The Polypeptide Composition of the Encephalomyocarditis Virus Particle

By A. T. H. BURNESS, SYLVIA M. FOX AND INGRID U. PARDOE
Memorial Sloan-Kettering Cancer Center, New York, New York 10021, U.S.A.

(Accepted 17 January 1974)

SUMMARY

The encephalomyocarditis (EMC) virus particle appears to contain about 60 chains each of four major polypeptides and 2 chains of a minor component. Two major polypeptides were assumed to have the same mol. wt. of 32,000 since they were not separated by electrophoresis on polyacrylamide gels containing SDS, although they are separable by calcium phosphate chromatography. The two other major polypeptides appeared to be homogeneous when examined by calcium phosphate chromatography; they have mol. wt. of 25,000 and 11,000, based upon gel electrophoresis, although examination of the latter polypeptide by gel filtration in the presence of 6 M-guanidine gave a mol. wt. of 6700. The minor component has a mol. wt., determined by gel electrophoresis, of about 42,000.

Similar results were obtained for the closely related Mouse–Elberfeld (ME) virus except that two polypeptides of mol. wt. 33,000 and 30,800, were found rather than two of 32,000. Since the ME and EMC viruses studied were both grown in Krebs II ascites tumour cells, the differences in mol. wt. of the polypeptides probably reflected differences either in the specificity of the cleavage enzyme or in the amino acid sequence in the cleavage region.

INTRODUCTION

It has already been established that there is more than one species of polypeptide in the encephalomyocarditis (EMC) virus particle (Work, 1964; Rueckert, 1965; Burness & Walter, 1967) and attempts have been made to determine the size of some separated polypeptides by sucrose density gradient sedimentation (Burness & Walter, 1967). The introduction of electrophoresis on polyacrylamide gels in the presence of SDS (Shapiro, Vinuela & Maizel, 1967), has provided better resolution and a more accurate estimate of molecular sizes. We report a re-examination of the polypeptides of EMC virus by this electrophoretic technique.

METHODS

EMC virus was a cloned, large plaque variant of the K2 strain (Hoskins & Sanders, 1957).

Mouse–Elberfeld (ME) virus was the M2 variant, the seed being kindly provided by Dr R. R. Rueckert.

Both EMC and ME virus were propagated in suspensions of Krebs II ascites tumour cells (Sanders, Huppert & Hoskins, 1958).
Virus growth. The method used was modified from those described previously (Bellett & Burness, 1963; Burness, 1969a). Krebs ascites tumour cells, at 10^6 cells/ml, were infected with either EMC or ME virus at a multiplicity of 3 to 10 p.f.u./cell and maintained at 4 °C for 1 h for virus adsorption to take place. The cells were next diluted to 10^7/ml in warm Earle's saline containing 1% (v/v) heat-inactivated calf serum, distributed in Erlenmeyer flasks so that one tenth of the flask vol. was occupied, and subsequently maintained in suspension at 37 °C for 18 h, or until at least 80% of the cells took up trypan blue (0.1%, w/v, in PBS), when virus release was then considered complete.

Radioactively labelled virus was prepared similarly except that 3 h after diluting the cells in warm Earle's saline the [3H], [35S] or [14C]-labelled material was added to a concentration of 5·0, 1·0 or 0·1 μCi/ml, respectively, and incubation continued as for the production of unlabelled virus.

Virus purification. A method previously shown (Burness, 1969a) to give highly purified EMC virus was used for both EMC and ME virus with the following modifications: (1) in the organic solvent extraction step the mixture used was 1 part butoxyethanol plus 2 parts ethoxyethanol; (2) the resuspension buffer, PP-NaCl, was replaced by a more soluble form, PP8-NaCl which contained 0·1 M-K_2HPO_4 + 0·2 M-Na_2P_2O_7 + 0·1 M-NaCl, the whole being adjusted to pH 8·0 with H_3PO_4 before making up to vol.; (3) the chromatographic step was omitted since the virus was sufficiently pure before this step for the experiments described in this report. The final purified product was resuspended in 0·1 M-NaCl + 0·02 M-phosphate buffer solution, pH 8·0.

Polyacrylamide gel electrophoresis. Virus was disrupted into its constituent polypeptides by warming to 44 °C for 30 min, or more recently by heating to 100 °C for 5 min, in an equal vol. of 2% (w/v) SDS + 2 mM-dithiothreitol (DTT) + 8 M-urea and the mixture subjected to electrophoresis in the presence of SDS (Shapiro et al. 1967) on 10% polyacrylamide gels as described by Dunker & Rueckert (1969).

The position of proteins on the gels was revealed either by staining with Coomassie brilliant blue (Chrambach et al. 1967) or by fractionating the gels and determining the radioactivity in each fraction. The earlier gels were cut into 1 mm thick slices using a device fitted with razor blades 1 mm apart and solubilized by warming at 60 °C for 3 h in 30% (v/v) H_2O_2 (Young & Fulhorst, 1965) before addition of scintillation fluid for radioactivity measurements. More recently the gels were pulverized using a Gilson gel fractionator (Gilson Medical Electronics, Middleton, Wisconsin 53562, U.S.A.) and the fractions stored overnight in 0·1% (w/v) SDS before adding scintillation fluid. Essentially similar results were obtained by either method.

Chromatography on calcium phosphate. Following gel-electrophoresis, the homogeneity of the separated components was checked by chromatography on calcium phosphate in the presence of SDS (Burness, Pardoe & Fox, 1972). Calcium phosphate (brushite), prepared and packed in a 2·5 × 10 cm column as described previously (Burness, 1967, 1969b), was washed with two column vol. of 0·005 M-phosphate buffer solution, pH 7·0, containing 1·0% (w/v) SDS + 1 mM-DTT. Sodium phosphate buffer solutions were used throughout since potassium salts would have resulted in precipitation of potassium dodecyl sulphate. The pulverized gel fractions in 0·1% (w/v) SDS comprising each electrophoretic component were combined, the gel particles removed by low speed sedimentation (2000 g for 5 min) and the supernatant fluid transferred directly to the top of the column which was then washed with about 20 ml of 0·005 M-phosphate buffer solution followed by a 200 ml linear gradient of 0·005 M- to 0·3 M-phosphate buffer solution, pH 7·0; all solutions contained 1% (w/v) SDS + 1 mM-DTT. The flow rate was 1 ml/min and 2 ml samples were collected, the
EMC virus polypeptides

phosphate concentrations of which were determined by the method of Fiske & Subbarow (1925) as previously described (Burness, 1967, 1969b) and the radioactivity in 1.5 ml portions measured in cocktail D (see below).

**Gel filtration on Bio Gel A5.** The method used was that described by Fish, Mann & Tanford (1969). [3H]-leucine-labelled EMC virus (0.1 ml) was disrupted by heating at 100 °C for 5 min with an equal vol. of a buffer solution at pH 8.3, containing 8 M-guanidine HCl + 10 mM-DTT + 0.01 M-EDTA + 0.05 M-tris-HCl. To this disrupted virus was added 0.2 ml of a mixture containing 2 mg each of ovalbumin, α-chymotrypsinogen, cytochrome C and bacitracin and 0.4 ml of a mixture of blue dextran (0.6 % w/v) and dinitrophenylalanine (0.1 %, w/v). These mol. wt. markers and dyes were dissolved in 6 M-guanidine HCl at pH 6.5 containing 1 mM-DTT. This complex mixture was transferred to the top of a column of Bio Gel A5 which was subjected to elution with 6 M-guanidine HCl at pH 6.5 + 1 mM-DTT. The elution vol. of EMC virus polypeptides were determined by radioactivity measurements in cocktail X (see below) after extinction measurements at 280 nm, 360 nm and 630 nm had located the protein mol. wt. markers, dinitrophenylalanine and blue dextran. Molecular weights were calculated using both Kd and Ve/Vo plots (Fish et al. 1969).

**Radioactivity.** Measurements were made in a Beckman LS 250 scintillation spectrometer. Gel electrophoresis samples were first diluted to 1 ml with either water or 0.1 % (w/v) SDS before addition of 10 ml cocktail T (5 g diphenyloxazole, PPO, per 1 l toluene) plus 2 ml Bio-Solv BBS-3. Calcium phosphate chromatography samples (0.5 ml) were counted in 10 ml cocktail D (380 g naphthalene and 19 g PPO made up to 3.7 l with dioxane). Gel filtration samples (0.5 ml) were diluted with 1 ml water and counted in 15 ml cocktail X (8.25 g PPO in 1 l toluene plus 500 ml Triton X). Radioactivity, in d/min, due to [3H] and [14C] when present together, or separately, was computed from ct/min using a Beckman Omega Data Reduction System and quench curves prepared using [3H]- and [14C]-toluene standards.

**Materials.** [2-14C]-uridine (50 mCi/mmol); [4,5-3H]-L-leucine (30 Ci/mmol); [35S]-L-methionine (25 Ci/mmol); and a reconstituted mixture of [U-14C]-labelled-L-amino acids (52 µCi/matom carbon) were purchased from Amersham/Searle Co., Arlington Heights, Illinois 60005; reconstituted mixtures of [G-3H]-labelled-L-amino acids were from New England Nuclear, Boston, Massachusetts 02118; Bio-Solv BBS-3 and 2,5-diphenyloxazole were obtained from Beckman Instruments, Inc., Fullerton, California 92634; Coomasie brilliant blue was from Colab Labs., Inc., Chicago, Illinois; dithiothreitol was from Calbiochem, La Jolla, California 92037; protein mol. wt. markers were from Schwarz/Mann, Orangeburg, New York 10962 and all other chemicals from Fisher Scientific Co., Springfield, New Jersey 07081.

**RESULTS**

*Polyacrylamide gel electrophoresis of disrupted EMC virus*

Three components were revealed when disrupted EMC virus was analysed by electrophoresis on polyacrylamide gel containing SDS and subsequently stained with Coomasie brilliant blue (Chrambach et al. 1967); two components, G2 and G3, were fairly close together with sharp outlines whilst the fastest moving component, G4, was indistinct (Fig. 1). EMC virus grown in the presence of [3H]-leucine or [3H]-labelled amino acids gave an analogous radioactive profile on fractionating except that in addition to these three components, one or more minor components (e.g. G1) were frequently detected (Fig. 1).

Component G2 contained about 60 % of either the [3H]-leucine or [3H]-labelled-amino
Fig. 1. Polyacrylamide gel electrophoresis of disrupted [PH]-leucine-labelled EMC virus. A virus preparation containing 35000 ct/min was disrupted by boiling for 5 min in 1% (w/v) SDS+4 M-urea+1 mm-DTT and electrophoresed at 6 mA per gel for 15 h on a 15 cm long × 0.6 cm diam, 10% (w/v) polyacrylamide gel in the presence of SDS. The gel was then fractionated for radioactivity measurements. Non-radioactive virus was treated similarly except that after electrophoresis the gel was stained with Coomassie brilliant blue (see insert).

Table 1. Properties of EMC virus components separated by SDS-polyacrylamide gel electrophoresis

<table>
<thead>
<tr>
<th>Component</th>
<th>[PH]-labelled amino acids</th>
<th>[PH]-leucine</th>
<th>[³⁵S]-methionine</th>
<th>Mol. wt.†</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>1.6</td>
<td>2.1</td>
<td>0.5</td>
<td>42000±1100</td>
</tr>
<tr>
<td>G2</td>
<td>63.9</td>
<td>61.8</td>
<td>36.9</td>
<td>32000±400</td>
</tr>
<tr>
<td>G3</td>
<td>26.9</td>
<td>26.6</td>
<td>55.5</td>
<td>25500±100</td>
</tr>
<tr>
<td>G4</td>
<td>7.6</td>
<td>9.4</td>
<td>7.0</td>
<td>11000±650</td>
</tr>
</tbody>
</table>

* Three determinations were made for each isotope.
† Mean and standard error of mean for 9 determinations.

acid-derived radioactivity, component G3 contained almost 27%, G4 less than 10% and G1 about 1 to 2% (Table 1). Gel electrophoresis of disrupted [³⁵S]-methionine-labelled virus revealed the same components (Fig. 2) but with the major proportion of the radioactivity located in G3 suggesting this component was richer in methionine than were the other components (Table 1).
Mol. wt. for EMC virus components (Table 1) were calculated from their mobilities, compared with those of bovine serum albumin, ovalbumin, chymotrypsinogen and cytochrome C, on electrophoresis in SDS-gels (Shapiro et al. 1967) which can give mol. wt. with better than 10% accuracy (Dunker & Rueckert, 1969; Weber & Osborn, 1969).

From these results, it might be concluded that EMC virus contained three major and at least one minor polypeptides. However, since separation on SDS-gel electrophoresis appears to depend upon size only, polypeptides of the same size would migrate as a single component. Thus, the number of components detected represents the minimum number of polypeptides present. However, gel electrophoresis combined with radioactive labelling is a very sensitive technique and the minor components detected may be host cell or non-capsid virus protein contaminants or aggregates, so that the number of polypeptides thought to be present could possibly be falsely high.

**Minimum number of polypeptides present**

**Comparison of EMC and ME viruses**

Rueckert, Dunker & Stolzfus (1969) examined ME virus by SDS-gel electrophoresis and detected four and not three major components that we describe here for closely related EMC virus. Two of the ME virus components, polypeptides α and β of mol. wt. 33,000 and 30,500, respectively, together contained 61.7% of the [3H]-leucine-derived radioactivity (Dunker & Rueckert, 1971). Since EMC virus component G2, of mol. wt. 32,000, contained 61.8% of the [3H]-leucine-derived radioactivity (Table 1), the possibility was considered that G2 may contain two polypeptides. Therefore, in order to obtain maximum separation of polypeptides possibly comprising this component, disrupted EMC virus was electro-
A. T. H. BURNESS, S. M. FOX AND I. U. PARDOE

Fig. 3. Polyacrylamide gel electrophoresis of a mixture of disrupted EMC and ME viruses containing $^3$H-leucine and $^{14}$C-labelled amino acids, respectively. Details as for Fig. 1.

Table 2. Electrophoresis of mixture of $^3$H-leucine-labelled EMC virus and $^{14}$C-amino acid-labelled ME virus

<table>
<thead>
<tr>
<th>Component</th>
<th>Radioactivity (%$^3$H)</th>
<th>Mol. wt.</th>
<th>Component</th>
<th>Radioactivity (%$^{14}$C)</th>
<th>Mol. wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.9</td>
<td>42000</td>
<td>ε</td>
<td>1.3</td>
<td>41000</td>
</tr>
<tr>
<td>G2</td>
<td>63.9</td>
<td>32000</td>
<td>α</td>
<td>61.9</td>
<td>33000</td>
</tr>
<tr>
<td>G3</td>
<td>25.0</td>
<td>25500</td>
<td>β</td>
<td>29.9</td>
<td>25300</td>
</tr>
<tr>
<td>G4</td>
<td>10.2</td>
<td>11000</td>
<td>γ</td>
<td>6.9</td>
<td>10900</td>
</tr>
</tbody>
</table>

phoresed on 20 cm long gels until it was estimated that G2 had traversed almost the length of the gel and, in addition, β-mercaptoethanol was added to the reservoir buffers to minimize disulphide interaction (Hall, 1970). However, even under these conditions, the G2 component was not resolved into two.

To exclude differences between the experimental procedures used by Rueckert and co-workers and ourselves, a mixture of EMC and ME viruses containing $^3$H-leucine and $^{14}$C-labelled amino acids, respectively, was degraded and analysed by gel electrophoresis. The fractionated gel showed that the EMC virus component G2 ran as a single symmetrical peak containing 63.9% of the $^3$H-radioactivity whereas the ME virus α and β polypeptides were resolved clearly and together contained 61.9% of the $^{14}$C-radioactivity (Fig. 3 and Table 2). The other two major components of EMC virus, G3 and G4, were similar in apparent mol. wt. and relative abundance to the ME virus polypeptides γ and δ, respectively, although slightly more radioactivity was associated with G4 than with δ (Fig. 3 and Table 2).
EMC virus polypeptides

Fig. 4. Calcium phosphate chromatography of disrupted EMC virus or its components. (a) Virus containing [\(^{14}\)C]-labelled amino acids disrupted as described in Fig. 1; (b) component G4; (c) component G3 and (d) component G2 obtained by SDS-gel electrophoresis of \([^{3}H]\)-leucine-labelled virus. Each preparation was transferred to the top of a column of calcium phosphate which was then washed with a linear gradient of 0.005 M- to 0.3 M-phosphate buffer solution containing 1% (w/v) SDS + 1 mM-DTT throughout.

Thus these isolates of ME and EMC viruses were different. However, it was not clear whether G2 or any other component of EMC virus contained two or more entities of the same mol. wt. which were not separated by electrophoresis. EMC virus components were therefore examined for heterogeneity by chromatography on calcium phosphate in the presence of SDS, which is a method not depending solely on molecular size differences (Burness et al. 1972).

Calcium phosphate chromatography of EMC virus components

The chromatographic profile for disrupted EMC virus containing \([^{14}\)C]-labelled amino acids showed an early eluting peak (C1), a broad, heterogeneous peak (C2) and a late eluting, sharp peak (C3) (Fig. 4a); these contained approx. 9%, 60% and 31% of the \([^{14}\)C]-labelled amino acid-derived radioactivity, respectively. Disrupted, \([^{3}H]\)-leucine-labelled virus was subjected to SDS-gel electrophoresis and portions of the separated components were examined by the same chromatographic technique. Gel component G4 eluted as a single peak corresponding in position and shape of elution pattern to chromatographic peak C1 (Fig. 4b). Gel component G3 eluted generally in the region expected for chromatographic peak C2 but there was slight contamination with peak C3 (Fig. 4c). In contrast, gel component G2 was resolved into two distinct peaks eluting in the region expected for chromatographic peaks C2 and C3 and containing 48.8% and 51.2%, respectively, of the radioactivity (Fig. 4d). This suggested, as suspected, that gel component G2 comprised two polypeptides in approximately equimolar amounts and presumably corresponding to polypeptides \(\alpha\) and \(\beta\) of ME virus. Which of the polypeptides in G2 is equivalent to \(\alpha\) and \(\beta\) of ME virus is presently being studied.
Maximum number of components in EMC virus

The minor components frequently detected in SDS-gel electropherograms of EMC virus each constitute only about 1 to 2% of the total radioactivity recovered. Such components may have been real virus particle constituents, contaminating host cell proteins, aggregates of the major virus particle polypeptides, artefacts resulting from incomplete degradation of the virus particles or may have arisen in other ways. Thus we have shown that the radioactivity in either $[^{14}C]$- or $[^{3}H]$-glucosamine was incorporated efficiently into EMC virus RNA (Burness, Pardoe & Fox, 1973). Since glycine, histidine and serine (Buchanan & Hartman, 1959) are known to be nucleic acid precursors, the possibility existed that some of the radioactivity added to infected cells as amino acids was metabolized and incorporated into RNA, a fragment of which entered the SDS-gel and was mistaken for a minor protein component. It was important, therefore, to determine the fate of the RNA of virus subjected to polyacrylamide gel electrophoresis. For this purpose $[^{14}C]$-uridine-labelled virus, disrupted with SDS, was analysed by electrophoresis on SDS gels; all of the radioactivity was trapped at the origin and none entered the gel. Thus none of the radioactive components detected by electrophoresis were poly nucleotide in nature and all were presumed to be polypeptides.

We have shown (Burness & Pardoe, 1971) that our preparations of purified virus were contaminated, if at all, with less than 1% of host cell or non-virus protein. Therefore, the remaining possibilities explaining the presence of minor components were that they were either real virus particle constituents, or artefacts composed of the major virus particle proteins. The two most frequently detected minor components were G1 and a component of mol. wt. about 65,000. Whereas G1 contained a fairly constant 1 to 2% of the total radioactivity (Table I), the amount of the component of mol. wt. 65,000 varied from 0 to 5% of the total. Because of the constancy in proportion of G1 it was considered to be a real virus particle polypeptide, whereas the 65,000 mol. wt. component and other minor components containing variable amounts of the total radioactivity were probably artefacts.

Molecular weights of virus components

Dunker & Rueckert (1969) have discussed the difficulties of determining the mol. wt. of proteins of about 10,000 or less by electrophoresis on 10% polyacrylamide gels. Therefore, although the values we obtained for the mol. wt. of G1, G2, and G3 were probably reliable, that of 11,000 for G4 may be in error. For this reason, we used the alternative procedure of gel filtration on Bio Gel in the presence of guanidine HCl (Fish et al. 1969) to estimate the size of component G4 in comparison with that of mol. wt. markers. The elution vol. of the markers were determined by extinction measurements at 280 nm, while the proteins were maintained in a reduced state with DTT which exhibits low u.v. absorbance when freshly prepared, unlike β-mercaptopethanol. Component G4 was derived from disrupted $[^{3}H]$-leucine-labelled EMC virus which was run at the same time as the mol. wt. markers; the elution vol. of the virus components were determined by radioactivity measurements. Cytochrome C of mol. wt. 12,400 was consistently eluted ahead of G4 (Fig. 5) which, therefore, must be distinctly smaller. Plots from three experiments of the logarithms of mol. wt. against either $V_e/V_o$ or $K_d$ (Fish et al. 1969) indicated similar values of 6700 for the mol. wt. of G4. This was adopted as a more realistic value than that of 11,000 obtained by SDS-gel electrophoresis (Table I). Under the conditions used for gel filtration components G2 and G3 were eluted as a single peak (Fig. 5).
EMC virus polypeptides

Fig. 5. Gel filtration on Bio Gel A5 in the presence of a solution containing 6 M-guanidine hydrochloride (adjusted to pH 6.5) + 1 mM-DTT of disrupted [3H]-leucine-labelled EMC virus (broken line) and a mixture of mol. wt. marker proteins and blue dextran, the elution vol. of which were determined by $E_{280}$ measurements (continuous line).

Number of polypeptides per virus particle

$N_i$, the number of chains of polypeptide $i$ per virus particle, can be calculated from the relationship:

$$N_i = \frac{W}{M_i} \times \frac{m_i}{100},$$

where $W$ is the total mol. wt. (in daltons) of the protein in the virus particle, $m_i$ is the mass percentage of polypeptide $i$ in the total protein and $M_i$ is the mol. wt. of polypeptide $i$. $W$ was calculated to be $5.8 \times 10^6$ for EMC virus which has a particle weight of $8.5 \times 10^6$ (Burness & Clothier, 1970) of which $68.3\%$ is protein (Burness, 1970).

The mass percentages of each polypeptide present were calculated from the [3H]-labelled amino acid contents determined by SDS-gel electrophoresis (Table 1); this assumes that none of the polypeptides in the virus particle has an unusual amino acid composition. The percentage radioactivity in the two polypeptides of gel component G2 was calculated to be $31.2\%$ and $32.7\%$ from the combined value of $63.9\%$, derived from electrophoretic results (Table 1) and assuming that the polypeptides were present in the ratio found by calcium phosphate chromatography of $48.8$ to $51.2$.

The mol. wt., $M_i$, of the components were estimated by SDS-gel electrophoresis (Table 1). However, as previously discussed, the more reliable mol. wt. obtained by gel filtration was used in the calculation for component G4.

The combination of these results leads to the conclusion that each virus particle comprises about 60 chains of each of the four major polypeptides and possibly 2 chains of polypeptide G1 (Table 3).
Table 3. Polypeptide composition of EMC virus

<table>
<thead>
<tr>
<th>Component</th>
<th>Mol. wt. (M)</th>
<th>Mass percentage of polypeptide in virus protein (m)</th>
<th>Polypeptide chains/virus particle (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>42,000</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>G2</td>
<td>32,000</td>
<td>31.2</td>
<td>57</td>
</tr>
<tr>
<td>G3</td>
<td>25,500</td>
<td>32.7</td>
<td>59</td>
</tr>
<tr>
<td>G4</td>
<td>67,000</td>
<td>26.9</td>
<td>61</td>
</tr>
</tbody>
</table>

DISCUSSION

The work on EMC virus polypeptides described in this report owes much to the elegant studies by Rueckert and co-workers on the closely related Mouse-Elberfeld (ME) virus (Rueckert, 1971). Since the compositions of EMC and ME viruses appear to be similar it is appropriate to adopt the nomenclature of Rueckert and to assume gel component G1 = α, the two polypeptides of gel component G2 = α and β, component G3 = γ and component G4 = δ.

Since the particle weight of ME virus was uncertain, Rueckert et al. (1969) assumed that there were 60 chains each per virus particle of α, β, and γ polypeptides. No assumptions were necessary for EMC virus since its particle weight (Burness & Clothier, 1970) and percentage composition (Burness, 1970) were known and allowed calculation with confidence of the number of chains of each component present. The similarity between EMC and ME viruses in the composition of the α, β, and γ polypeptides suggests that the assumptions concerning ME virus were justified.

Although the corresponding polypeptides of both ME and EMC viruses were generally alike they were not identical. Thus for our clone of EMC virus the α and β chains were so similar in mol. wt. that they could not be separated by SDS-gel electrophoresis, whereas little difficulty was experienced in separating the α and β polypeptides of ME virus; some clones of EMC virus contain α and β chains which can be separated by SDS-gel electrophoresis (Rueckert, 1971). The picornavirus genome is believed to be translated into a single large precursor protein which is subsequently cleaved to yield the stable virus particle proteins and other products (Jacobson & Baltimore, 1968; Summers & Maizel, 1968; Butterworth et al. 1971; Dobos & Martin, 1972). Since both ME and EMC viruses were grown in Krebs II ascites cells, the differences between their α and β polypeptides probably reflect differences in the specificities of the cleavage enzymes induced or in the amino acid sequences in the precursor proteins of these viruses.

Another apparent difference between ME and EMC viruses concerns the number of δ chains present. We conclude that there are about 60 δ chains per EMC virus particle, whereas Rueckert et al. (1969) calculated that there were 34 δ chains in the ME virus particle. However, this estimate for the ME virus was based solely on results obtained by SDS-gel electrophoresis and if we use analogous electrophoretic data (Table 1) we would obtain an estimate of 40 δ chains per EMC virus particle. These differences result from the uncertainty in the size of polypeptide δ. The mol. wt. of EMC virus δ polypeptide was found to be 11,000 by SDS-gel electrophoresis but 6,700 by gel filtration. Since the polypeptides of this size may show anomalous electrophoretic mobility on 10% polyacrylamide gels (Dunker &
EMC virus polypeptides

Rueckert, 1969) and thus give unreliable mol. wt., we have more confidence in the gel filtration estimate of 6700 and the corresponding conclusion of 60 δ chain per virus particle. The mol. wt. of ME virus δ polypeptide has since been re-examined and is now considered to be 7300 (Stolzfus & Rueckert, 1972).

The electrophoretic results in this paper are similar to those reported for mengo virus (O’Callaghan, Mak & Colter, 1970) which, like EMC and ME viruses, is classified in the Columbia SK or cardiovirus sub group (Fenner, 1968) of the picornaviruses. Disrupted mengo virus was found to contain three major components, I, II, and III of mol. wt. 31000, 28000 and 20000 which comprised about 60%, 25% and 8% of the virus particle, respectively. Based on mol. wt. and relative abundance, mengo virus components I and II appeared to correspond to EMC virus components G2 and G3. We have demonstrated here that a single peak of radioactivity detected by SDS-gel electrophoresis does not necessarily indicate a single polypeptide in that peak since molecules of the same size may migrate together. It is probable, therefore, that component I of mengo virus contains two distinct polypeptides, shown here as EMC virus peak G2, corresponding to the α and β polypeptides of ME virus.

Theories of virus structure developed by Crick & Watson (1956, 1957) and Caspar & Klug (1962) propose that the virus shell (capsid) comprises protein subunits which are distributed to conform with icosahedral symmetry and which must, therefore, be present as 60, or multiples of 60, subunits. Allowing for slight errors in determination of mol. wt. and percentage abundance, our results suggest that there are 60 chains each of polypeptides α, β, γ, and δ in the EMC virus particle; it is probable that all four major polypeptides comprise the EMC capsid. We are now attempting to dissociate the virus into subunits to determine the location of polypeptides in the capsid.

We thank F. Kingsley Sanders for his interest and constructive criticism and Barbara Parcinski for growing the virus. The work was supported in part by NCI grant CA-08748.

REFERENCES


A. T. H. BURNESS, S. M. FOX AND I. U. PARDOE


(Received 26 October 1973)