Isolation and Characterization of Trypsin-resistant O₁ Variants of Foot-and-Mouth Disease Virus

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SUMMARY

Strains of foot-and-mouth disease virus of types O₁ and A₁₀ were isolated which showed no significant loss of infectivity upon trypsinization. These ‘trypsin-resistant’ (TR) viruses were obtained by serial passage in BHK cells of virus that was trypsin-treated before inoculation of the cells. Three O₁ isolates were cloned and studied further. Cell attachment of those TR O₁ variants (OTR₁) was not reduced by trypsinization, unlike that of parent virus.

The polypeptide compositions of TR viruses as determined by SDS-polyacrylamide gel electrophoresis were identical with those of parent virus, with the exception of OTR₁ which contained an additional polypeptide approx. 3000 daltons larger than VP₁. After trypsinization, which normally cleaves VP₁, the polypeptide composition of the three TR viruses (including OTR₁) and of parent virus did not show any significant difference. In OTR₁ both the additional virus protein and VP₁ were cleaved into a P₁₈ molecule and smaller fragments. The surface location of this additional polypeptide was confirmed by iodination experiments. It was shown by immunodiffusion experiments that only OTR₁ differed from the parent virus. This antigenic change was present on the trypsin-sensitive part of the virus since trypsinized TR viruses (including OTR₁) were antigenically identical to trypsinized parent virus.

The electrophoretic mobilities of the three OTR viruses isolated, and of parent virus, differed somewhat before trypsinization. After trypsin-treatment, the mobilities of TR viruses were all increased to the same level; however, their rate of migration was lower than that of trypsin-treated parent virus. This lower mobility of trypsin-treated OTR viruses was the only difference which could be associated with retained infectivity.

INTRODUCTION

Unlike most other picornaviruses, foot-and-mouth disease virus (FMDV) is sensitive to the action of trypsin (Matheka et al. 1962), but although infectivity is reduced by trypsinization, the virus particles retain complement fixing activity and contain infectious RNA (Brown et al. 1963). Trypsin-treated virus particles still sediment at 146S and the buoyant density is only slightly increased (Wild & Brown, 1967). Loss of infectivity is due to lack of adsorption of trypsin-treated virus particles to susceptible cells (Brown et al. 1963; Wild & Brown, 1967; Rowlands et al. 1971). It was demonstrated by immunodiffusion that an antigenic site was lost after trypsinization (Cowan, 1969; Wild et al. 1969; Meloen, 1976). The immunogenicity of inactivated FMDV type O₁ is also strongly reduced by the action
of trypsin (Rowlands et al. 1971; Cavanagh et al. 1977). Since polyacrylamide gel electrophoresis shows that only VP₁ (mol. wt. approx. 29 K), one of the four major polypeptides of the virion, is hydrolysed by trypsin (Wild et al. 1969; Burroughs et al. 1971) and only this trypsin-sensitive polypeptide stimulates the production of neutralizing antibody (Laporte et al. 1973; Bachrach et al. 1975), both cell attachment and immunogenicity must be associated with VP₁.

Infectivity is not completely destroyed by trypsinization. The residual infectivity was at first considered to be due to virus particles which had escaped from the action of the enzyme (Wild et al. 1969). However, Rowlands et al. (1971) subsequently showed by sucrose gradient electrophoresis that the infectivity of trypsin-treated O₁-virus preparations was associated with the trypsin-altered virus particles which show a markedly increased negative charge in comparison with untreated virus.

Reduction in infectivity may vary strongly between different FMDV strains: the titre of an O₁ virus was reduced over three logs, while a titre reduction of only one log was observed for a type C (997) virus which had received the same treatment and was titrated in the same cell system (Rowlands et al. 1971). Such variation in sensitivity to trypsin might also exist within an uncloned virus population. If viruses could be isolated which do not show a reduction of infectivity after treatment with trypsin, such viruses might contribute to a better understanding of the process of virus–cell attachment and of immunogenicity.

Such ‘trypsin-resistant’ (TR) virus populations could be produced by serial passage, giving the successive virus harvests a trypsin-treatment before use as inoculum for succeeding passages. These propagations in which TR virus was given a selective advantage during inoculation resulted in virus populations which did not show a significant reduction in infectivity upon trypsinization. The effect of trypsinization upon some of the immunological, biochemical and physical properties of the isolated TR viruses was studied.

**METHODS**

**Reagents and materials.** Trypsin type XII, soybean trypsin inhibitor, α-chymotrypsin type II, bovine serum albumin (BSA), ovalbumin, chymotrypsinogen and cytochrome c were purchased from Sigma, SDS from Serva, polyethylene glycol 6000 (PEG) from Hoechst. Hyflo supercell filter-aid from Johns Manville and agarose type HSA and HSC from Litex Glostrup, Denmark, NCS cell solubilizer was from Nuclear, Chicago.

**Cells and viruses.** FMDV type O (subtype I, strain BFS 1860, kindly supplied by the Animal Virus Research Institute, Pirbright, U.K.) had been passaged nine times in BHK 21 Cl 13 monolayers and twice in BHK suspension cell cultures before it was used for the isolation of ‘trypsin-resistant’ strains. The same O₁ virus was also cloned by plaque purification three times (O₁cl). FMDV type A (subtype Ia, strain Holland), maintained by passage in cattle, was passaged three times in pigs and four times in BHK monolayer cells. BHK monolayers were maintained in Eagle’s medium and BHK suspension cultures in enriched Eagle’s medium containing 5% PEG-treated serum (Barteling, 1974).

**Virus titration.** Infectivity was measured by the agarose-cell suspension plaque assay using BHK suspension culture cells stored over liquid nitrogen (Barteling, 1972). Virus was allowed to adsorb to cells for 1 h at 37 °C before they were mixed with agarose medium. Agarose HSA was used instead of EDTA-washed agar. Virus yields were estimated by sucrose gradient centrifugation and u.v. analysis of the gradients as described previously (Barteling & Meloen, 1974).

**Enzyme treatment.** Trypsinization of purified as well as non-purified virus was performed at 37 °C for 15 min, using an enzyme concentration of 1 mg/ml. The presence of 5% PEG-treated bovine serum in crude virus preparations did not inhibit enzyme activity, as estimated
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by plaque titration. The reaction was stopped by addition of 1.5 mg/ml soybean trypsin inhibitor.

Chymotrypticization leading to a limited hydrolysis of VP1 and to a limited reduction in infectivity was performed according to Cavanagh et al. (1977). Virus and chymotrypsin were incubated for 1 h at 37 °C in the presence of 2 mg/ml BSA.

Isolation of 'trypsin-resistant' viruses. Non-cloned O and A virus and cloned O virus were trypsin-treated and after membrane filtration used to inoculate 200 ml BHK suspension cultures containing 106 cells per ml. Harvests were taken after 24 h incubation at 36 °C. One ml of each progeny virus was again trypsin-treated and used as inoculum for the next passage. This 'selective advantage inoculation' type of propagation was repeated a number of times until 'trypsin-resistant' (TR) virus had been obtained which showed less than 0.5 log reduction. Trypsin-resistance at the different passage levels was estimated by plaque titration. Parent virus was used as a control in all trypsinization experiments. Three TR viruses were plaque purified. The cloned progenies of two experiments with non-cloned O parent virus and of one experiment with cloned O parent virus were designated OTR1, OTR2 and OTR3 respectively. Finally, the viruses were propagated in 6 or 40 l scale BHK suspension culture in Eagle's MEM containing 5 % of PEG-treated bovine serum (Barteling, 1974). The virus was added in the log growth phase of the cells and harvested when cell viability, as estimated by staining with trypan blue, had dropped below 20 %.

Virus purification. Virus was precipitated with 7 % PEG and collected with 0.8 % filter-aid on a Büchner funnel. The virus was eluted in 1/10 of the original volume either immediately or after storage of the dry filter cake at −70 °C. Elution was performed with 0.05 m-Na-phosphate buffer (PB) containing 0.2 m-NaCl, pH 7.5. The virus suspension was clarified by filtration and precipitated with 4 % PEG. After centrifugation for 20 min at 5000 g, the supernatant was discarded and the pellet stored in small portions at −70 °C. After elution of the virus in PB it was finally purified by sucrose density gradient centrifugation or by isopycnic banding in CsCl. The purified virus was dialysed against PB. Virus concentration and relative purity were determined spectrophotometrically (Bachrach et al. 1964). Virus preparations were stored at 4 °C in the presence of 0.1 % sodium azide.

Radioactive labelling. BHK monolayers in square, milk dilution bottles were inoculated with virus at a multiplicity of approx. 50 p.f.u./cell in 3 ml methionine-free Eagle's MEM containing 2 % PEG-treated calf serum. Adsorption was allowed to proceed for 90 min at 37 °C; subsequently cells were washed twice with PBS before 3 ml of methionine-free EMEM containing 50 μCi/ml 35S-methionine and 5 % dialysed PEG-treated calf serum were added to the monolayers. Labelling of the surface proteins of purified virus with 35S was performed using the lactoperoxidase reaction (Marchalonis, 1969).

Measurement of attachment of virus to cells. Untreated and trypsin-treated 35S-methionine labelled O and OTR1 virus (about 5000 ct/min) were added to 107 BHK cells suspended in 1 ml Eagle's medium containing 2 % horse serum. The virus cell suspension was stirred gently for 30 min at 4 °C, after which the cells were removed by centrifugation. The radioactivity of the cell pellet was estimated after treatment with 0.5 ml NCS and was compared with the activity of the supernatant.

Immunodiffusion. The double diffusion (Ouchterlony) method was performed on coverslips with an agarose layer about 1 mm thick. The wells had a diam. of 6 mm and distances between the wells were 4 mm. The other conditions were as described elsewhere (Meloen, 1976). Early anti-O1 and anti-OT1 antisera were obtained by infecting FMDV-susceptible pigs in the foot-pad and collecting serum 7 days post infection (p.i.). Sera were stored at −20 °C.

SDS-polyacrylamide gel electrophoresis (PAGE). Purified virus was dissociated by the addition of sample buffer (Laemmli, 1970) supplemented with 6 m-urea and the virus poly-
peptides were analysed on 10% polyacrylamide gel slabs using the discontinuous buffer system of Laemmli (1970). Radioactivity was visualized by fluorography (Bonner & Laskey, 1974).

Agarose gel electrophoresis. The electrophoretic mobilities of purified virus before and after trypsinization were investigated using a cooled flat bed electrophoresis apparatus. For this purpose type HSC agarose was applied which showed low electro-endosmosis. A glass plate of 9 x 11 cm was placed on a horizontal table. Fifteen ml of a hot 0.8% agarose solution in electrophoresis buffer, to which 10% sucrose had been added, was poured on to the plate and a vertical comb placed immediately over it in the agarose. The comb divided the agarose gel into wells of 4 x 1.5 x 1.3 mm, leaving a gel layer of 0.2 mm under the wells. After the agarose had set, 6 µl of samples which had been dialysed against electrophoresis buffer without sucrose, and which contained approx. 0.3 mg/ml of purified virus, were added to the wells. The plates were left for 30 min at room temperature and the wells then refilled with 5 µl agarose buffer without sucrose. Electrophoresis was performed at 8 °C and 100 V (approx. 15 V/cm) for 120 min. Phenol red solution 0.01% in electrophoresis buffer was used as a reference. At the end of the run, the position of the phenol red was marked and the plates were dried overnight at 37 °C and stained in a 0.2% Coomassie brilliant blue solution. In general, electrophoresis was performed with a tris-HCl buffer, pH 8.8, containing 25 mM-tris, 10 mM-NaCl and 1 mM-EDTA.

RESULTS

Isolation of 'trypsin-resistant' virus

'Trypsin-resistant' virus was obtained by a 'selective advantage inoculation' type propagation. Six such isolations were made, three starting with uncloned O virus, two with cloned O virus and one with A virus (Fig. 1). Titres of cloned and uncloned parent O virus were reduced by more than three logs by trypsinization, while infectivity of A virus dropped between 2.5 and 3 logs. After three or four such passages in BHK suspension cultures the infectivity of progeny from the uncloned O virus was only slightly reduced by the action of the enzyme. A10 virus was trypsin-resistant after five passages, but when plaque-purified O virus was used, five to seven passages were necessary for the development of resistance (Fig. 1). Some of the 'trypsin-resistant' viruses obtained were plaque purified. Clones OTR1 and OTR3 were obtained from 'trypsin-resistant' populations derived from uncloned parent virus, while OTR3 originated from cloned parent virus. In general, titre reductions of less than 0.5 log were found for these viruses on treatment with trypsin. In this study no further attention will be given to the A-type isolate.

Attachment of virus to BHK cells

Attachment of FMDV to susceptible cells is strongly reduced on trypsinization of the virus (Brown et al. 1963; Rowlands et al. 1971). The effect of trypsinization on the attachment of parent virus and of TR progeny virus was investigated with two different BHK cell populations, fresh cells from a suspension culture as used for isolation and propagation of the virus and BHK cells from our liquid nitrogen stock as used for the virus titration. For this purpose 35S-labelled viruses were used. Table 1 shows that attachment of parent virus is markedly reduced by trypsin treatment while only a slight reduction was observed with OTR1 virus.

Immunodiffusion tests

Differences between complete and trypsin-treated virus can be demonstrated by immunodiffusion against 'early' convalescent antisera (Cowan, 1969; Wild et al. 1969; Meloen,
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Fig. 1. Titre reduction by trypsinization of viruses at different passage levels of a 'selective advantage inoculation' type propagation for trypsin-resistant virus starting with crude O virus (○---○), plaque purified O virus (●-○) and A10 virus (▲-▲). △--△, OTR\(_1\); □--□, OTR\(_2\); ■---■, OTR\(_3\).

Table 1. Effect of trypsin treatment on the adsorption to BHK cells

<table>
<thead>
<tr>
<th>Virus</th>
<th>Before trypsinization</th>
<th>After trypsinization</th>
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<tbody>
<tr>
<td></td>
<td>Fresh cells (%*)</td>
<td>N(_2)-stock cells (%*)</td>
</tr>
<tr>
<td>O</td>
<td>86 * * * * * *</td>
<td>80</td>
</tr>
<tr>
<td>OTR(_1)</td>
<td>85</td>
<td>80</td>
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\* Percentage of \(^{35}\)S-labelled virus adsorbed to \(10^7\) cells (mean of two experiments).

Fig. 2. Immunodiffusion with an 'early' convalescent antiserum which also reacts with a newly exposed antigen of trypsin-treated (tt) virus. (a) Comparison of non-treated (O) and trypsin-treated (ttO) virus. The same results were obtained if trypsin-treated O was replaced by trypsinized OTR. (b) Comparison of trypsin-treated O and OTR viruses.

1976). In addition, differences between variants can be detected with these sera (Cowan, 1969). In our immunodiffusion studies, 'early' (7 days p.i.) convalescent pig sera prepared against parent (O) virus and TR progeny viruses were used. Only OTR\(_1\) differed from untreated parent virus. OTR\(_2\) and OTR\(_3\) were identical to the parent virus (data not shown).

With all antisera, untreated viruses spurred on their trypsin-treated counterparts and all trypsinized viruses demonstrated complete lines of identity. With some of these antisera (against O as well as against TR viruses) all trypsin-treated viruses also spurred on complete viruses, yielding a cross (Fig. 2a). This indicates the presence of a newly exposed antigen.
present only after trypsinization. With these antisera also, only lines of identity were obtained between trypsin-treated viruses (Fig. 2b). Thus no antigenic differences were observed between the trypsin-treated parent virus with low infectivity and the infectious trypsinized TR progeny viruses.

**Effect of trypsin on polypeptide composition**

As trypsin cleaves VP₁ into a piece with a mol.wt. of approx. 18 K (P₁₈) and one or more smaller fragments (Wild *et al.* 1969; Burroughs *et al.* 1971; Strohmaier & Adam, 1974; Matheka & Bachrach, 1975), the effect of trypsinization on the composition of 'trypsin-resistant' viruses was studied by SDS-PAGE electrophoresis (Fig. 3). Only untreated OTR₁ virus differed clearly from parent virus. It showed the presence of an additional polypeptide (designated VP₄₅), approx. 3 K larger than VP₁ (Fig. 3a). Scanning of the gel patterns of parent virus and of OTR₁ (Fig. 3b) shows that the area of the VP₁ peak of parent virus is
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Fig. 4. Autoradiogram of SDS-polyacrylamide gel electrophoresis of different virus preparations labelled with $^{131}$I by lactoperoxidase. (a) Iodinated O virus; (b) iodinated OTR virus; (c) iodinated trypsin-treated O virus; (d) iodinated trypsin-treated OTR virus; (e) trypsin-treated iodinated O virus; (f) trypsin-treated iodinated OTR virus.

about the same as that of the sum of VP1 and VP₄ of OTR₁. This suggests that in OTR₁ part of VP₁ is replaced by VP₄. Whether this additional polypeptide was situated at the surface of the virion or in a more internal position, was investigated by lactoperoxidase labelling with $^{131}$I (Laporte & Lenoir, 1973; Talbot et al. 1973). In the case of parent virus mainly VP₁ was labelled, while in OTR₁, VP₄ was also accessible to the isotope (Fig. 4a and b), which suggests an exterior position for both VP₁ and VP₄.

After trypsinization, all three TR viruses showed the same polypeptide composition as trypsinized parent virus (Fig. 3). In the case of OTR₁, VP₄ disappeared and, like VP₄, was cleaved into P18 and a 6 K fragment.

If iodinated parent and OTR₁ virus were trypsinized, radioactivity was found in P18; the small amount of iodine in VP₂ and VP₃ could still be detected there after trypsin treatment (Fig. 4e and f). Differences in infectivity may have been due to changed conformations of the polypeptides. Therefore parent and OTR₁ virus were also labelled after trypsinization. Again the same patterns were observed (Fig. 4c and d). These results indicate that at least part of the P18 fragment of VP₁ is in an exterior position in parent virus and TR virus both before and after trypsinization.

The effect of chymotrypsin treatment on the polypeptide composition of parent virus was also studied. VP₁ is hydrolysed to two polypeptides, both of which are about 1 to 2 K larger than the corresponding VP₁ fragments which remain after trypsinization (Fig. 3a). This suggests that, by trypsin treatment, VP₁ is cleaved into at least three fragments.

Agarose gel electrophoresis

Electrophoretic mobilities of the different isolates and the influence of trypsinization upon the charge of the virus particles were compared using cooled flat bed agarose gel electrophoresis. Untreated viruses all showed different mobilities; even cloned parent virus differed somewhat from uncloned virus. Relative to the mobility of phenol red at pH 8·8, RF values were estimated to be 0·29, 0·33 and 0·30 for O, Ocl and OTR₁ respectively. OTR₂ and OTR₃ did not migrate at this pH. After trypsinization the mobilities of both parent virus and TR viruses increased markedly. Although the mobilities of the untreated TR viruses differed, after trypsinization they showed the same migration (RF 0·52), which was lower than that.
of trypsin-treated parent virus (RF 0.57). The same was observed in other buffer systems. Treatment of parent virus with chymotrypsin also increased the rate of migration of the virus particle, but not to the same extent as did trypsinization (Fig. 5).

DISCUSSION

The electrophoretic mobility (at pH 8.8) of FMDV type O1 is greatly increased and antigenicity is markedly reduced by trypsin treatment (Rowlands et al. 1971; Cavanagh et al. 1977). Depending on the cell system used for titration, infectivity drops from 1 to more than 3 logs. Under the same conditions, infectivity of a strain of type C virus was only reduced by 0.2 to 1.2 logs, although the negative charge of the virus particle was also markedly increased (Rowlands et al. 1971). Thus FMDV transition to a low pl status caused by trypsinization is not necessarily accompanied by a large drop in infectivity. The experiments reported here indicate that in normal FMDV populations virus particles may be present which retain about the same infectivity for BHK cells on trypsinization. By a 'selective advantage inoculation' type propagation, after only three to four passages a virus population was obtained which did not show a significant reduction in infectivity by treatment with the enzyme.

Starting with a cloned trypsin-sensitive virus, 'trypsin-resistant' progeny could also be obtained although in this case it took five to seven passages. This suggests that 'trypsin-resistance' can be the result of mutation. The work of Brown and co-workers (Brown et al. 1963; Wild & Brown, 1967; Rowlands et al. 1971; Cavanagh et al. 1977) showed that the reduced infectivity of trypsin-treated FMDV was due to lack of adsorption to susceptible cells. As might be expected from the retained infectivity, we found that, contrary to that of parent virus, cell attachment of OTR virus was not markedly lowered by trypsinization. Although cell attachment and infectivity were not reduced significantly by trypsinization of the TR isolates, immunodiffusion, SDS-PAGE analysis and agarose gel electrophoresis showed clearly that these viruses were affected by trypsin.
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1D tests with specific early convalescent sera raised against parent and TR viruses demonstrated that the antigenic sites of trypsin-treated TR viruses were changed in the same way as in trypsinized parent virus. Even OTR₁, which before treatment was slightly different from parent virus, appeared the same after trypsinization (Fig. 2). Thus, after treatment, the parent virus and the TR variants appear antigenically identical when diffused against serum known to react against the newly exposed sites of trypsin-treated parent virus. Therefore, the antigenic changes that result from trypsinization of O₁ virus are not necessarily associated with a significant reduction in infectivity as was suggested by results obtained by Rowlands et al. (1971).

In common with other picornaviruses, FMDV contains four major polypeptides (Burroughs et al. 1971; Laporte & Lenoir, 1973). The SDS-PAGE patterns described in this study are in agreement with these findings (Fig. 3) with the exception of OTR₁ virus, which always showed an additional polypeptide (VP₄). This property was genetically stable: extensive plaque purification (up to five times) and passage in a pig followed by 10 passages in BHK or pig kidney cells did not change the SDS-PAGE pattern. Scanning of the gel patterns suggests a replacement of part of VP₁ by VP₄ (Fig. 3 b).

As found by others (Wild et al. 1969; Burroughs et al. 1971; Cavanagh et al. 1977) the VP₁ molecule of O₁ (parent) virus is sensitive (in situ) to the action of trypsin, leaving polypeptides of 18 K and 6 K mol.wt. This was also the case with TR viruses (Fig. 3 a). In OTR₁ both VP₁ and VP₄ were cleaved to P₁₈. In addition it was shown that when intact OTR₁ virus was labelled with ^125I, both polypeptides were accessible to the label, which demonstrates their external position in the virion. Normally in FMDV only VP₁ is iodinated (Laporte & Lenoir, 1973; Talbot et al. 1973). Trypsinization of ^125I-labelled O and OTR₁ virus results in identical SDS-PAGE polypeptide patterns with a highly labelled P₁₈ molecule in both cases. Labelling of trypsin-treated virus resulted in practically the same pattern (Fig. 4). No label was observed in or near the buffer front, which suggests that only the P₁₈ parts of VP₁ and VP₄ are situated in an external position. The other fragments are either not exposed or do not contain tyrosine, the amino acid residue to which the ^125I is attached. Thus VP₄ has much in common with VP₁ and may be an elongated VP₁. How this virus protein is genetically coded remains an open question.

Working with the same strain of FMDV, Cavanagh et al. (1977) observed that chymotrypsin, like trypsin, only cleaves VP₁. At low enzyme concentrations a P₂₀ cleavage product is obtained and infectivity is not affected significantly. These results have been confirmed by us. We observed two VP₁ cleavage products which were both about 1 to 2 K larger than those resulting from trypsinization (Fig. 3 a). Thus the sum of the mol.wt. as determined by SDS-PAGE analysis of the chymotrypsin cleavage products of VP₁ is about 3 K higher than that of the virion associated VP₁ fragments after trypsinization. No fragments were found in the buffer front. This was also the case if a system for the detection of oligopeptides (Swank & Munkres, 1971) was used (data not shown). Thus a fraction of VP₁ of about 30 amino acids seems to be lost upon trypsinization. This also indicates that trypsin splits VP₁ at least at two sites and not at one as was suggested by results of others (Strohmaier & Adam, 1974; Matheka & Bachrach, 1975). Chymotrypsin treatment of parent virus reduces infectivity by 0.25 log. At high chymotrypsin concentrations VP₁ is cleaved to P₁₈ and infectivity reduced to the level of trypsin-treated virus (Cavanagh et al. 1977). Attachment is further reduced if cleavage of VP₁ proceeds beyond P₂₀ to P₁₈, suggesting that the surface configuration of the virus required for attachment is largely conserved in P₂₀. Our ‘trypsin-resistant’ variants also show that when VP₁ is cleaved to P₁₈, cell attachment can be conserved. Neither antigenic changes induced by trypsinization nor cleavage of VP₁ to P₁₈ are necessarily correlated with reduced infectivity. It is interesting to consider which other
factors may be responsible for the conserved infectivity. The discrete difference in electrophoretic mobility of the trypsin-treated parent virus in comparison to that of the trypsinized 'resistant' variants (which all show the same mobility) was our only observation which could be associated with the difference in cell attachment and in infectivity. This suggests that the charge of the virus particle may play a critical role in the early stages of infection. The work of others also points in this direction. Large and small plaque variants of Mengo virus were found to differ in preferential isoelectric points. This difference was associated with the relative rates of attachment of these viruses to L cells and their differing capacity to haemagglutinate human erythrocytes at pH 8.0 (Chlumecka et al. 1973). Crowell (1976) reported a relationship between electrophoretic mobilities of the six Coxsackie B viruses and pH optima for adsorption to HeLa cells which also implies a critical role for the electrical charge of the virus particle. This role may be due to the charge as such, e.g. for selecting the proper virus receptors, but it is also possible that the charge is only the expression of certain (critical) surface configurations.

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Note added. A critical role of the charge with respect to infectivity of trypsin-treated type O virus is further suggested by our finding that 'trypsin-resistant' viruses can be isolated directly from uncloned virus which has been electrophoresed. This 'trypsin-resistant' virus can only be eluted from the position in the gel corresponding to trypsin-treated TR virus mobility and not from other areas (S. Barteling, unpublished data).

REFERENCES


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