}$H-IAA</td>
<td>14.2</td>
</tr>
<tr>
<td>Modified with $^{14}$C-NEM</td>
<td>0</td>
</tr>
<tr>
<td>Labelled with $^{3}$H-lysine</td>
<td>39.6</td>
</tr>
<tr>
<td>Modified with Bolton Hunter reagent</td>
<td>30.3</td>
</tr>
<tr>
<td>Modified with SIP</td>
<td>56.5</td>
</tr>
</tbody>
</table>

* The percentages of the radioactivity were derived from SDS polyacrylamide gel electrophoresis.

Table 2. Calculation of the lysine residues in poliovirus proteins

and calculation of the modified lysine residues

<table>
<thead>
<tr>
<th>Capsid proteins</th>
<th>VP1</th>
<th>VP2</th>
<th>VP3</th>
<th>VP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Lysine residues*</td>
<td>15.1</td>
<td>5.6</td>
<td>11.7</td>
<td>5.5</td>
</tr>
<tr>
<td>(ii) Lysine residues modified with SIP†</td>
<td>3.4</td>
<td>0.6</td>
<td>2.0</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Amino acid analysis was carried out after hydrolysis of the polypeptides in 6 N-HCl (see Methods), which yielded 4.41 mol of lysine and 93.53 mol of the other amino acids (Cys and Trp were not determined). The moles are expressed as mol per 100 mol of amino acids recovered. The mol. wt. of a structural unit was assumed to be 95 K (Baltimore, 1969), which corresponds to 863 amino acid residues. On this basis, 38 lysine residues per structural unit were computed, which is equivalent to 100% radioactivity of $^{3}$H-lysine-labelled protein (Fig. 2, Table 1). The number of lysine residues of each polypeptide was derived from the percentage of radioactivity (Table 1).

† The number of modified lysine residues was calculated as follows: $^{3}$H-SIP, sp. act. of 66 Ci/mmol was used. 1 $A_{660}$ unit of virus corresponds to $9.4 \times 10^{10}$ virions (Rueckert, 1971) and was determined to be equivalent to $3.54 \times 10^{3}$ d/min of $^{14}$C-amino acid-labelled virus. After modification, the resulting ratio of $^{3}$H:$^{14}$C radioactivity was $6.79 \times 10^{2}$ d/min:$2.46 \times 10^{5}$ d/min. The results of the calculations show that 365 mol of SIP were introduced per 1 mol of virus. Since there are 60 copies of each polypeptide in a virion and since the percentage of modification was determined (Table 1), the number of modified lysine residues could be calculated.

Chemical modification of the amino groups in the intact virion was performed either with Bolton Hunter reagent or with SIP (Fig. 3). The sedimentation rate of the treated virus in a sucrose gradient was unchanged. Furthermore, no influence on the infectivity was detectable after modification.

With both reagents, VP1 and VP3 were modified to a greater extent than VP2, while VP4 reacted to only a small extent with SIP (Table 1). If $^{14}$C-protein-labelled virus was treated with increasing amounts of SIP, the ratio of incorporated $^{3}$H radioactivity to $^{14}$C radioactivity was not enhanced, indicating that the reaction was complete within 0.5 h under these conditions. Comparing the modification with SIP and Bolton Hunter reagent, the relative amount of $^{3}$H and $^{131}$I radioactivity bound to VP1 or VP3 was different (Fig. 3).

From the specific radioactivity of SIP (66 Ci/mmol) and from the assumption that one absorbance unit corresponds to $9.4 \times 12^{13}$ virions (Rueckert, 1971), it is possible to calculate the number of modified amino groups in each virus protein (Table 2).
Chemical cross-linking

Protein neighbourhoods were subsequently examined with DMS. In a control experiment, cross-linked material was mixed with 14C-protein-labelled virus as a marker immediately before centrifugation through a sucrose gradient. The cross-linked 3H-virus and the 14C-labelled virus gave bands at identical positions (160S; Fig. 4). There was no radioactivity detectable in front of the virus peak, indicating that no cross-linking between virus particles took place and no radioactivity remained on top of the gradient, indicating that the particles were not partially fragmented.

The cross-linked material was denatured and subjected to electrophoresis on polyacrylamide gels in SDS. The four polypeptides (VP1 to VP4) can be seen on the electrophoresis pattern (Fig. 5) and a new slower migrating component (mol. wt. 63 K) appeared (Fig. 6). The use of radioactive labelled material allows the distribution of the virus polypeptides in the gels to be quantified. After cross-linking, VP1 and VP3 are diminished by approx. 25% as compared with VP2 and VP4 which were not affected by the reagent. The amount of radioactivity in the 63 K complex is one half that of VP2. About 25% of the total of VP1 and VP3 are cross-linked. Since there are 60 copies of each protein in one virion, there should therefore be 15 cross-links per virion.

Two further complexes were observed on the electrophoresis pattern migrating more slowly than the 63 K complex, but their yield was too low for isolation and proper characterization. When DMA was used for cross-linking instead of DMS, the results were similar. Therefore, DMS was used in all following experiments. The infectivity of the virus after cross-linking was determined. Virus which had been submitted to the reaction conditions, omitting DMS, served as a control. The infectivity decreased from \(9.5 \times 10^8\) p.f.u./ml (control) to \(0.22 \times 10^8\) p.f.u./ml (virus after cross-linking). The specific infectivity decreased from 730 p.f.u./d/min (control) to 15 p.f.u./d/min (cross-linked virus).
Fig. 5. SDS polyacrylamide gel electrophoresis of poliovirus polypeptides uniformly labelled in vivo with $^3$H-amino acids after cross-linking with DMS. Electrophoresis was performed as described in Methods. The direction of migration is from left to right.

Fig. 6. Plot of log (mol. wt.) versus migration of the cross-linked complex (63 K). Marker proteins: VP1, 35 K; VP2, 28 K; VP3, 24 K (Baltimore, 1969); bovine serum albumin (BSA) 67 K. Electrophoresis was performed on 12.5 % polyacrylamide gels as described in Methods. Migration distance of BSA was determined in a Gilford spectral photometer equipped with a gel scanner at 280 nm.

Fig. 7. SDS polyacrylamide gel electrophoresis of poliovirus polypeptides after cleavage of the 63 K complex, labelled with $^3$H (●—●) and poliovirus polypeptides uniformly labelled with $^{14}$C-amino acids (○—○). Electrophoresis was performed as described in Methods. The migration is from left to right.

Cleavage of the cross-link and identification of the proteins

To identify the cross-linked proteins, the 63 K complex was subjected to ammonolysis, causing cleavage of amidine groups. The split complex was re-electrophoresed in the presence of $^{14}$C-labelled virus proteins (Fig. 7). Two proteins, namely VP1 and VP3, in nearly equal amounts, migrated exactly to the positions of the marker proteins. VP2 and VP4 were not detectable on the $^3$H electrophoresis pattern. In some experiments there was still a small amount of radioactivity precisely at the position of the complex, indicating that ammonolysis was not complete under these conditions. There were also a few fast-migrating small peaks (Fig. 7), probably virus protein fragments originating from the ammonolysis, which might hydrolyse weak peptide bonds.
DISCUSSION

VP1 and VP3 are adjacent proteins in the poliovirus capsid, as indicated by about 15 links per virion between these two proteins with the bifunctional reagents DMS and DMA, binding covalently to ε-amino groups of lysine or N-terminal amino groups. The estimation of the mol. wt. of the uncleaved cross-link complex still excludes this complex consisting of VP1–VP1 or VP3–VP3, although the mol. wt. may be overestimated by 10%. VP2 and VP4 are not involved in cross-linking as they are not detectable on the electrophoresis pattern of the split complex. There is support from the modification with the monofunctional SIP and Bolton Hunter reagent that VP1 and VP3 are exposed proteins. The lactoperoxidase-catalysed iodination of VP1 (Beneke et al. 1977), exclusively modifying surface-directed tyrosine residues (Michalski & Sells, 1974), proves this protein to be a surface protein. The acetylation of VP1 with acetic anhydride (Lonberg-Holm & Butterworth, 1976) also points in this direction. Since the distance of the covalent linkage with the bivalent reagent is only 0.8 nm in the case of DMA, it seems likely that cross-linking takes place at the surface. Therefore, it is reasonable to assume that VP1 and VP3 are neighbouring proteins in contact with the surface of the shell. With the Bolton Hunter reagent, VP3 clearly reacts to a higher extent than VP1, which may reflect steric effects or an incomplete reaction of VP1.

From dissociation of the virus by alkali degradation (Katagiri et al. 1971), resulting in a loss of VP4 and VP2, it seems likely that the basic matrix of the capsid is constructed mainly from VP1 and VP3. The cross-linking experiment supports this idea. VP1 and VP3 also have adjacent cistrons on the RNA, indicating that they are neighbouring polypeptides in the protein precursor NCVPI of the capsid proteins. The adjacent arrangement of VP1 and VP3 may be an important key in determination of the fine structure of poliovirus.

It is unlikely that all lysine residues in the four polypeptides are reactive for chemical modification. It is generally accepted that lysine groups vary in their reactivity depending on their environment. Those which are hidden in the interior of a protein, between a protein–protein interface or protein–RNA interface are in general not accessible, whereas those which are positioned towards the outside or on the protein surface are more reactive. Chemical modification with SIP indeed demonstrates that only a proportion of all lysine residues is reactive. The bulk of 11.7 out of 15.1 lysines of VP1, 5.0 out of 5.6 lysines of VP2, 9.7 out of 11.7 lysines of VP3 and the total of 5.5 lysines of VP4 are probably buried. The number of lysines per protein reacted with SIP might be overestimated by one residue, taking into account the possible reaction of the N-terminal amino group of a peptide chain.

From the relative inaccessibility of VP2 and VP4 for chemical modification one might assume that both are interior proteins with VP4 being more deeply buried than VP2. This should be applicable for proteins with spherical shapes. The easy release of VP4 after adsorption of the virion to a cell or after heat treatment of the virus may be caused by a conformational change of the protein shell (Rueckert, 1976). However, if VP2 and VP4 have elongated shapes with only the lysine residues buried, VP2 and to a lesser extent VP4, might contribute to the surface of the shell as well.

Chemical modification with the two monofunctional reagents has no effect on the infectivity of the virus. Obviously, lysine groups are not necessarily an integral factor in virus adsorption to a cell. Contrary to this result the infectivity is reduced by more than 90% after cross-linking with the bifunctional reagent.

The number of lysines in poliovirus is similar to that in Mengo virus (Ziola & Scraba, 1975) and mouse Elberfeld virus (Stoltzfus & Rueckert, 1972). The total number of lysines is in reasonable agreement with the amino acid analysis of poliovirus proteins of Cooper & Bennett (1973). However, this calculation of the total number of lysines may be in error by up to 10%, taking into account that (i) the calculation of the number of amino acids over-
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looks several amino acids (Cys, Trp) and (ii) the determination of the mol. wt. of the four polypeptides is still accompanied by some uncertainties, e.g. that of VP4, which varies from 5 K (Maizel & Summers, 1968) to 11 K (Wouters & Vandekerckove, 1976). The number of lysines in each of the four polypeptides presented here agrees satisfactorily with that calculated by Wouters & Vandekerckove (1976), except in the case of VP2.

The presence of cysteine in the three major polypeptides and its absence in the smallest has been reported for several other picornaviruses: e.g. Mengo virus (Ziola & Scraba, 1975), mouse Elberfeld virus (Stoltzfus & Rueckert, 1972) and Coxsackie B3 virus (Phillipson et al. 1973). In the intact virion, however, all cysteine residues are inaccessible, since they are not reactive with either IAA or NEM, confirming earlier results that cysteines are not reactive in the intact virion (Lonberg-Holm & Butterworth, 1976).

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