Biochemical Studies on a Virus Associated with Egg Drop Syndrome 1976

By D. TODD AND M. S. McNULTY
Veterinary Research Laboratories, Stormont, Belfast BT4 3SD, Northern Ireland

(Accepted 12 January 1978)

SUMMARY

A number of hitherto undescribed, serologically identical viruses have been isolated from a syndrome of depressed egg production in broiler breeder flocks (Egg Drop Syndrome 1976). One of these, 127 virus, was purified after growth in chick embryo liver cells. Three particle types B1, B2 and B3 with densities of 1.32, 1.30 and 1.28 g/ml, respectively, were separated in CsCl equilibrium density gradients. B1 and B2 particles possessed infectivity and were labelled with $^3$H-thymidine. However, they differed morphologically and B2 particles agglutinated chicken erythrocytes whereas B1 particles did not. B3 particles were not infectious, were not labelled with $^3$H-thymidine but agglutinated chicken erythrocytes. They were penetrated by stain when examined by electron microscopy and probably correspond to empty particles. B1, B2 and B3 particles differed in their polypeptide compositions; seven of the polypeptides in B1 and B2 particles had counterparts in purified fowl adenovirus type 1.

The soluble haemagglutinin from 127 virus, which did not sediment under centrifugation conditions sufficient to pellet virus particles, was purified using DEAE cellulose chromatography and gel filtration. Purified soluble haemagglutinin contained two polypeptides (mol. wt. 67000 and 65000), which co-migrated in polyacrylamide gels with two of those present in purified 127 virus particles. The soluble haemagglutinin sedimented heterogeneously (20 to 50S) in sucrose density gradients and had a density of 1.24 g/ml in CsCl. It was inactivated by trypsin, urea and pyridine. Electron microscopy of purified soluble haemagglutinin showed rod-like structures with lengths of 25 to 30 nm, which radiated from a central area measuring approx. 10 nm in diam. It is suggested that 127 virus is an adenovirus.

INTRODUCTION

During the past few years an economically important disease of laying birds has been recognized in Western Europe. This disease has now been called Egg Drop Syndrome 1976 (EDS 76). A number of serologically indistinguishable viruses were isolated from broiler breeder flocks with this depressed egg production syndrome in Northern Ireland (McFerran et al. 1978). One of these viruses, isolate 127, was selected for further study and was found to have some properties in common with adenoviruses. It replicated in the nuclei of infected cells, was inhibited by iododeoxyuridine and was insensitive to chloroform (J. B. McFerran, personal communication). However, while 127 virus was similar to adenoviruses in size it did not show classical adenovirus morphology. Furthermore, 127 virus preparations...
agglutinated fowl erythrocytes to high titres (McFerran et al. 1978). Although the property of haemagglutination is common to all human adenoviruses (see review by Philipson et al. 1975), as yet only viruses belonging to fowl adenovirus type I have been shown to haemagglutinate and this has been restricted to rat (Burke et al. 1968) and sheep (Fadly & Winterfield, 1975) erythrocytes. Moreover, 127 virus was not neutralized by antisera to 11 prototype fowl adenovirus serotypes (McFerran & Connor, 1978; McFerran et al. 1978).

Evidence for this being a new virus also came from serological studies which showed that infection had not occurred in fowl prior to 1976 (McFerran et al. 1977).

In view of its recent appearance in fowl and the difficulty in assigning it to any known taxonomic group, we considered that it was of interest to biochemically investigate 127 virus and to compare some of its properties with those of fowl adenovirus type I (FAV-I). In this paper we describe the purification and polypeptide compositions of three types of particles associated with 127 virus infection of chick embryo liver cells. In addition, the purification and properties of a soluble virus haemagglutinin are reported.

METHODS

Cell cultures. Chick embryo liver cells were prepared by the method described previously (McFerran et al. 1978). Livers from 12- to 14-day-old embryos were dispersed using 0.05% trypsin solution. The cells were grown in M199 with 10% foetal calf serum and maintained in Earle’s lactalbumin (LAE) with 2% foetal calf serum.

Viruses, virus growth and infectivity. A stock pool of 127 virus was prepared after growing the virus three times at limiting dilution in primary chick embryo liver cells. For purification purposes, monolayer cultures of the same cells in 12 oz bottles were infected with the stock pool of virus (diluted × 10²) and the cultures maintained at 37 °C in LAE with 2% foetal calf serum. The virus was harvested after 5 to 6 days, when almost all the cells had become detached from the glass. In labelling experiments, ³H-thymidine (20 μCi/ml; Radiochemical Centre, Amersham) was added to the maintenance medium at 24 h post infection. Virus samples were titrated for infectivity by inoculation of tenfold serial dilutions into cultures of primary chick embryo liver cells in tubes. The tubes were rolled at 37 °C and examined daily for evidence of c.p.e. After 5 or 6 days the cells were disrupted by a cycle of freezing and thawing and the lysate tested for haemagglutination using fowl erythrocytes.

The CELO (Phelps) strain of FAV-I was inoculated into the allantoic cavity of fertile 10-day-old hens’ eggs. The allantoic fluid was harvested after 5 to 6 days incubation at 37 °C.

Virus purification. Following infection with 127 virus, the cells which remained attached to the glass were shaken into the medium and the mixture subjected to one cycle of freezing and thawing. The resulting cell lysate was sonicated for 10 to 15 s in 20 ml volumes, pooled, and extracted in 20 ml volumes by shaking with 2 ml Arcton 113 (I.C.I. Ltd.). Following centrifugation at 2000 g for 10 min the aqueous phases were pooled. Centrifugation of this mixture at 80000 g for 2 h at 4 °C allowed a crude virus pellet to be collected. The supernatant (SN1) was retained for the purification of the soluble haemagglutinin (HA). The crude virus pellet was resuspended in approx. 6 to 8 ml of 0.1 M-phosphate buffered saline (PBS) using a 5 s period of sonication and further fractionated using CsCl equilibrium density gradient centrifugation.

The allantoic fluid from eggs infected with FAV-I was clarified by centrifugation at 2000 g for 10 min and a crude virus pellet collected by centrifugation at 80000 g for 2 h at 4 °C. The crude virus pellet, after resuspension in PBS, was fractionated using CsCl density gradients.
**Egg Drop Syndrome 1976 virus**

CsCl equilibrium density gradient centrifugation. Ten ml vol. of CsCl in PBS (density 1.31 g/ml) in 14 ml polycarbonate tubes were overlaid with the suspensions of crude pellets of 127 virus and FAV-1 (3 ml) and the tubes centrifuged at 130000 g for 17 h at 10 °C in a swing-out rotor. Alternatively, 1 ml samples of purified soluble HA were layered on to 12 ml volumes of CsCl (density 1.26 g/ml) and the tubes centrifuged at 130000 g for 20 h at 10 °C. After centrifugation, fractions (0.25 ml) were collected by puncture of the bottom of the tube. The density profile was determined by refractometry. Prior to measurement of absorbance, HA and radioactivity, the fractions were diluted by adding 1 ml of PBS. Particles which banded at different densities were pelleted by pooling the appropriate fractions and centrifuging the pools at 130000 g for 2 h at 4 °C. Pellets were resuspended in PBS. Alternatively, opalescent bands in CsCl gradients were carefully drawn off using hypodermic needles, diluted with PBS and particles collected by centrifugation as described above.

**Purification of 127 virus soluble haemagglutinin.** SN1 was concentrated to approx. 10 ml by dialysis against polyethylene glycol (Aquacide III, Calbiochem) and dialysed overnight against 0.05 M-phosphate buffer, pH 7.2 (PB). This material was applied to a DEAE cellulose (Whatman DE52) column (20 × 0.9 cm) which had been equilibrated with PB. The sample was eluted using a 100 ml linear salt gradient prepared by mixing 50 ml amounts of PB and PB containing 0.5 M-NaCl. The absorbance at 280 nm of the eluate was recorded using a LKB Uvicord II linked to an Anachem chart-recorder and fractions (3 ml) were collected. Those fractions containing peak HA were pooled and concentrated to a volume of 1 ml using polyethylene glycol. The concentrated sample obtained after ion-exchange chromatography was filtered through a column (50 × 0.9 cm) packed with Sepharose 4B (Pharmacia Ltd.) which had been equilibrated with PB. The absorbance was recorded as described above and fractions of approx. 1.5 ml were collected.

**Haemagglutination titrations.** The HA titre was determined by mixing 0.2 ml volumes of serial twofold dilutions of the sample in 0.145 M-NaCl solution with 0.2 ml of 0.8 % fowl erythrocytes in plastic WHO plates. Titres were read after 30 min at room temperature.

**Polyacrylamide gel electrophoresis.** The polypeptides present in purified virus preparations were analysed by electrophoresis on gels containing 12.5 % acrylamide and 0.375 % bis-acrylamide as described by Todd & McNulty (1977). Coomassie brilliant blue stained gels were photographed or scanned at 590 nm as previously described (Todd & McNulty, 1977). The mol. wt. of the virus polypeptides were determined by comparing their mobilities relative to the tracker dye with those of the reference proteins β-galactosidase (mol. wt. 130000), lactoperoxidase (mol. wt. 92000), bovine serum albumin (mol. wt. 67000), immunoglobulin G (heavy and light chains with mol. wt. of 55000 and 23000 respectively), lactic dehydrogenase (mol. wt. 38000) and cytochrome c (mol. wt. 13000).

**Estimation of protein.** Protein estimations were performed as described by Lowry et al. (1951) using bovine serum albumin as standard. Alternatively, the protein concentration of purified soluble HA samples was determined by comparing the areas on absorbance scans of stained gels containing fractionated soluble HA polypeptides and known amounts (1 to 10 μg) of bovine serum albumin, which were electrophoresed, stained and scanned under identical conditions.

**Sucrose density gradient sedimentation.** Samples (200 to 500 μl) of purified soluble HA were layered on to pre-formed 5 to 25 % (w/v) sucrose density gradients in PBS and the gradients centrifuged at 150000 g for 6 h at 4 °C in a 6 × 14 ml MSE swing-out rotor. Catalase (Boehringer Mannheim) and porcine immunoglobulin (Koch-Light Laboratories) were sedimented on parallel gradients.
Fig. 1. CsCl equilibrium density gradient centrifugation of crude 127 virus pellets. (a) — , $A_{260}$; — , HA titre; — , density of CsCl (g/ml). (b) — , $A_{260}$; — , $^3$H-thymidine ct/min.

Heat treatment. Duplicate samples of purified soluble HA, which had been diluted with 9 vol. of PB, were incubated for 30 min periods at different temperatures in the range 30 to 90 °C at 10 °C intervals. Samples were titrated for HA after immediate cooling. Alternatively, purified soluble HA was incubated at 56 °C. Samples were removed after different periods of time and assayed for HA.

Trypsin treatment. Purified soluble HA samples which had been diluted in 0·1 M-tris acetate (pH 8·5) buffer containing 0·002 M-CaCl$_2$ were incubated at 37 °C for 1 h with equal
Table 1. Infectivity and haemagglutination titres of suspensions of B1, B2 and B3 particles of 127 virus*

<table>
<thead>
<tr>
<th>Particle type</th>
<th>Density (g/ml)</th>
<th>Protein concn. (µg/ml)</th>
<th>Infectivity titre (log₁₀ TCID₅₀/ml)</th>
<th>HA titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>1.32</td>
<td>2000</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>B2</td>
<td>1.30</td>
<td>650</td>
<td>6.5</td>
<td>320</td>
</tr>
<tr>
<td>B3</td>
<td>1.28</td>
<td>4200</td>
<td>0</td>
<td>5120</td>
</tr>
</tbody>
</table>

* The starting material for virus purification was 120 ml cell lysate with an infectivity titre of 10⁶.⁵ TCID₅₀/ml and an HA titre of 4096.

Fig. 2. Electron micrographs of purified particles and purified soluble haemagglutinin associated with 127 virus infection. (a) B1 particles; (b) B2 particles; (c) B3 particles; (d) soluble haemagglutinin.

 volumes of bovine trypsin (Sigma; Type III) at final concentrations of 0.01, 0.1, 1.0, 10 and 100 µg/ml. Titrations for HA were performed immediately after cooling.

Pyridine and urea treatment. Purified soluble HA samples were incubated with 8% pyridine and 6 M-urea at 37 °C for 30 min and HA titrations carried out immediately.

Electron microscopy. Preparations of purified 127 virus particles and soluble HA were mounted on carbon-coated grids and negatively stained with 4% sodium phosphotungstate, pH 7.1.
Table 2. Molecular weights of 127 virus polypeptides

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Mol. wt. × 10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>11</td>
</tr>
</tbody>
</table>

RESULTS

Purification of 127 virus

The HA titre and infectivity were determined at each step in the purification scheme. Neither HA titre nor infectivity was affected by sonication and extraction with Arcton 113. Following centrifugation at 80,000 g for 2 h approximately half of the HA activity remained in SN1. Electron microscopic examination of SN1 revealed few virus particles and those that were observed were usually disrupted. The HA activity which did not sediment with the virus particles will be referred to as soluble HA.

Three well-defined opalescent bands B1, B2 and B3 were visible when the crude virus pellet was subjected to equilibrium density gradient centrifugation in CsCl. These bands had densities of 1.32 (B1), 1.30 (B2) and 1.28 g/ml (B3). HA activity was associated with B2 and B3, but not with B1 (Fig. 1a). A large peak of HA was also present at lower densities (1.22 to 1.26 g/ml).

When ³H-thymidine-labelled virus preparations were similarly fractionated (Fig. 1b), peaks of radioactivity were coincident with B1 and B2, but not with B3. The peak of radioactivity at densities 1.23 to 1.26 g/ml may represent contaminating cellular material.

Infectivity of the three particles

B1 and B2 particles possessed similar levels of infectivity (Table 1). B3 particles, which contained the greatest amount of HA activity, had no detectable infectivity. Lysates of cell cultures which had been infected with B1 particles agglutinated fowl erythrocytes, even though purified B1 particles were incapable of haemagglutination.

Electron microscopy of B1, B2 and B3 particles

Although B1 and B2 particles were similar in size (78 nm), they differed in appearance (Fig. 2a and b). B2 particles resembled adenoviruses more closely but the triangular facets indicative of icosahedral structure were not obvious (Fig. 2a and b). The particles present in B3 were usually penetrated by stain and often showed signs of disruption (Fig. 2c). Rod-like structures corresponding to the adenovirus fibre were not observed in any of these samples.
Egg Drop Syndrome 1976 virus

Fig. 3. Electrophoresis on 12.5% polyacrylamide gels of purified virus preparations. (a) FAV-1, 1.32 to 1.33 g/ml; (b) B1 particles of 127 virus; (c) B2 particles of 127 virus; (d) B3 particles of 127 virus; (e) 127 virus soluble haemagglutinin. Broken lines join 127 virus polypeptides and their proposed counterparts in the FAV-1 profile. (f), (g) and (h) Co-electrophoresis experiment: (f) purified soluble haemagglutinin; (g) purified soluble haemagglutinin and B3 particles of 127 virus; (h) B3 particles of 127 virus at three times the amount used in (g).

Polypeptide composition of B1, B2 and B3 particles

The polypeptide profiles of B1, B2 and B3 particles, obtained by photographing Coomassie blue stained gels, are shown in Fig. 3(b, c and d). B2 particles contained the greatest number of distinct polypeptide bands (P1 to P13) and these were in the mol. wt. range 126,000 to 11,000. B1 particles contained polypeptides corresponding to all of those in B2 except one (P13), but P6, P7 and P8 were present in much smaller amounts. In the six purified preparations examined the minor polypeptide (P2) was barely detectable in B1 particles and appeared to be present in greater amounts in B3 and B2 particles. The polypeptide profile of B3 particles revealed an almost total absence of P9, P10 and P13 and much reduced amounts of P8. The background staining between P1 and P4, which in B3 is resolved into additional bands, varied from preparation to preparation. Re-banding of B1, B2 and B3 particles on CsCl gradients did not change the observed polypeptide compositions. The mol. wt. of P1 to P13 are shown in Table 2.
Purification and polypeptide composition of FAV-1

Two sharply defined opalescent bands were observed when crude FAV-t pellets were centrifuged in CsCl equilibrium density gradients. These possessed densities of 1.29 g/ml and 1.32 to 1.33 g/ml. Virus particles banding at 1.29 g/ml were penetrated by stain when examined in the electron microscope, while those banding at 1.32 to 1.33 g/ml were not. The 1.32 to 1.33 g/ml particles possessed infectivity (approx. 10^8 TCID_{50}/mg protein).

The polypeptides of purified FAV-1 (density 1.32 to 1.33 g/ml) were analysed in an identical manner to those of B1, B2 and B3 127 virus particles (Fig. 3a). At least 7 of the polypeptides
in 127 virus B1 particles appeared to have counterparts in the FAV-1 polypeptide profile (Fig. 3). These were P1, P3, P4, P5, P10, P11 and P12. In addition P13 in B2 particles may correspond to the smallest polypeptide of FAV-1.

**Purification of 127 virus soluble haemagglutinin**

SN1 was used as a source of soluble HA. After concentration and dialysis, the supernatant solution was fractionated by DEAE cellulose chromatography (Fig. 4a). The fractions containing peak HA activity eluted at a salt concentration of approx. 0.125 M-NaCl. This peak corresponded in part to a broad spread of material with high absorbance at 280 nm. The peak HA-containing fractions were pooled, concentrated and filtered through
Sepharose 4B. The fractionation profile (Fig. 4b) shows that the HA eluted in the void volume and was well separated from the material present in the large peak of absorbance. Peak HA-containing fractions were pooled and stored at $-20\,^\circ\text{C}$. This purified soluble HA usually possessed an HA titre of 640 to 1280 units and contained 10 to 20 $\mu$g protein/ml.

Properties of 127 virus soluble haemagglutinin

Polypeptide composition

Electrophoresis on 12.5% polyacrylamide gels (Fig. 3e) of purified soluble HA revealed the presence of one major (mol. wt. 67,000) and one minor polypeptide (mol. wt. 65,000). Co-electrophoresis experiments (Fig. 3f, g and h) showed that the major and minor polypeptides of the soluble HA co-migrated with 127 virus polypeptides P2 and P3 respectively.

Buoyant density

Samples of the purified soluble HA banded at a density of 1.24 g/ml in CsCl equilibrium density gradients (Fig. 5a).

Sedimentation behaviour

Samples of purified, soluble HA were sedimented through 5% to 25% (w/v) sucrose density gradients in PBS. The fractionation profile (Fig. 5b) shows that, although the major HA peak possessed a sedimentation coefficient of approx. 25S relative to catalase (11S) and porcine immunoglobulin (7S), the HA activity sedimented heterogeneously with coefficients in the range 20 to 50S.

Stability to heat, trypsin and chemical treatments

The HA titre of purified soluble HA was not altered by incubation for periods of 30 min at temperatures up to 70 $^\circ\text{C}$ but was totally destroyed at 80 $^\circ\text{C}$ and above. Purified soluble HA samples retained their activity at 56 $^\circ\text{C}$ for up to 24 h.

Samples of purified soluble HA were inactivated by incubation (1 h at 37 $^\circ\text{C}$) with trypsin at concentrations above 1 $\mu$g/ml.

Urea (6 M) and pyridine (8%) both inactivated the purified soluble HA after incubation at 37 $^\circ\text{C}$ for 30 min.

Electron microscopy of soluble haemagglutinin

Purified soluble HA samples were examined by negative contrast electron microscopy. Structures similar to those shown in (Fig. 2d) were consistently observed. These consisted of up to 11 rod-like elements with lengths of 25 to 30 nm which radiated from a central area measuring approx. 10 nm in diam.

DISCUSSION

The labelling of preparations of infectious 127 virus with $^3$H-thymidine demonstrates that this virus is a DNA virus. Additional evidence for this is provided by the inhibition of the virus by iododeoxyuridine and its replication in the nucleus (J. B. McFerran, personal communication).

There was a close similarity between the polypeptide profiles of 127 virus preparations which banded in CsCl density gradients at a density of 1.32 g/ml (Br) and those of FAV-1 banding at a similar density. At least 7 of the 127 virus polypeptides appeared to have counterparts in the FAV-1 polypeptide profiles (Fig. 3). The polypeptide profiles of both
FAV-1 and 127 virus displayed features characteristic of those described for human adenoviruses (Maizel et al. 1968; Everitt et al. 1973) and FAV-1 (Laver et al. 1971; Yasue & Ishibashi, 1977). These included the presence of a prominent polypeptide of mol. wt. approx. 120,000, two polypeptides in the mol. wt. range 65,000 to 70,000, a polypeptide of approx. 19,000 mol. wt. and several polypeptides below mol. wt. 14,000. The behaviour of 127 virus and FAV-1 in CsCl density gradients was also similar. In each case, infectious virus banded at a density of 1.32 to 1.33 g/ml, while empty particles banded at 1.28 to 1.29 g/ml. However, with 127 virus, an additional band of infectious virus was present at a density of 1.30 g/ml. On the basis of these similarities and other properties (see Introduction) shared in common with adenoviruses, we suggest that 127 virus is an adenovirus. The fact that 127 virus is not neutralized by antisera to any of the 11 prototype fowl adenovirus serotypes (McFerran & Connor, 1978; McFerran et al. 1978) suggests that it is either a new fowl serotype or an adenovirus from an avian species other than domestic fowl.

The detection of three populations of 127 virus particles differing in their densities in CsCl requires some comment. The particles banding at 1.28 g/ml (B3) were not infectious, were not labelled with H-thymidine but were penetrated by stain when examined in the electron microscope. These therefore probably correspond to empty particles. Infectious particles banded at densities of 1.30 g/ml (B2) and 1.32 g/ml (B1). These were morphologically different, and also differed in their ability to agglutinate fowl erythrocytes. Furthermore, there were differences in the polypeptide compositions of B1 and B2 particles. Human adenoviruses 2, 12 and 16 have been separated on CsCl density gradients into 4, 5 and 8 populations of particles respectively (Burlingham & Doerfler, 1969; Wadell et al. 1973). Particles of adenovirus 16 which differed in density were similar in morphology, contained DNA and were infective, but possessed different polypeptide compositions (Wadell et al. 1973). Wadell et al. (1973) considered that the bands at lower densities consisted of incomplete virus particles which were precursor forms rather than breakdown forms of complete virus. By analogy, it is therefore possible that 127 virus B2 particles are precursors of B1 particles.

If 127 virus is indeed an adenovirus, it is probable that P1 corresponds to the hexon polypeptide. This conclusion is made on the basis of the molecular size of the polypeptide and also on the amount present relative to the other polypeptides. As P10 is virtually absent from B3 particles, which are considered to be empty particles, it is probably the major internal DNA-associated polypeptide.

The soluble HA associated with 127 virus infection did not sediment under centrifugation conditions which were sufficient to pellet B1, B2 and B3 particles. Purified soluble HA contained one major and one minor polypeptide. Co-electrophoresis experiments showed that these polypeptides co-migrated with 127 virus polypeptides P2 and P3 respectively. The mol. wt. of these polypeptides (67,000 and 65,000) are in the range recorded for the fibre and penton base polypeptides of human adenoviruses (Philipson et al. 1975). The exclusion of soluble HA from Sepharose 4B suggests that its component polypeptides are present in highly aggregated forms. Similarly, the heterogeneous sedimentation behaviour of purified soluble HA may be the result of differing degrees of aggregation. The 25S sedimentation coefficient of the HA peak was higher than that (8.3S) recorded for the fibre dimers of human adenovirus (Wadell et al. 1969) and somewhat lower than the 50 to 100S values reported for the aggregates of 12 pentons, which are termed dodecons (Norrby, 1969).

Examination of purified soluble HA in the electron microscope revealed the presence of aggregated rod-like components (25 to 30 nm in length) which resemble adenovirus fibres. However, the central core to which the fibres were attached was both smaller than the
aggregate of 12 penton bases in the dodecon and did not possess its subunit structure (Norrby, 1968). In addition, the marked heat stability of purified soluble HA contrasted with that recorded for dodecahedral aggregates (Norrby & Wadell, 1967). The inactivation of soluble HA by trypsin and pyridine treatments may indicate the presence of penton base in this component, since in studies with human adenoviruses the penton base was found to be trypsin sensitive (Wadell & Norrby, 1969) and pyridine has been shown to dissociate fibres from the penton bases (Pettersson & Hoglund, 1969).

On the basis of these results, we suggest that the structures present in soluble HA consist of aggregates of small numbers of penton bases to which more than one fibre is attached as has been postulated for FAV-1 (Laver et al. 1971), or aggregates of a number of fibres held together by small amounts of penton base or another ‘cementing’ protein which is trypsin and pyridine sensitive. These model structures would be consistent with the relative amounts of the polypeptides in the soluble HA if the major polypeptide represents fibre and the minor polypeptide the penton base or ‘cementing’ protein. Although this hypothesis would necessitate the fibre polypeptide (P2) having an electrophoretic mobility less than that of the penton base (P3) it would provide an explanation for the presence of larger amounts of P2 on the haemagglutinating B2 and B3 particles than on the non-haemagglutinating B1 particles.

We are grateful to Miss I. Moreland for skilled technical assistance and to Mr W. Curran for the electron micrographs.

REFERENCES


Egg Drop Syndrome 1976 virus


(Received 7 October 1977)