Characterization of Human Parainfluenza Viruses.

I. The Structural Proteins of Parainfluenza Virus 2 and their Synthesis in Infected Cells

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SUMMARY

Two strains of human parainfluenza virus 2 (HPV2), P2 1972/6 and P2 1980, grow to high titre in MEK3 cells, and their structural proteins and virus-induced protein synthesis have been characterized by gel electrophoresis and immunoprecipitation. Purified viruses contain seven polypeptides, including cellular actin: L (175K mol. wt.), HN (72K to 74K), NP (66K to 67K), F1 (52K to 58K), P (49K), A (44.5K) and M (39K). Virus-induced polypeptide synthesis was first detected at 8 h post-infection with the appearance of NP; other major structural proteins were detected from 10 to 12 h after infection and onwards. The synthesis of both the structural glycoproteins was demonstrated, although proteolytic processing could not be detected. Reproducible differences in the gel migration of the HN, F1 and NP polypeptides were found in whole virus, in infected cells and cells subjected to immunoprecipitation. These differences may reflect genetic diversity within HPV2 and provide a means of probing the molecular epidemiology of these viruses.

INTRODUCTION

Human parainfluenza viruses are important pathogens, associated with mild upper respiratory tract illness in older children and adults; in infants and young children they are a major cause of morbidity, producing lower respiratory tract illnesses such as croup, broncheolitis and pneumonia (Glezen et al., 1976). Isolated and identified over 25 years ago, the human parainfluenza viruses are subdivided into four major serotypes: 1, 2, 3 and 4 (Chanock, 1956; Chanock et al., 1958; Johnson et al., 1960). Homotypic re-infection occurs, most commonly with type 3, slightly less often with type 1, and rarely with type 2 (Welliver et al., 1982). All serotypes are ubiquitous, and infections are reported at all times of the year; in addition, there are epidemics such that in the U.K. epidemics of types 1 and 2 occur predominantly in the winter, while epidemics of type 3 have a distinct summer peak.

Little is known of the structure and replication of human parainfluenza viruses, or whether or not they bear any genetic relationship to each other or to other members of the Paramyxoviridae. A single report for parainfluenza virus 3 indicates that it contains eight or nine major virion peptides, ranging in mol. wt. from $17 \times 10^3$ to $125 \times 10^3$ (Guskey & Bergtrom, 1981). Immunoprecipitation experiments indicate that the internal polypeptide, NP, of parainfluenza 2 is antigenically similar to the corresponding polypeptide of simian virus 5 (SV5); some antigenic similarity exists also between the NP peptides of parainfluenza 1 and 3 viruses (Goswami & Russell, 1982). Apart from these studies, the experimental investigation of human parainfluenza viruses has been neglected, probably because of their inability to grow adequately in most established cell lines and because it has been assumed that they are similar to the well studied, non-human paramyxoviruses such as Newcastle disease virus, Sendai virus and SV5.

Whether or not the major serotypes are related genetically, infections by parainfluenza viruses are so common and widespread that significant genetic variation within types might be anticipated, particularly among strains isolated at different times or from different localities.
an attempt to characterize the human parainfluenza viruses (HPV) and investigate their molecular epidemiology, we have begun a study of 13 HPV strains isolated between 1959 and 2118 and representative of all major serotypes; a continuous line of monkey embryo kidney cells, MEK₃ (Kennett et al., 1972), is permissive for the growth of all strains. In this paper, we compare the structural proteins of two strains of parainfluenza virus 2 (isolated in 1972 and 1980) and their synthesis in infected cells, using gel electrophoresis and immunoprecipitation.

METHODS

**Cells and viruses.** MEK₃ cells and two isolates of HPV2, P₂ 1972/6 and P₂ 1980, were obtained from Drs I. D. Gust and M. L. Kennett, Fairfield Hospital for Communicable Diseases, Victoria, Australia. MEK₃, a continuous heteroploid epithelial cell line, was derived from a cynomolgus monkey foetus (Kennett et al., 1972) and was routinely cultured as monolayers in 75 cm² flasks (1 × 10⁷ cells/flask) with Hanks’ Medium 199 containing 10% foetal calf serum (FCS), 10 mM-HEPES pH 7.4, penicillin (50 units/ml) and streptomycin (50 μg/ml). Both P₂ 1972/6 and P₂ 1980 were cloned, and seed stocks prepared by infecting MEK₃ cells maintained on Hanks’ Medium 199 containing 2% FCS. The supernatant fluid was harvested on the development of + 3 (75%) to + 4 (100%) c.p.e. (cytopathic effect) and stored at −80 °C in small aliquots.

**Virus titration and growth.** Virus infectivity was determined by plaque assay in MEK₃ cells. Serial 10-fold dilutions of supernatant fluid were prepared in phosphate-buffered saline (PBS) pH 7.2. Samples (0.2 ml) from appropriate dilutions were inoculated into confluent monolayers of MEK₃ cells on six-well (9.6 cm²) multicluster plates. After adsorption for 1 h at room temperature, the cells were overlaid with 3 ml of Hanks’ Medium 199 containing 2% FCS, 0-7% (w/v) agarose and antibiotics. After 6 days incubation at 34 °C plaques were visualized by adding 3 ml of overlay medium containing 0-005% (w/v) neutral red. Plaque titres were determined after incubation overnight at 34 °C.

One-step growth curves were performed as follows. Confluent monolayers of MEK₃ cells (approx. 1.2 × 10⁶ cells in 9-6 cm² Petri dishes) were warmed with warm PBS and inoculated with 0-2 ml PBS containing 2 × 10⁶ p.f.u. of virus. After adsorption at room temperature, the inoculum was replaced by maintenance medium and incubated at 34 °C. Zero time post-infection corresponds to the addition of medium; single dishes were sampled at 4-hourly intervals for extracellular virus, measured by plaque assay. Haemagglutinin (HA) titrations were performed using guinea-pig erythrocytes according to the method of Shimokata et al. (1980).

**Isotopic labelling and purification of virus.** MEK₃ cell monolayers in 75 cm² flasks were inoculated with either HPV2 strain P₁ 1972/6 or P₁ 1980 at a multiplicity of 1 p.f.u./cell. At the appearance of c.p.e. (16 to 18 h after infection), the culture medium was replaced with 5 ml of methionine-free Medium 199 supplemented with 20 μCi/ml [³⁵S]methionine (1200 Ci/mmol). Cells were incubated at 34 °C until the development of + 3 to + 4 c.p.e., when the medium was removed and clarified at 2000 g for 15 min at 4 °C. The supernatant fluid was carefully layered onto a 0-25 ml cushion of 60% (w/v) sucrose in NTE buffer (100 mM-NaCl, 10 mM-Tris-HCl pH 7.4, 2 mM-EDTA) and centrifuged at 40000 rev/min for 1 h at 4 °C in a SW50.1 rotor. Virus resting on the cushion was diluted to 2.5 ml with NTE buffer, layered onto a 10 ml linear 15 to 60% (w/v) sucrose-NTE gradient and centrifuged at 36000 rev/min for 3 h at 4 °C in a SW41Ti rotor. The single visible band was removed, diluted to 5 ml with NTE buffer and the virus pelleted at 40000 rev/min for 1 h at 4 °C in a SW50.1 rotor. Virus pellets were dissociated in 0-15 ml electrophoresis sample buffer (50 mM-Tris–HCl pH 6-8, 2% SDS, 3% 2-mercaptoethanol, 20% glycerol, 0-001% bromophenol blue), boiled for 2 min and stored at −20 °C until analysed by SDS–polyacrylamide gel electrophoresis (PAGE).

**Labelling of infected cells.** Confluent monolayers of MEK₃ cells in 9-6 cm² Petri dishes (1.2 × 10⁶ cells) were infected with HPV2 strains P₁ 1972/6 and P₁ 1980 at an estimated multiplicity of 10 p.f.u./cell. At various times after infection, the medium was replaced with fresh medium containing 0-15 M-NaCl and the cells incubated at 34 °C for 15 min to reduce host cell protein synthesis (Lamb et al., 1978; Bernstein & Hruska, 1981). This medium was replaced with either 0-5 ml methionine-free Medium 199 supplemented with [³⁵S]methionine (20 μCi/ml) or 0-5 ml glucose-free Medium 199 supplemented with [³H]glucosamine (25 μCi/ml) containing 0-15 m-NaCl and the cells incubated for 1 h. The cells were washed with ice-cold PBS, scraped off the dish and dissociated in 0-2 ml electrophoresis sample buffer. Samples were repeatedly forced through a 21-gauge needle to fragment the DNA, boiled for 2 min and stored at −20 °C until analysed by SDS–PAGE.

**Preparation of radiolabelled antigens and immunoprecipitation.** Confluent monolayers of MEK₃ cells in 75 cm² flasks were inoculated with 1 p.f.u./cell of HPV2 strains P₁ 1972/6 or P₁ 1980. At 16 h post-infection, the medium was replaced with 5 ml methionine-free Medium 199 supplemented with [³⁵S]methionine and the cells incubated until + 2 (50%) to + 3 (75%) c.p.e. had formed (6 h). The cells were washed with PBS, scraped off and pelleted at 2800 rev/min for 10 min at 4 °C. Cells were resuspended in PBS, re-pelleted and dissociated in 5-0 ml buffer A (0-15 m-NaCl, 10 mM-Tris–HCl pH 7-4, 1 mM-EDTA, 0-01% sodium azide; 1% Nonidet P40, 500 units
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Aprotinin/ml, 0-2 mg phenylmethylsulphonyl fluoride/ml and stored overnight at 4 °C (Stephenson & ter Meulen, 1979). The lysates were mixed vigorously on a Vortex mixer for 1 min, repeatedly forced through a 23-gauge needle, centrifuged at 100000 g for 1 h at 4 °C and the supernatants stored at −80 °C.

Immunoprecipitation of HPV2 proteins was carried out by the method of Kessler (1975) as modified by Stephenson & ter Meulen (1979). Briefly, clarified cell lysates (200 µl) and antiserum (40 µl) were mixed and incubated overnight at 4 °C. A 30 µl amount of a 1:1 slurry of Protein A-Sepharose CL4B in buffer A was added, and incubation continued for 3 h with frequent mixing. Immune complexes were pelleted in a Beckman B microfuge for 2 min and washed three times by resuspending in fresh buffer A and re-pelleting. The final pellet was dissolved in 0-15 ml electrophoresis sample buffer, incubated at 37 °C for 10 min, boiled for 2 min and stored at −20 °C.

*Polycrylamide gel electrophoresis.* Samples were analysed on 12-5% SDS-polyacrylamide gels using a continuous Tris-glycine buffer system as described by Lamb et al. (1976). Proteins were visualized by staining with Coomassie Brilliant Blue, and [35S]methionine- and [3H]glucosamine-labelled proteins were detected by fluorography using the water-soluble fluor, sodium salicylate, as described by Chamberlain (1979). Dried gels were exposed to Kodak X-Omat XRP-5 films at −80 °C.

Antisera. Hyperimmune antisera specific for HPV2 strains P2 1972/6 and P2 1980 grown in MEK3 cells were raised in rabbits by four intramuscular, eight intradermal and two footpad injections of sucrose gradient-purified virus in an emulsion with Freund’s complete adjuvant. Rabbits were boosted after 4 weeks by four intramuscular injections of the same inoculum and the blood was collected 2 weeks after the second inoculation.

Reagents. L-[35S]Methionine (> 1200 Ci/mmol) and D-[6-3H]glucosamine (30 Ci/mmol) were purchased from Amersham; aprotinin from Sigma; phenylmethylsulphonyl fluoride from Merck; protein markers (mol. wt. × 10−3) thyroglobulin (330), ferritin (220, 18.5), phosphorylase b (94), bovine serum albumin (67), catalase (60), ovalbumin (43), lactate dehydrogenase (36), carbonic anhydrase (30), trypsin inhibitor (20-1) and α-lactalbumin (14.4) from Pharmacia. All other reagents were analytical grade.

**RESULTS**

*Growth of HPV2 in MEK3 cells*  
Plaque-purified stocks of strains P2 1972/6 and P2 1980 grown in MEK3 cells from dilute inocula for 72 h at 34 °C had plaque and HA titres of approx. 10^7.5 and 10^8.2 p.f.u./ml and 160 and 320 HAU/ml respectively. One-step growth curves were obtained by infecting monolayer cultures at an estimated multiplicity of 1 p.f.u./cell and subsequently determining the amount of infectious virus accumulating in the medium. The result obtained for P2 1972/6 (Fig. 1) was indistinguishable from that obtained for P2 1980. Under these conditions, infectious virus was detectable from 8 h post-infection and release was virtually complete by 36 h. At 24 h, the cell monolayers showed extensive cell fusion and by 36 h had almost completely disintegrated (+4 c.p.e.).

*The structural polypeptides of HPV2*  
Purified virus strains grown in MEK3 cells were labelled with [35S]methionine and analysed by polyacrylamide gel electrophoresis. A representative fluorograph is shown in Fig. 2. At least six virus-specific polypeptides are resolved, as well as a peptide with the migrational characteristics of cellular actin, which is thought to interact with the M polypeptides of paramyxoviruses (Giuffre et al., 1982). Because HPV2 polypeptides have similar migrational properties to those of SV5 (Etkind et al., 1980; Goswami & Russell, 1982) and mumps virus (Herrler & Compans, 1982), we have used similar designations, namely L, HN, NP, F1, P and M; both HN and F1 polypeptides are glycosylated (see below). Small, but consistent, differences between strains were observed for at least three polypeptides, namely HN, NP and F1. To determine the mol. wt. of the structural polypeptides, denatured virus polypeptide preparations were electrophoresed in a 12.5% gel with marker proteins of known mol. wt. The migration distances of the marker proteins were plotted against their mol. wt. on a logarithmic scale, although a linear relationship was not obtained for the largest markers. Only the L protein of HPV2 migrated in the non-linear portion of the curve, so that its mol. wt. is estimated. The mol. wt. determinations of the other polypeptides are listed in Table 1.
Fig. 1. Single-cycle growth curve of HPV2 strain P₂ 1972/6. Confluent MEK₃ cell monolayers were infected at a multiplicity of 1 p.f.u./cell and cultivated at 34 °C in Hanks' Medium 199 containing 2% FCS. Infectious virus released in the medium was determined by plaque assay as described in Methods.

Fig. 2. Fluorograph after SDS-PAGE of the polypeptides of purified HPV2 strains (a) P₂ 1972/6 and (b) P₂ 1980 grown in MEK₃ cells in the presence of [³⁵S]methionine. Virions were labelled, purified and subjected to electrophoresis on a 12.5% SDS-polyacrylamide gel as described in Methods.

Table 1. Molecular weight estimations for HPV2 polypeptides

<table>
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<th>Polypeptide</th>
<th>P₂ 1972/6</th>
<th>P₂ 1980</th>
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<tr>
<td>L</td>
<td>~175000</td>
<td>~175000</td>
</tr>
<tr>
<td>HN</td>
<td>74000</td>
<td>72000</td>
</tr>
<tr>
<td>NP</td>
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<tr>
<td>F₁</td>
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<tr>
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<td>44500</td>
</tr>
<tr>
<td>M</td>
<td>39000</td>
<td>38500</td>
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Polypeptide synthesis in infected cells

To determine the time course of synthesis of HPV2 virus-specific polypeptides, MEK₃ cells were labelled with [³⁵S]methionine at different times after infection and cell lysates were analysed by SDS-PAGE (Fig. 3). Virus infection did not appear to shut off host cell protein synthesis, so that under the conditions of this experiment only three polypeptides (HN, NP and M) could be identified with certainty. Densitometer tracings of each track indicated that NP could just be detected above cellular protein background at 8 h post-infection, the M polypeptide appeared at 10 h while the HN protein was detected from 12 h onwards. From 16 h there was increased intensity of a polypeptide whose migration corresponded to that of the virion P polypeptide. At later times in infection (24 to 28 h) an additional polypeptide, labelled X, running ahead of the NP appeared, which could be a cleavage product of the NP
Fig. 3. Time course of polypeptide synthesis in MEK3 cells infected with HPV2 strain P2 1980. At the indicated intervals after infection (4, 6, 8, 10, 12, 16, 20, 24, 28 h) cells were labelled with [35S]methionine for 1 h. The cells were disrupted, subjected to SDS-PAGE on a 12-5% polyacrylamide gel and labelled polypeptides were detected by autoradiography. UI, Uninfected MEK3 cells.

(Mountcastle et al., 1974; Hightower et al., 1975). Low mol. wt. polypeptides not present in purified virions have been found in cells infected with SV5 (Peluso et al., 1977) and mumps (Herrler & Compans, 1982); we have been unable to detect any virus-induced polypeptides of mol. wt. lower than that of the M polypeptide in cells infected with HPV2.

Increased resolution was obtained in experiments in which virus-induced polypeptide synthesis by P2 1972/6 and P2 1980 was compared in the presence of hypertonic salt concentrations (Saborio et al., 1974). Virus-induced polypeptide synthesis at 20 h after infection is shown in Fig. 4. In addition to HN, NP and M, the synthesis of the L and P polypeptides can be detected; the locations of the fusion (F0) polypeptide or any cleavage products of F0 have not been detected. Strain differences in the gel migration of the HN and NP polypeptides observed in purified virions (Fig. 2) were also detected in infected cells (Fig. 4).

To detect the synthesis and possible processing of virus-induced glycoproteins, MEK3 cells infected with either P2 1972/6 or P2 1980 were labelled for 1 h with [3H]glucosamine at 22 h. After electrophoresis, labelled polypeptides were visualized by fluorography, and recorded by densitometer tracing (Fig. 5). For each virus strain two major peaks of radioactivity were observed, the first of which has been labelled HN and co-migrated with an abundant virion polypeptide, labelled HN in Fig. 2. The second peak is assumed to be the cleaved fusion protein (F1) and corresponds for each strain exactly with a minor polypeptide detected by 35S-labelling of whole virus (Fig. 2). The minor peak of activity running behind the HN in Fig. 5 is also found in fluorographs of uninfected cells, labelled with [3H]glucosamine, and assumed to be a cell glycoprotein.

Immunoprecipitation

Sera from rabbits hyperimmunized with either P2 1972/6 or P2 1980 precipitated the same polypeptides whether the antigens were from cells infected with the homologous or heterologous
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Fig. 4. Comparison of the polypeptides synthesized in MEK3 cells infected with HPV2 strains P2 1972/6 and P2 1980 in the presence of hypertonic salt concentrations. At 20 h post-infection, cells were incubated for 15 min in medium containing 150 mM-NaCl and labelled with [35S]methionine for 1 h in maintained salt concentrations. UI, Uninfected MEK3 cells; (a) P2 1972/6-infected cells; (b) P2 1980-infected cells.

Fig. 5. Densitometer tracing of the fluorogram from SDS-PAGE of [3H]glucosamine-labelled HPV2 polypeptides synthesized in MEK3 cells. At 22 h after infection, MEK3 cells infected with HPV2 strains P2 1972/6 and P2 1980 were subjected to a hypertonic salt concentration and labelled with [3H]glucosamine for 1 h as described in Methods.

Fig. 6. Fluorograph, from SDS-PAGE of immunoprecipitates from cytoplasmic extracts of [35S]methionine-labelled HPV2-infected MEK3 cells. Cells were infected with P2 1972/6 (a, c) or P2 1980 (b, d), labelled for 6 h from 16 h post-infection and extracts were precipitated with antisera against P2 1972/6 (a, b) or P2 1980 (c, d). The designations of the immunoprecipitated polypeptides are given for P2 1972/6 on the left side and for P2 1980 on the right side.

strain of virus (Fig. 6). Thus, when P2 1972/6 antiserum was reacted with P2 1972/6 (Fig. 6a), six polypeptides (including actin) with migrational characteristics identical to virus structural polypeptides (Fig. 2) were detected; when reacted with P2 1980-infected cells (Fig. 6b), HN, NP, P, A and M were precipitated. Similarly, P2 1980 antiserum precipitated polypeptides of both strains, including the F1 polypeptide of P2 1972/6 (Fig. 6c, d). The strain differences in gel migration rate of the NP, and the glycoproteins HN and F1 noted previously with purified virus and in infected cells are also observed under the conditions of immunoprecipitation.

DISCUSSION

Human parainfluenza viruses are usually associated with poor yield in tissue culture and extreme lability (Kingsbury, 1977; Goswami & Russell, 1982), factors that no doubt contribute
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to their experimental neglect. In this study, we report that representative strains of HPV2 grow to high titre in MK3 cells, a heteroploid epithelial cell line derived from a cynomolgus monkey foetus (Kennett et al., 1972). Similar unpublished data indicate that MEK3 cells are permisse for all human parainfluenza virus serotypes. The availability of a suitable cell type provides sufficient material for the characterization of virus structural proteins, the analysis of virus-induced polypeptides synthesis, and the detection of strain variation as manifest by gel migrational differences.

Purified HPV2 strains comprise at least seven proteins, bearing a general similarity to those found in the simian paramyxovirus SV5 (Buetti & Choppin, 1977; Etkind et al., 1980) and human mumps virus (Herrler & Compans, 1982). Accordingly, the HPV2 polypeptides have been designated L, HN, NP, F1, P, A and M. Cellular actin (A) is found in all paramyxoviruses, and is thought to be associated with the M polypeptide (Giuffre et al., 1982). The [35S]methionine-labelled F1 protein of HPV2 was present in low levels compared with that in [3H]leucine-labelled mumps virus (Herrler & Compans, 1982) or 14C-mixed amino acid-labelled SV5 (Buetti & Choppin, 1977) which suggests that it contains relatively small quantities of methionine. Each of the strains analysed, P2 1972/6 and P2 1980, had a characteristic and reproducible polypeptide profile, as obtained from purified virus (Fig. 2), or infected cells (Fig. 4 and 6). Gel migrational differences were observed for the two glycosylated polypeptides HN and F1 and for the nucleoprotein NP. Whether such variation reflects genuine size or structural differences and consequent genetic diversity has yet to be established. The results presented above indicate, however, that strain variation exists, as detected by gel migration, and might be a useful, in only empirical, means of investigating the molecular epidemiology of human parainfluenza 2 viruses.

The mol. wt. for the HPV2 structural proteins have been determined (Table 1) and, allowing for the differences in gel conditions, compare reasonably well with those published for SV5 (Caligiuri et al., 1969; Peluso et al., 1977). However, our results suggest that the NP of HPV2 is significantly larger than the corresponding polypeptide of SV5, while both the HN and P polypeptides may also be larger. This result supports the findings of Goswami & Russell (1982) for HPV2 and HPV3, who noted particularly that human viruses appear to contain larger nucleoproteins than other paramyxoviruses.

The synthesis of virus-induced polypeptides in HPV2-infected MEK3 cells was investigated in the absence (Fig. 3) or presence (Fig. 4) of hypertonic salt concentrations. In the latter case, diminished levels of cellular protein synthesis afforded relatively higher incorporation of [35S]methionine in virus-induced polypeptides, with all but the F1 protein being clearly determinable. They also appeared to be synthesized in approximately the same unequal proportions as found in purified virions. This apparent control of viral polypeptide synthesis has also been noted for SV5 (Peluso et al., 1977), Newcastle disease virus (Hightower & Bratt, 1974) and Sendai virus (Lamb et al., 1976).

Glycoprotein synthesis, as determined by the incorporation of [3H]glucosamine, could be detected in infected cells (Fig. 5). Two glycosylated polypeptides corresponding in their migrational properties to the virion structural polypeptides labelled HN and F1 were prominent. The minor peak occurring to the left of the HN polypeptide in Fig. 5 is also found in similar amounts in uninfected cells and is assumed to be a cell glycoprotein. Under our experimental conditions a relatively low level of incorporation of glucosamine was obtained. This has reduced the chance of establishing the existence and size of a transient precursor F0, commonly found in paramyxovirus-infected cells, and enzymically cleaved to yield F1 and F2 (Scheid & Choppin, 1977). Although we have been unable to detect a low mol. wt. glycoprotein equivalent to F2, we believe that the HPV polypeptide labelled F1 is a cleavage product and that its precursor F0 is either processed very rapidly or obscured by the HN peak. The apparently large difference in mol. wt. for the F1 of P2 1972/6 (58K) compared with that of P2 1980 (52K) may indicate a difference in the site at which the F0 of each strain is cleaved.

Immunoprecipitation is an elegant technique in widespread use for the detection of antigenic similarities between corresponding virus structural and virus-induced proteins. When applied to HPV2 (Fig. 6), the anticipated result is obtained, namely that antiserum to one strain not only
precipitates probably all homologous virus-induced proteins but precipitates the induced proteins of a heterologous strain. HPV2 strains are conventionally typed by neutralization or by haemagglutination-inhibition, tests that involve only the surface proteins. As a more sensitive indicator of immunological (and presumably genetic) relatedness, immunoprecipitation might be expected to supercede other methods in the assignment of recent isolates of human parainfluenza viruses to particular subgroups.

The findings presented in this paper indicate that the structure and cell biology of human parainfluenza 2 viruses are similar to those of the well studied, non-human parainfluenza viruses.

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REFERENCES


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