A Comparison of the Structural Polypeptides of Five Strains of Mumps Virus

By MICHELINE MCCARTHY AND RICHARD T. JOHNSON

Department of Neurology, Johns Hopkins University School of Medicine, Traylor Building, 1721 E. Madison Street, Baltimore, Maryland 21205, U.S.A.

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SUMMARY

The polypeptide patterns of five strains of mumps virus have been compared under uniform electrophoresis conditions. These strains included the MJ and RW strains, recently isolated from human cerebrospinal fluid, the neuro-adapted Kilham strain and the chick embryo-adapted Enders and Jeryl Lynn strains. A similar pattern of polypeptides, featuring six common and virus-specific species, was obtained with purified virus grown in ovo, in chick embryo fibroblasts (CEF) or in Vero cells. These polypeptides have been designated VPI (mol. wt. 200,000), VP2 (mol. wt. 80,000 or 75,000), VP3 (mol. wt. 68,000), VP4 (mol. wt. 58,000), VP5 (mol. wt. 45,000), VP6 (mol. wt. 39,000). The virus glycoproteins were identified as VP2 and VP4; the nucleocapsid-associated proteins as VPI, VP3 and VP5. Additional polypeptides occurring both in uninfected Vero cells and in uninfected CEFs appeared in polypeptide profiles of purified mumps virus preparations, and could not be designated virus-specific polypeptides. Although strain differences were minimal, we did observe (1) an extra protein, nucleocapsid-associated, in patterns from the chick-adapted Enders strain of mumps virus and (2) strain-dependent variability in the size of VP2.

INTRODUCTION

Recent electrophoresis studies of mumps virus polypeptide profiles have yielded results which vary as to both the numbers and molecular weights of virus-specific proteins. Jensik & Silver (1976) described six major polypeptides for the chick embryo grown Jo Ann strain of mumps virus, ranging from 40,000 to 66,000 mol. wt. and including two glycoproteins of mol. wt. 66,000 and 56,000 respectively. Huppertz et al. (1977) resolved seven major polypeptides for the chick embryo grown Enders strain, ranging from 47,000 to 68,000 mol. wt. In that study glycoproteins were not identified. Orvell (1978a, b) resolved eight polypeptides for the Enders strain ranging from mol. wt. 40,000 to 75,000. He identified a haemagglutinin glycoprotein (HN) of mol. wt. 75,000 and a fusion (F) glycoprotein of mol. wt. 61,000. All studies have suggested that there is a major nucleocapsid protein and a membrane-associated (M) protein.

The variability of these reports may derive from the use of different mumps virus strains or from the use of different electrophoresis methodology. Direct comparisons of several strains of Newcastle disease virus (NDV) have indicated strain differences in the susceptibility of virus glycoproteins to proteolytic cleavage, yielding strain differences in polypeptide profiles (Nagai et al. 1976). In this study, we compare five strains of mumps virus under uniform electrophoresis conditions. These strains originate from different clinical disease and isolation sites. Therefore, they allow a comprehensive re-evaluation and comparison of mumps virus polypeptides.
METHODS

Virus stocks and cell cultures. Stocks of the Kilham neuro-adapted strain of mumps virus (Kilham & Overman, 1953) and the MJ and RW strains recently isolated from human cerebrospinal fluid were grown and titrated in Vero cells. Virus-infected as well as non-infected Vero cell cultures were negative for the presence of SV5 antigen when assayed by fluorescent staining using specific goat antiserum (Microbiological Associates, Walkersville, Md., U.S.A.). Stocks of the Enders strain of virus (Enders et al. 1946) were grown and titrated in chick embryo via the allantoic route and stocks of the Jeryl Lynn strain of virus (Buynak & Hillemann, 1966) were grown and titrated in chick embryo fibroblasts (CEF).

Growth of virus for polypeptide analysis. Radioactively labelled Kilham, MJ or RW mumps virus was grown in Vero cell monolayers inoculated with 1 TCID₅₀ stock virus per cell. After a 2 h adsorption period, inoculum was removed and monolayers were overlaid with minimal essential medium (MEM) plus 2% foetal calf serum (FCS). Radioactive label was added 24 h p.i. Glucosamine label (D-6-³H-glucosamine hydrochloride; New England Nuclear) was used at a final concentration of 10 µCi/ml. Mixed ¹⁴C-amino acid label (New England Nuclear) was used at a final concentration of 2 µCi/ml; mixed ³H-amino acid label (New England Nuclear) was used at a final concentration of 5 µCi/ml. Prior to addition of mixed amino acid label, the medium was removed and replaced with amino acid-deficient medium (90%, v/v, MEM lacking all amino acids except glutamine plus 10%, v/v, normal MEM and 2% FCS). Seventy-two h p.i. half the medium was removed and replaced with fresh medium and half the original amount of radioactive label. Culture fluids were harvested at 5 days p.i.

Unlabelled Kilham, MJ or RW was grown on Vero cell monolayers inoculated with 0·1 TCID₅₀ of stock virus per cell. Culture fluids were harvested 5 days p.i. and combined with fluids from radioactively labelled, infected cells at a volume ratio of 5 parts unlabelled fluids to 1 part labelled fluids.

Radioactively labelled and unlabelled Enders and Jeryl Lynn mumps viruses were grown on CEF monolayers inoculated with 1 EID₅₀ Enders stock virus per cell or 0·002 TCID₅₀ Jeryl Lynn stock virus per cell. Infected cultures were maintained in MEM or amino acid-deficient MEM plus 2% tryptose phosphate broth (TPB). Cultures were labelled with glucosamine or mixed amino acids as described above. Radioactively labelled and unlabelled Enders virus were also grown in 6- to 7-day-old chick embryos inoculated with 10⁴ or 10⁶ EID₅₀ (0·2 ml) Enders plaque-purified stock virus. Infected eggs were incubated at 37 °C. To radioactively label virus, 100 µCi ³H-glucosamine or 500 µCi ³H-uridine (5,6-³H-uridine; New England Nuclear) in a vol. of 0·2 ml was injected into each infected egg 24 h p.i. Allantoic fluids were harvested 5 days post-inoculation.

Purification of virus. Culture fluids were clarified by centrifugation at 6000 gₛ for 15 min. Virus was pelleted at 96000 gₛ for 1 h in the AH-627 swinging bucket rotor (Dupont-Sorvall). Pellets were resuspended in 0·2 m-NaCl, 0·01 m-tris-HCl, pH 7·4, 0·001 m-EDTA, and subjected to discontinuous sucrose gradient centrifugation at 96000 gₛ for 2 h. Discontinuous gradients consisted of a 3 ml cushion of 60% sucrose in TSV buffer (0·1 m-NaCl, 0·001 m-EDTA, 0·01 m-tris-HCl, pH 7·4) plus a 5 to 6 ml shelf of 25% sucrose-TSV. The material banding at the cushion-shelf interface was collected, dialysed against or diluted with TSV buffer and further purified by isopycnic gradient centrifugation for 5 h at 96000 gₛ on continuous, 25% to 50% linear sucrose-TSV gradients. Gradients were monitored for absorbance at 254 nm in the ISCO model UA-5 absorbance monitor equipped with 5 mm flow cells. Samples from each fraction were analysed for radioactivity by liquid scintillation counting and for haemagglutinating activity (HA) by the microtitre pattern method. Haemagglutinating units (HAU) equal the reciprocal of endpoint dilution values.
Strain comparison of mumps virus polypeptides

Fig. 1. Sucrose density gradient profiles of (a) Kilham virus grown in Vero cells and labelled with $^3$H-amino acids and of (b) Enders virus grown in ovo and labelled with $^3$H-uridine. $^3$H ct/min (●—●), HA units (○—○) and density (□—□) are shown. Sedimentation is from right to left.

Allantoic fluid collected from infected eggs was clarified by centrifugation at 6000 $g_{av}$ for 15 min. Fluids were subjected to discontinuous gradient centrifugation to concentrate virus. Virus was further purified by isopycnic gradient centrifugation.

Detergent treatment of virus and purification of nucleocapsids. Nucleocapsids were isolated from purified virions by either the method of Mountcastle et al. (1970), using Triton X-100 and sodium deoxycholate at neutral pH, or the method of Jensik & Silver (1976) using Triton X-100 with high salt at high pH. After incubation with detergent, reaction mixtures were centrifuged on discontinuous CsCl gradients as described by Mountcastle et al. (1970). Nucleocapsid material was then mixed with 30% CsCl (w/v) in TSV, and centrifuged to equilibrium for 16 h at 169,000 $g_{av}$, 4 °C. Nucleocapsids banded at a density of 1.28 to 1.30 g/ml. Detergent extracted material was precipitated from Triton-containing solutions with n-butanol (Scheid & Choppin, 1973).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis using sodium dodecyl sulphate (SDS–PAGE) was conducted essentially according to Laemmli (1970) in either cylindrical gels, 0.6 cm in diam., or slab gels, 0.3 cm thick. Final acrylamide concentration in all resolving gels was 8% (w/v), that in stacking gels was 3% or 5%. Discontinuous tris buffer systems were used as further described under Results. Slab gel buffers included 0.5 M-urea plus 2 mM-EDTA; running buffer for slab gel electrophoresis included 0.05 M-urea plus 2 mM-EDTA. Samples were dissolved in electrophoresis sample buffer (0.01 M-sodium phosphate buffer, pH 7.4, 1% (w/v) SDS, 10% (v/v) glycerol, 0.001% (w/v) bromophenol blue with 2.5% (v/v) β-mercaptoethanol] and boiled 2 to 3 min. Electrophoresis in cylindrical gels was conducted at a constant current of 1.25 mA/gel and electrophoresis in slab gels at a constant voltage of 125 V in a water-cooled slab gel apparatus (Model #221, Bio-Rad, Richmond, Calif., U.S.A.). To process gels for staining of protein, gels were fixed in 12.5% (w/v) trichloroacetic acid, stained with
Coomassie brilliant blue R-250, and clarified with 20% (v/v) methanol plus 7% (v/v) glacial acetic acid. Radioactivity in slab gels was detected by fluorography (Bonner & Laskey, 1974).

To estimate the mol. wt. of virus polypeptides, virus preparations were subjected to co-electrophoresis on slab gels with marker proteins which included: RNA polymerase (mol. wt. 165,000, 155,000, 39,000), phosphorylase A (mol. wt. 91,000), bovine serum albumin (mol. wt. 68,000), Sindbis virus proteins (mol. wt. 52,000, 48,000, 30,000), and β-galactosidase (mol. wt. 135,000).

RESULTS

Purification of virus

Upon continuous density gradient purification of radioactively labelled mumps virus, radioactivity and HA were distributed in a broad band across sucrose–TSV density gradients (Fig. 1). With Vero cell-grown preparations, maximum values of both parameters occurred in the density range 1.15 to 1.20 g/ml, with modal values occurring at a density of approx. 1.19 g/ml. Egg-grown virus was similar in its density gradient profile, with maximum ct/min and HA occurring in the density range 1.16 to 1.20 g/ml, and with modal values occurring at approx. 1.185 g/ml. Additional radioactivity and HA often concentrated at the interface between the 60% sucrose–TSV cushion and the gradient. Electron microscopy indicated that this material was nucleocapsid and aggregated virus.
Electrophoresis methodology and the polypeptide pattern of mumps virus

To evaluate how discontinuous electrophoresis methodology might affect the mumps virus polypeptide pattern, we subjected purified egg-grown Enders mumps virus to SDS-PAGE on 8% polyacrylamide gels using both the Laemmli (1970) and the Maizel (1971) tris and tris-glycine formulations (Fig. 2). The Laemmli method resolved nine distinct polypeptides in the mol. wt. region from 40,000 to 80,000, while the Maizel method resolved seven distinct polypeptides. If the leading shoulders of peaks 1 and 3 of the Maizel pattern (arrows in Fig. 2) are included as separate polypeptides, the total number of polypeptides and the mol. wt. determined by the two methods are the same. Definition and separation of different species is clearly less ambiguous with the Laemmli method, which we subsequently used for all gels.

Polypeptide patterns of purified virions

Mumps virus strains Kilham, RW and MJ, labelled with mixed 3H- or 14C-amino acids, were first subjected to SDS-PAGE on cylindrical gels (data not shown). The patterns repeatedly contained nine polypeptides with estimated mol. wt. of 140,000, 80,000 (Kilham, RW) or 75,000 (MJ), 68,000, 58,000, 54,000, 48,000, 45,000, 43,000 and 39,000. Glucosamine labelled the 140,000, 80,000 or 75,000 and the 58,000 mol. wt. polypeptides. Several additional polypeptides appeared variable in different virus preparations (e.g. mol. wt. 90,000 and 36,000). Slab gel electrophoresis and fluorography of labelled virus preparations yielded essentially the same patterns of regular or variable glycosylated and non-glycosylated polypeptides but with the addition of two species (Fig. 3), a non-glycosylated polypeptide of mol. wt. 200,000 and a non-glycosylated 72,000 mol. wt. polypeptide. The latter polypeptide migrated as a thin, sharp, weakly-labelled band just behind the heavily labelled 68,000 mol. wt. protein.

To determine whether polypeptides were virus-specific, we compared virus polypeptide patterns with patterns of material purified from cells mock-infected with HBSS and labelled with 3H-glucosamine and mixed 14C-amino acids. The yield of 14C-amino acid label in this cellular material was low, making detection of proteins by fluorography difficult, but Coomassie staining followed by fluorography and detection of 3H-glucosamine label showed the following Vero cell polypeptides co-migrated with polypeptides in the mumps virus patterns: a very large (> 200,000 mol. wt.) glycosylated polypeptide, a 140,000 mol. wt. glycoprotein and seven non-glycosylated proteins of mol. wt. 90,000, 72,000, 54,000, 48,000, 43,000, 36,000 and 34,000 (Fig. 3). Thus, the number of polypeptides unique to the Kilham, MJ or RW mumps viruses was reduced to six including two glycoproteins (Table 1).

The polypeptide pattern of egg-grown Enders virus differed (Fig. 2 and 4). The 140,000 mol. wt. glycoprotein was not present. Cylindrical and slab gel patterns repeatedly contained ten polypeptides of mol. wt. 200,000, 75,000, 68,000, 59,000, 57,000, 54,000, 48,000, 45,000, 43,000 and 39,000. The Enders mumps virus was also subjected to SDS-PAGE on cylindrical and slab gels containing 10% acrylamide with an acrylamide:bisacrylamide ratio of 10:0.18, the ratio used by Orvell (1978b) to resolve Enders virus proteins. Again, the same ten polypeptides were observed, although their separation and relative migration rates were altered by the change in gel composition (data not shown). This led to a shift in the mol. wt. of some polypeptides to slightly higher values, which were in better agreement with those reported by Orvell. Using the same set of mol. wt. standards, values obtained were: approx. 200,000, 75,000, 70,000 (versus 68,000), 62,000 (versus 59,000), 59,000 (versus 57,000), 57,000 (versus 54,000), 47,000, 44,000, 41,000 and 38,000. We did not observe a 73,000 mol. wt. protein like that reported by Orvell (1978b). However, we did observe on our other slab gels (8% acrylamide, 0.21% bis) a very thin, sharp band with
Fig. 3. Fluorogram from SDS–PAGE of purified, radioactively labelled mumps viruses, including RW (lane 1), Kilham (lanes 4 and 5), and MJ (lane 6) labelled with ³H-glucosamine. The strain variation in the size of the larger virus glycoprotein is clearly shown. Lane 2 is Kilham virus labelled with ³H-amino acids and the pattern includes the 200,000 and 72,000 mol. wt. polypeptides. Lane 3 is ³H-glucosamine labelled Vero cell material; arrows are superimposed to show positions of non-glycosylated proteins detected by Coomassie stain. Positions and sizes of mol. wt. standards are indicated at right.
Strain comparison of mumps virus polypeptides

Table 1. Polypeptides of mumps virus

<table>
<thead>
<tr>
<th>Polypeptide designation</th>
<th>Mol. wt. $\times 10^{-3}$</th>
<th>Tentative identity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP 1</td>
<td>200</td>
<td>L</td>
</tr>
<tr>
<td>VP 2</td>
<td>80 (Kilham, RW)</td>
<td>gp1 (HN?)</td>
</tr>
<tr>
<td>VP 3</td>
<td>75 (Enders, MJ)</td>
<td>gp (F?)</td>
</tr>
<tr>
<td>VP 4</td>
<td>58</td>
<td>NP</td>
</tr>
<tr>
<td>VP 5</td>
<td>45</td>
<td></td>
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<tr>
<td>VP 6</td>
<td>39</td>
<td>M</td>
</tr>
</tbody>
</table>

* Tentative identity of the polypeptides is based on experiments performed in this study and/or analogy to other paramyxoviruses. See text for details.
† Glycoprotein.

mol. wt. 72,000. This polypeptide was resolved only on slab gels, similar to the species observed with Vero cell-derived mumps viruses. Variable amounts of 34,000 and/or 36,000 mol. wt. polypeptides occurred in most virus preparations, again as with Vero cell-grown virus. The Enders virus glycoproteins were the 75,000 and the 57,000 mol. wt. proteins as determined by SDS–PAGE of $^3$H-glucosamine-labelled egg-grown virus on cylindrical gels (data not shown).

Glucosamine- and amino acid-labelled Jeryl Lynn or Enders mumps virus, grown in CEF, were subjected to SDS–PAGE on slab gels (Fig. 5 and 6). This host cell was chosen since it is the adapted host of the Jeryl Lynn strain of mumps virus (Buynak & Hilleman, 1966) and since it allowed for direct comparison of *in vitro*– and *in ovo*-grown Enders virus polypeptides. The polypeptides of egg-grown Enders virus were all present in CEF-grown Enders virus, with either exact correspondence or only slight change in mol. wt., e.g. the 54,000 protein shifted to a heavily labelled 52,000 protein. The major glycoproteins of egg and CEF Enders viruses were the same. The polypeptides of CEF-grown Jeryl Lynn virus (Fig. 6) were similar to those of Enders virus. However, there did not appear to be a separate 59,000 mol. wt. protein. The smaller of the two Jeryl Lynn glycoproteins migrated just between the positions of the 59,000 and the 57,000 mol. wt. proteins of Enders virus. A 54,000 mol. wt. protein was present and co-migrated with the 54,000 protein of egg-grown Enders virus. The 45,000 protein was usually detectable only in overloaded gels.

To determine which polypeptides derived from the chick cell host, we examined material purified from CEF and the allantoic fluids of chick embryos which were mock-infected with HBSS, labelled with $^3$H-glucosamine and mixed $^{14}$C-amino acids. No material was recovered from allantoic fluids. The material isolated from CEF was completely lacking in HA. It was subjected along with egg-grown Enders (Fig. 4) and CEF-grown Jeryl Lynn (Fig. 6) viruses to SDS–PAGE on slab gels. Most of the CEF polypeptides co-migrated with polypeptides in the virus patterns, including the 200,000, 72,000, 59,000, 54,000, 48,000, 43,000 and 36,000 mol. wt. proteins. However, certain polypeptides appeared to be more heavily labelled in virus preparations than in material from uninfected cells. These included the 200,000, the 59,000 and the 54,000 mol. wt. (52,000 in CEF-Enders virus) proteins. Thus, determination of virus-specific proteins was very difficult. These proteins may be virus-specific proteins which overlap in size and electrophoretic mobility with host proteins, or they may be host proteins specifically amplified during virus protein synthesis. By comparison with CEF polypeptides, the number of proteins which could be unambiguously identified as unique to Enders or Jeryl Lynn mumps viruses was reduced to five. The virus-specific glycoproteins were the same in egg-grown and CEF-grown virus preparations; there was no evidence for additional uncleaved virus-specific glycoproteins in the *in vitro* virus.
The unique and common polypeptides of these five strains of mumps virus have been designated VP1 to VP6 (Table 1). The 200,000 mol. wt. protein has been included in this group since it appeared to be unique in three Vero cell-grown virus strains. Although its origin in chick-adapted virus strains was ambiguous, it is unlikely that the protein is virus-specific in one case and not the other, given the overall similarity in virion structural polypeptides.
Strain comparison of mumps virus polypeptides

Fig. 5. Fluorogram from SDS-PAGE of CEF-grown Enders virus labelled with ³H-glucosamine (left lane) or ¹⁴C amino acids (centre lane), plus unlabelled egg grown Enders virus (right lane). Arrows are superimposed on the right hand lane to show the positions of unlabelled proteins detected by Coomassie stain.

Fig. 6. Fluorogram from SDS-PAGE of ³H-glucosamine labelled material from uninfected CEF (lane 1) and Jeryl Lynn virus (lane 2), ¹⁴C-amino acid labelled Jeryl Lynn virus (lane 3) and material from uninfected CEF (lane 4) plus unlabelled, egg-grown Enders virus (lane 5). Arrows are superimposed on lane 5 to show the positions of unlabelled proteins detected by Coomassie stain.
Fig. 7. Coomassie-stained slab SDS–PAGE of purified egg-grown Enders virus (lane 3), extracted proteins (lane 1) and nucleocapsids (lane 5) from virus treated with Triton X-100 at high salt and high pH, plus extracted proteins (lane 2) and nucleocapsids (lane 4) from virus treated with Triton X-100 and sodium deoxycholate at neutral pH.
**Strain comparison of mumps virus polypeptides**

The proteins of purified mumps virus nucleocapsids

Purified nucleocapsids from egg-grown Enders virus had a buoyant density on CsCl gradients of 1.30 g/ml, similar to that of measles and other paramyxoviruses (Blair & Duesberg, 1970; Hall & Martin, 1973). Slab gel SDS-PAGE indicated that the most prominent polypeptide in the nucleocapsid preparation was VP3, the 68,000 mol. wt. protein (Fig. 7). Minor nucleocapsid components included the 200,000, 59,000, 54,000 and 45,000 mol. wt. proteins. Preparations also contained reduced amounts of other virus proteins, including both glycoproteins and VP6, the 39,000 mol. wt. protein. Extraction of whole virus at neutral pH with Triton plus sodium deoxycholate removed glycoprotein and VP6 slightly more efficiently than Triton used alone at high pH and high salt concentration (Fig. 7, compare lanes 4 and 5). Detergent-extracted material was predominantly the glycoproteins and VP6, the 39,000 mol. wt. polypeptide (Fig. 7, lanes 1 and 2). The VP6 polypeptide could be isolated from detergent extracts by first dialysing extracts thoroughly against 0.01 M-sodium phosphate buffer, pH 7.4, and then centrifuging at 65,000 g for 30 min, indicating that VP6 behaves like the paramyxoviruses M protein (Hewitt & Nermut, 1977).

Nucleocapsid preparations from MJ mumps virus banded diffusely on CsCl gradients, with 3H-uridine-labelled material ranging in buoyant density from 1.28 to 1.30 g/ml. Purified MJ mumps virus nucleocapsids contained predominantly VP3 (data not shown) with lesser amounts of the 54,000, 58,000, 45,000 and 39,000 mol. wt. proteins. Detergent-extracted material included predominantly the virus glycoproteins and the 140,000 mol. wt. glycoprotein. Thus, egg-grown mumps virus and tissue culture-grown, non-adapted mumps virus were similar in nucleocapsid composition and biochemical properties, except for the 59,000 mol. wt. protein peculiar to the chick-adapted Enders variant of the virus.

**DISCUSSION**

We have examined the polypeptide patterns of five different strains of mumps virus, two non-adapted, low-passage isolates (MJ and RW), one neuro-adapted strain (Kilham) and two chick-adapted strains (Enders and Jeryl Lynn). We have attempted to distinguish virus-specific polypeptides by comparison of virus proteins with those of uninfected host cells. A similar pattern of polypeptides was obtained with purified virus grown in ovo, on CEF or in Vero cells. This pattern resembles that found with other paramyxoviruses such as NDV (Mountcastle et al. 1971; Hightower et al. 1975) or especially SV5 (Caliguiri et al. 1969; Peluso et al. 1977). There is a very large, non-glycosylated protein of approx. 200,000 mol. wt. present in minor amounts, analogous to the L protein of paramyxoviruses. There are two major virus glycoproteins, having mol. wt. of 80,000 or 75,000 (VP2) and approx. 58,000 (VP4). A non-glycosylated polypeptide of mol. wt. 68,000 dominates the polypeptide patterns of both purified virions and purified nucleocapsids. This appears to be the major nucleocapsid protein (NP). Also associated with the nucleocapsid is a smaller, less prominent polypeptide, VP5, mol. wt. 45,000. In its size, its association with the nucleocapsid and its relative lack of prominence, VP5 is analogous to protein 5 of SV5 (Buetti & Choppin, 1977; Peluso et al. 1977) or the 47,000 mol. wt. unique polypeptide of NDV (Hightower et al. 1975). The smallest unique virus polypeptide has a mol. wt. of 39,000. In both its size and its biochemical properties, this protein resembles the M or membrane protein characterized for other paramyxoviruses (Hewitt & Nermut, 1977).

Several polypeptides occurring in both uninfected Vero cells and uninfected CEF appeared in polypeptide profiles of purified mumps virus preparations. Certain of these were more prominently labelled in virus preparations than in cellular preparations, e.g. the 59,000 mol.
wt. protein of Enders virus or the 54,000 mol. wt. protein. This suggests a virus-specific role for these species, either as a virus-coded protein or as a host protein amplified during virus synthesis. However, the role of these proteins cannot be determined from the present data.

Our comparative data on five strains of mumps virus suggest that strain differences in virus polypeptide profiles are minimal. Previous reported differences in virus polypeptide patterns and mol. wt. are more probably explained by different electrophoresis conditions, such as buffer composition, % acrylamide in resolving gels or the proteins employed as mol. wt. standards. For example, we demonstrated that slight changes in the composition of the electrophoresis gels and buffers can yield polypeptide patterns for the Enders strain with altered mol. wt. and resolution, especially in the separation of VP2 and VP3 (compare Fig. 2 and 4). This could explain the differences between the patterns obtained by Jensik & Silver (1976), Huppertz et al. (1977) and Orvell (1978a, b). While a consensus is developing as to the size and identity of the glycoproteins, the M protein and the NP protein of egg-grown mumps virus, there are still differences among reported studies as to the number, size and identity of the virus-specific polypeptides. It is likely that methodological rather than biological factors underlie these differences.

The five mumps virus strains did exhibit strain-dependent differences in (1) the presence of an extra nucleocapsid-associated protein and (2) the size of VP2. The 59,000 mol. wt. protein occurred only in patterns of egg-grown and CEF-grown Enders virus. This protein also appeared in uninfected CEF, but only as a very minor, weakly labelled species. Whether or not this protein is a unique virus gene product is unknown; it may be a modified version of the major nucleocapsid protein, as has been observed in NDV preparations (Hightower et al. 1975). It may be a host protein amplified during virus protein synthesis. Further study would be necessary to clarify the nature of this polypeptide species. The size of the smaller glycoprotein, VP4, identified as the fusion protein (Jensik & Silver, 1976; Orvell, 1978a, b), is similar for all the mumps virus strains. There is greater variability in the size of the larger glycoprotein, VP2, which has been described as the haemagglutinin (HN) protein (Jensik & Silver, 1976; Orvell, 1978a, b). This glycoprotein appears to have two mol. wt. variants, one of 75,000 and one of 80,000. The size of the glycoprotein does not correlate with the cytopathogenicity or host range of the virus strain in which it occurs. The non-adapted RW mumps virus, which is non-cytopathic in cell cultures (M. McCarthy, unpublished data), has the same 80,000 mol. wt. glycoprotein as the hamster brain-adapted Kilham virus, which is highly cytopathic in Vero cells and neurovirulent in hamster brain. The non-adapted MJ isolate has the same 75,000 mol. wt. glycoprotein as the egg-adapted Enders virus. There does not seem to be any variation among these mumps viruses in the ability to haemagglutinate or haemadsorb red blood cells. This suggests that both the larger and smaller versions of glycoprotein VP2, the putative haemagglutinin, are fully functional. The small difference in size observed between the two forms of VP2 is not likely to reflect a precursor–product relationship between the two. Rather, the variability in size is probably due to differences in glycosylation patterns resulting from a few amino acid sequence differences. The finding of both forms of the virus glycoprotein in non-adapted ‘street’ isolates of the virus suggests natural variation in the structure of VP2.

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