Dissection of Vesicular Stomatitis Virus into the Infective Ribonucleoprotein and Immunizing Components

By B. CARTWRIGHT, C. J. SMALE and F. BROWN

Animal Virus Research Institute, Pirbright, Surrey

(Accepted 14 November 1969)

SUMMARY

Treatment of the infective component of vesicular stomatitis virus with Nonidet P40 produces an infective skeleton-like structure which has the shape and approximate size of the intact virus particle. The infectivity of the skeleton is enhanced 100 to 1000 fold by mixing with DEAE-dextran. The skeleton lacks the outer envelope and fringe structure and in consequence does not produce neutralizing antibodies in guinea pigs. The density of the skeleton is 1.22 g./ml. in potassium tartrate gradients compared with 1.14 g./ml. for the virus. Sodium deoxycholate removes protein from the skeleton and releases the filamentous ribonucleoprotein in an infective form. As with the skeleton, the infectivity of the ribonucleoprotein is enhanced by DEAE-dextran. Ribonuclease has no effect on the ribonucleoprotein but trypsin destroys its infectivity. The ribonucleoprotein has a density of 1.22 g./ml. in tartrate gradients, sediments at about 40 s in sucrose gradients and does not produce neutralizing antibodies in guinea-pigs.

INTRODUCTION

Treatment of the infective component of vesicular stomatitis virus with Tween 80 and ether yields at least three types of particle: (1) a component with the size and shape of the virus but lacking most of the lipid envelope and fringe structure; (2) 'rosettes' similar to those found in unfractionated harvests of the virus and consisting presumably of envelope fragments; (3) a slowly sedimenting (3 to 6s) fraction possessing most of the immunizing activity (Brown et al. 1967b; Brown, Cartwright & Smale, 1967a). We have termed the first component the 'skeleton' because it is penetrated by phosphotungstic acid to reveal the helical structure of the virus. It is infective and can be separated cleanly from the virus by centrifugation in a potassium tartrate gradient. Electron microscopy showed that several of the skeleton particles had fragments of the virus envelope still attached. The presence of these fragments may account for the observation that the skeleton fraction will produce neutralizing antibodies in guinea-pigs (Brown et al. 1967a), because virus particles from which the fringe structure has been removed by trypsin will not produce neutralizing antibodies in the same host (Cartwright, Smale & Brown, 1969). Further, about 50% of the radioactivity of the skeleton fraction prepared from 32P-labelled virus was retained in the phenol layer when it was extracted with sodium dodecyl sulphate and phenol, suggesting that the skeleton prepared by Tween 80 and ether disruption of the virus contained significant quantities of surface phospholipid.

In the present work the virus has been dissected in a series of steps. Nonidet P40 removed the envelope and fringe structure cleanly, leaving a skeleton which could then be degraded to the filamentous ribonucleoprotein with sodium deoxycholate. The ribonucleoprotein was then cleaved into the free RNA and protein with sodium dodecyl sulphate.
METHODS

With the exception of the titration of the virus and detergent-disrupted virus in BHK 21 cells and the double labelling experiments with [³H]uridine and [¹⁴C]amino acids, all the methods used have been described (Cartwright et al. 1969). The virus and detergent-disrupted virus were titrated in BHK cells by adding 0.1 ml. amounts of tenfold dilutions to monolayers in Petri dishes (6 cm.), allowing 30 min for adsorption at room temperature, washing twice with phosphate-buffered saline and then overlaying with nutrient agar. The plaques were revealed by staining with 1//10,000 neutral red after incubating the monolayers for 24 hr at 37 °. For the titrations made in the presence of DEAE-dextran, the tenfold dilutions (in phosphate-buffered saline) were mixed with an equal volume of DEAE-dextran solution (1 mg./ml.), 0.2 ml. amounts added to the monolayers for 30 min. at room temperature and the same procedure as above then followed.

In the double-labelling experiments, virus was grown in Earle’s saline containing actinomycin D (0.1 μg./ml.) and [³H]uridine and [¹⁴C]amino acids. The amino acids were either the hydrolysate of Chlorella protein or a mixture of the individual amino acids supplied by the Radiochemical Centre, Amersham, Buckinghamshire. Samples were counted in a Packard Scintillation Counter (No. 3310) under conditions appropriate to discrimination of ¹⁴C and ³H.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Infectivity in mice log. ID 50/ml.</th>
<th>Infectivity in BHK 21 cells p.f.u./ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9.4</td>
<td>Without DEAE-dextran With DEAE-dextran</td>
</tr>
<tr>
<td>0.2 % Nonidet P₄₀</td>
<td>5.2</td>
<td>2.5 4.7</td>
</tr>
<tr>
<td>0.1 % sodium deoxycholate</td>
<td>4.8</td>
<td>1.8 4.3</td>
</tr>
<tr>
<td>0.1 % sodium dodecyl sulphate</td>
<td>3.6</td>
<td>2.5 2.8</td>
</tr>
<tr>
<td>0.3 % Tween 80 + ether</td>
<td>5.2</td>
<td>3.5 5.3</td>
</tr>
</tbody>
</table>

RESULTS

Effect of Nonidet P₄₀, sodium deoxycholate and sodium dodecyl sulphate on the infectivity of vesicular stomatitis virus

Unfractionated virus with an infectivity of 10⁹ ID₅₀/ml. in mice and 10⁶ p.f.u./ml. in BHK 21 cells was treated with 0.2 % Nonidet P₄₀ or 0.1 % sodium deoxycholate or 0.1 % sodium dodecyl sulphate. Dilutions of the preparations in phosphate-buffered saline were inoculated into mice or onto BHK 21 monolayers. The Nonidet and deoxycholate preparations gave infectivities in mice 4 log. lower than those obtained with the virus. The infectivities in BHK cells were 6 to 7 log. lower than those obtained for virus (Table 1). These results are similar to those with Tween 80 and ether disrupted virus (Brown et al. 1967b). Addition of an equal volume of DEAE-dextran (1 mg./ml.) to the dilutions before inoculation of the BHK cells enhanced the infectivities of the Nonidet and deoxycholate treated preparations by 2 to 3 log., whereas the infectivity of the virus was not
Dissection of vesicular stomatitis virus increased by more than 0·5 log. DEAE-dextran had a similar effect on the infectivity of Tween 80 and ether disrupted virus (Table 1).

Sodium dodecyl sulphate lowered the infectivity in both mice and BHK cells by 6 to 7 log. and the infectivity in BHK cells was not enhanced significantly by DEAE-dextran (Table 1). This suggested that the infectivity of the disrupted preparation was due to traces of residual virus rather than to a new structure. The infectivity was not due to free RNA because it was unaffected by ribonuclease.

![Graphs showing distribution of infectivity](image)

Fig. 1. Distribution of infectivity in sucrose gradients of vesicular stomatitis virus before and after treatment with 0·2% Nonidet P40 or 0·1% sodium deoxycholate. The tubes were centrifuged for 2 hr at 20,000 rev./min. in 15 to 45% gradients, using the SW 25·1 rotor. Consecutive 3 ml. fractions were titrated in mice. (a) Virus; (b) Nonidet-disrupted virus; (c) deoxycholate-disrupted virus.

Fractionation of the Nonidet and deoxycholate disrupted preparations in sucrose gradients confirmed that most of the infectivity was no longer associated with the virus (Fig. 1). Whereas the peak of virus infectivity was in fractions 10 to 12 of 15 to 45% gradients which had been centrifuged at 20,000 rev./min. for 2 hr (Fig. 1a), most of the infectivity of the Nonidet treated virus was found at the bottom of the tube (Fig. 1b) and the most active fractions of the deoxycholate treated preparation were in the upper part of the tube (Fig. 1c).
Fractionation of detergent disrupted virus in sucrose gradients

The observation that most of the infectivity of the Nonidet treated virus sedimented to the bottom of 15 to 45% sucrose gradients which had been centrifuged at 20,000 rev./min. for 2 hr suggested that aggregation had occurred. This was confirmed by the distribution of radioactivity in sucrose gradients of Nonidet treated virus which had been labelled by growing in the presence of [3H]uridine and [14C]amino acids. Conventional collection of the sucrose gradient fractions through a hole pierced in the bottom of the tube resulted in the loss of most of the 3H, together with approximately 40% of the 14C. This radioactive material could be recovered by rinsing the bottom of the tube with buffer solution. The remainder of the 14C was present in material which remained at the top of the tube during centrifugation (Fig. 2b). By sedimenting the Nonidet treated virus through a sucrose gradient formed on top of a cushion of potassium tartrate solution (d = 1.30 g./ml.), the 3H-containing structure could be collected at the sucrose-tartrate interface.

![Graph](image)

**Fig. 2.** Distribution of radioactivity in sucrose gradients of [3H]uridine and [14C]protein labelled vesicular stomatitis virus after disruption with 0.2% Nonidet P 40, 0.1% sodium deoxycholate or 0.1% sodium dodecyl sulphate. The tubes were centrifuged for 2 hr at 20,000 rev./min. in 15 to 45% gradients, using the SW 25'1 rotor. (a) Virus alone; (b) Nonidet-disrupted virus; (c) deoxycholate-disrupted virus; (d) dodecyl sulphate disrupted virus. × × × ×, 3H; ••••, 14C.

Radioactive virus which had been disrupted with 0.1% sodium deoxycholate gave a well defined peak containing 3H and 14C in fractions 16, 17 and 18 (Fig. 2c). This corresponds to the position of peak infectivity in Fig. 1. More than 60% of the 14C was at the top of the tube but there was no 3H (RNA) at this position.

Sodium dodecyl sulphate at concentrations ranging from 0.1% to 1.0% disrupted the virus into material which sedimented slowly in 15 to 45% sucrose gradients (Fig. 2d). However, centrifugation of the disrupted virus for 18 hr at 18,000 rev./min. in 5 to 25%
Dissection of vesicular stomatitis virus

Sucrose gradients gave the profile shown in Fig. 3. The virus RNA sedimented as a homogeneous peak at approximately 38s and was free from protein.

Fractionation of immunizing activity of disrupted virus preparations

Both 0.2% Nonidet P40 and 0.1% sodium deoxycholate slightly increased the immunizing activity of the virus in guinea-pigs (Table 2). The levels of neutralizing antibody in the sera of the animals receiving the Nonidet and deoxycholate disrupted preparations were almost as high as those obtained with Tween 80 and ether disrupted preparations. Virus disrupted with 0.1% sodium dodecyl sulphate, which separates all the protein from the RNA of the virus (Fig. 3), was much inferior in producing neutralizing antibodies (Table 2).

![Figure 3: Distribution of radioactivity in sucrose gradients of [3H]uridine and [14C]protein labelled vesicular stomatitis virus after disruption with 0.1% sodium dodecyl sulphate. The tubes were centrifuged for 18 hr at 18,000 rev./min. in 5 to 25% gradients in 0.1 M acetate, pH 5.0, and 0.1% sodium dodecyl sulphate, using the SW 25-1 rotor. × - - - ×, 3H; @-----@, 14C.]

Table 2. Effect of detergents on the immunizing activity of vesicular stomatitis virus particles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log. ID50 neutralized by 0.015 ml. serum Average for sera from four guinea-pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.9</td>
</tr>
<tr>
<td>0.3% Tween 80 + ether</td>
<td>5.0</td>
</tr>
<tr>
<td>0.2% Nonidet P40</td>
<td>4.6</td>
</tr>
<tr>
<td>0.1% Sodium deoxycholate</td>
<td>4.4</td>
</tr>
<tr>
<td>0.1% Sodium dodecyl sulphate</td>
<td>2.1</td>
</tr>
</tbody>
</table>
Centrifugation of the Nonidet and sodium deoxycholate disrupted preparations in sucrose gradients followed by inoculation of the fractions into guinea-pigs showed that all the immunizing activity was associated with the slowly sedimenting material (Table 3). This contrasts with the properties of Tween 80 and ether disrupted preparations which contain immunizing material sedimenting into the lower part of the tube (Brown et al. 1967a, and Table 3).

Table 3. Distribution of immunizing activity in sucrose gradient fractions of vesicular stomatitis virus disrupted with Tween 80 and ether, Nonidet P40 and sodium deoxycholate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–6</td>
</tr>
<tr>
<td>Control</td>
<td>0.5</td>
</tr>
<tr>
<td>0.2% Nonidet P40</td>
<td>0.3</td>
</tr>
<tr>
<td>0.1% sodium deoxycholate</td>
<td>0.4</td>
</tr>
<tr>
<td>0.3% Tween 80 + ether</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Characterization of the products in Nonidet and deoxycholate disrupted virus

Electron microscopy. Nonidet removed all the envelope and surface projections to leave a skeleton-like structure penetrated by phosphotungstic acid. The skeletons were always present as aggregates usually containing at least four particles (Fig. 4a).

The only recognizable product after deoxycholate treatment was similar to the filamentous material seen in harvests of the virus (Fig. 4b). The material had approximately the same width as the strands of the helical structure seen in virus particles penetrated by phosphotungstic acid. (Fig. 4c).

Sedimentation in tartrate density gradients. After disruption with Nonidet, all the $^3$H of virus labelled with $[^3]$H]uridine and $[^14]$C]amino acids sedimented to a density of 1.22 g./ml. in tartrate gradients (Fig. 5). About 50% of the $^{14}$C (protein) was associated with the $^3$H peak and the remainder was at the top of the tube. All of the $^3$H of deoxycholate disrupted virus sedimented to a density of 1.22 g./ml. and about 30% of the $^{14}$C counts were associated with this peak. In addition, a $^{14}$C peak was found at 1.18 g./ml. The remainder of the $^{14}$C was at the top of the gradient. The high density of the RNA-containing material suggests that most of the lipid has been removed from the virus by the detergents.

Use of virus labelled with $^{32}$P. When virus was grown in the presence of $^{32}$PO$_4$, both the RNA and phospholipid fractions were labelled. After treatment of $[^{32}]$P]virus with Nonidet or sodium deoxycholate and centrifugation for 2 hr at 20,000 rev./min. in 15 to 45% sucrose gradients, more than 90% of the radioactivity remained at the top of the gradients. This slowly sedimenting material is presumably mainly phospholipid because most of the radioactivity remained in the phenol layer after extraction with 0.1% sodium dodecyl sulphate and phenol. The remaining 10% of the $^{32}$P of the Nonidet preparation sedimented to the bottom of the sucrose gradient. Extraction of this pelleted fraction with 0.1% sodium dodecyl sulphate + phenol did not leave more than 70% of the counts in the aqueous layer, suggesting that some phospholipid may still be associated with the skeleton structure. In contrast, all of the $^{32}$P of the peak found at fractions 17 and 18 of the deoxycholate disrupted preparations was retained in the aqueous layer after sodium dodecyl sulphate + phenol extraction, suggesting that this peak consisted of ribonucleoprotein only.
Dissection of vesicular stomatitis virus

Fig. 4. Electron micrographs of vesicular stomatitis virus demonstrating the effect of Nonidet and sodium deoxycholate. (a) Virus treated with 0.2% Nonidet P40 showing removal of the envelope and surface projections; (b) filaments of ribonucleoprotein released by treating the virus with 0.1% sodium deoxycholate; (c) a single particle from an untreated virus harvest; the particle has been penetrated by phosphotungstic acid, showing the internal helical structure. The maximum width of the filaments, 100 Å, is similar to that of the strands of the helical structure seen in (c).
Physical state of the virus RNA in the disrupted preparations. As shown above (Fig. 3), the RNA of vesicular stomatitis virus can be separated completely from protein by mixing the virus with 0.1% sodium dodecyl sulphate in 0.1 M acetate, pH 5.0, followed by centrifugation in a 5 to 25% sucrose gradient at 20,000 rev./min. for 18 hr. Virus labelled with [3H]-
Dissection of vesicular stomatitis virus

uridine was disrupted with 0.2% Nonidet P40 or 0.1% sodium deoxycholate and each preparation then mixed with 0.1% sodium dodecyl sulphate in 0.1 M acetate, pH 5.0. BHK cell RNA was added before centrifugation in 5 to 25% gradients. The distribution of 3H in the gradients showed that the RNA present in the disrupted preparations had the same sedimentation coefficient as that of the intact virus (Fig. 6).

![Figure 6](image)

Fig. 6. Sucrose gradient centrifugation of RNA isolated from [3H]uridine labelled vesicular stomatitis virus and from 0.2% Nonidet P40 and 0.1% deoxycholate disrupted preparations. The RNA preparations were mixed with BHK cell RNA and centrifuged for 18 hr at 20,000 rev./min. in 5 to 25% sucrose gradients prepared in 0.1 M acetate, pH 5.0, containing 0.1% sodium dodecyl sulphate. (a) RNA from virus; (b) RNA from Nonidet-disrupted virus; (c) RNA from deoxycholate-disrupted virus.

Effect of ribonuclease and trypsin on the infectivity and sedimentation characteristics. Ribonuclease, 10 μg./ml., had no effect on the infectivity of either preparation but trypsin, 0.1 mg./ml., reduced the infectivity by more than 2 log. (Table 4). Sedimentation studies showed that the effect of the trypsin was due to degradation of the RNA. Virus labelled with [3H]uridine was treated with 0.2% Nonidet P40 or 0.1% sodium deoxycholate and these dis-
Table 4. Effect of trypsin and ribonuclease on the infectivity of vesicular stomatitis virus disrupted with Nonidet P40, deoxycholate or Tween 80 and ether

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Infectivity (log. ID₅₀/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Nonidet virus</td>
<td>5.8</td>
</tr>
<tr>
<td>Deoxycholate virus</td>
<td>5.8</td>
</tr>
<tr>
<td>Tween-ether virus</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Fig. 7. Sucrose gradient centrifugation of RNA isolated from trypsin treated preparations of (a) [³H]uridine labelled virus, (b) 0.2% Nonidet-disrupted [³H]uridine labelled virus and (c) 0.1% deoxycholate-disrupted [³H]uridine labelled virus. The RNA was centrifuged under the conditions described in Fig. 6.

Ruptured preparations diluted tenfold in Eagle's medium before adding trypsin. Samples were used for titration in mice to check that the trypsin was active in the presence of Nonidet or deoxycholate and the remainder was made 0.1% with respect to sodium dodecyl sulphate in
Dissection of vesicular stomatitis virus

0.1 M acetate and centrifuged in 5 to 25% sucrose gradients. The distribution of radioactivity in the gradients is compared in Fig. 7 with that obtained with the RNA isolated from intact virus which had been treated with trypsin. The RNA from trypsin-treated virus sedimented to the same position as that from untreated virus but the RNA from the enzyme-treated disrupted preparations sedimented very slowly. The RNA of Nonidet or deoxycholate-treated preparations has the same sedimentation coefficient as virus RNA (Fig. 6). Degradation of the RNA in the trypsin-treated preparations was presumably caused by cleavage of the protein by the enzyme, leaving the RNA susceptible to traces of ribonuclease in the preparations.

Fig. 8. Effect of trypsin on the sedimentation of [3H]uridine and [14C] protein labelled virus ribonucleoprotein. The preparations were centrifuged for 3.5 hr at 25,000 rev./min. in 15 to 45% gradients, using the SW 25-1 rotor. (a) Control; (b) trypsin treated. × --- ×, 3H; ● --- ●, 14C.

Despite the loss of a considerable proportion of the infectivity and degradation of the RNA, the sedimentation behaviour of the filamentous ribonucleoprotein, obtained by fractionation of deoxycholate-treated virus, was not altered by incubating with trypsin. Using virus labelled with [3H]uridine and [14C]amino acids, more than 70% of the 3H and 14C of the ribonucleoprotein structure still sedimented to its usual position in sucrose gradients.
gradients after trypsin treatment. The remainder of the radioactivity was present at the top of the gradient (Fig. 8).

**Stepwise disruption of virus with Nonidet P40 and sodium deoxycholate.** Virus labelled with \( ^{[3}H\)uridine and \( ^{[14}C\)amino acids was mixed with 0.2% Nonidet P40 and centrifuged in a 15 to 45% sucrose gradient for 2 hr at 20,000 rev./min. The resulting pellet, which contained almost all the \(^{3}H\) and 40% of the \(^{14}C\) of the virus, was mixed with 0.1% sodium deoxycholate and sedimented in a second gradient under the same conditions. The distribution of radioactivity in the gradient shows that deoxycholate released the ribonucleoprotein from the skeleton by removing protein, which then remained at the top of the gradient (Fig. 9).

![Fig. 9. Effect of 0.1% sodium deoxycholate on the sedimentation of the RNA-containing substructure isolated from Nonidet-disrupted virus.](image)

DISCUSSION

We have shown previously (Brown et al. 1967a, b); that vesicular stomatitis virus can be converted into an infective skeleton-like structure by treatment with Tween 80 and ether. This process appeared to separate most of the envelope from the skeleton so that most of the immunizing activity, which is associated with the fringe-like projections, could be separated from the infective core of the virus by sucrose gradient centrifugation of the Tween-ether disrupted virus. All our preparations of the skeleton, however, produced significant amounts of neutralizing antibody when inoculated into guinea-pigs. This was probably caused by the incomplete removal of the envelope from the skeletons (Brown et al. 1967a) but the possibility remained that the skeleton structure possessed immunizing activity. To resolve this problem and at the same time obtain more information on the biological
Dissection of vesicular stomatitis virus

activities of the various components of the virus particle we have explored the use of other detergents which disrupt the virus.

Three detergents were examined. Sodium dodecyl sulphate (0.1%) released the virus RNA, entirely free of protein, but the protein had low immunizing activity (Table 2). Nonidet P40 appeared to remove the envelope structure completely (Fig. 4a) and the separated skeleton did not produce neutralizing antibodies in guinea pigs. The separated envelope and fringe structure produced higher levels of neutralizing antibody than the untreated virus (Table 2). The skeleton was infective in mice and BHK 21 cells and the infectivity was enhanced by up to 3 log. by DEAE-dextran. The infectivity of untreated virus was increased only two- to threefold under the same conditions. Further evidence that the infectivity of the skeleton was not due to residual virus was obtained from its sedimentation characteristics in sucrose and tartrate gradients (Fig. 1 and 5).

Sodium deoxycholate (0.1%) also produced an infective sub-structure, the infectivity of which was enhanced by 2 to 3 log. by DEAE-dextran. In this case, however, the infective structure sedimented at 140s compared with 625s for the virus (Bradish, Brooksby & Dillon, 1956) and the properties of the material suggest that it is the ribonucleoprotein component of the virus. This is supported by the appearance in the electron micrograph of the deoxycholate-disrupted preparation (Fig. 4b) which is identical with that described by McCombs, Benyesh-Melnick & Brunswig (1966), Klimenko, Uvarov & Gajdamovich (~1966), Simpson & Hauser (1966), Bergold & Munz, (1967), Nakai & Howatson (~1968), and Wagner et al. (1969). The protein released from the virus produced activities of antibody in guinea-pigs as high as those obtained with the untreated virus.

Electron micrographs suggest that successive treatments with Nonidet P40, sodium deoxycholate and sodium dodecyl sulphate produce a stepwise dissection of the virus into structural components which retain biological activity. Nonidet removes the envelope and fringe structure but leaves an infective skeleton which has the shape and approximate size of the virus particle. The bullet shape of the skeleton is destroyed by sodium deoxycholate, presumably owing to removal of a protein. Fractionation of deoxycholate-treated virus in a tartrate gradient gave, in addition to the filamentous ribonucleoprotein (~22 g./ml.), a protein at density 1.18 g./ml. (Fig. 5c). We have assumed that this protein holds the filamentous ribonucleoprotein in the rigid bullet-shape of the skeleton. Sodium dodecyl sulphate removes the remaining protein of the ribonucleoprotein and releases the RNA entirely free from other constituents of the virus.

Recent work by Kang & Prevec (1969) and Wagner and his colleagues (Wagner, Schnaitman & Snyder, 1969; Wagner et al. 1969) has shown that the intact virus contains at least four proteins which can be separated by polyacrylamide gel electrophoresis, whereas the ribonucleoprotein contains only one of these proteins. Examination of the proteins present in the biologically active degradation products described here should establish whether the individual products are formed as a result of the stepwise removal of distinct protein entities and subsequently define the functions of the individual proteins.

We wish to thank Miss Joan Crick for suggesting the use of DEAE-dextran in the titration of the disrupted virus preparations.
REFERENCES


(Received 10 October 1969)