Particle Weight and other Biophysical Properties of Encephalomyocarditis Virus

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SUMMARY

A particle weight of $8.50 \times 10^8$ for encephalomyocarditis virus was derived by combining results from (a), sedimentation velocity measurements on highly purified preparations of virus (0.1 to 5 mg./ml.) in 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0, which gave an $S_{20, w}^0$ of 162.3 S, (b), differential sedimentation studies of virus in the same solvent but containing various ratios of D$_2$O to H$_2$O which gave a partial specific volume of 0.678 ml./g., with results from (c), analysis of boundary spreading during low speed centrifugation of dilute virus solutions which led to a diffusion coefficient $D_{20, w}$ of $1.44 \times 10^{-7}$ cm.$^2$/sec. Sedimentation equilibrium studies on similar virus preparations in the same solvent gave a particle weight of $8.52 \times 10^8$. This suggested a hydrated particle diameter for the virus of 29.8 nm. and a frictional ratio $f/f_0$ of 1.130 which was consistent with that of a hydrated sphere containing 0.29 g. water/g. dry virus.

INTRODUCTION

Structural features of animal viruses have frequently been deduced from the more plentiful data on purified plant viruses which can of course be obtained in larger quantities than purified animal viruses. That it is dangerous to equate the structure of even simple plant and animal viruses was shown by the finding of Burness & Walter (1967) that highly purified EMC virus contained several structural proteins, like other picornaviruses (Maizel, 1963; Rueckert, 1964; Work, 1964), but unlike turnip yellow mosaic virus (Harris & Hindley, 1961) which is often regarded as a model for all small spherical viruses. Techniques developed in this laboratory can, however, supply large qualities of purified encephalomyocarditis (EMC) virus (Sanders, Huppert & Hoskins, 1958; Burness, 1969) which can be used for detailed biophysical and biochemical study. This report describes the measurement of the particle weight of such purified EMC virus by two methods, the first using sedimentation velocity and diffusion studies and the second involving sedimentation equilibrium.

METHODS

Virus growth and purification. The EMC virus used was a cloned, large plaque variant of the K2 strain (Hoskins & Sanders, 1957) grown and purified as previously described (Burness, 1969).

Virus concentration was estimated using a specific extinction coefficient, $E_{260}^{\%}$, at 260 nm. = 77.4 for virus preparations in 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0 (Burness, 1970).

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Ultracentrifugal analysis. Sedimentation coefficients were determined at 29,500 rev./min. and at constant temperatures close to 20° using an AnD rotor with 12 mm. 4° Kel F cells in a Spinco model E ultracentrifuge, equipped with both schlieren and ultraviolet optics, modified as recommended by Markham (1963). Virus concentrations ranged from 0.1 to 5 mg./ml. in 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0.

Fig. 1. Characteristic response curves for Kodak Commercial (□—□), CF8 (●—●) and Royal Blue X-ray films (○—○). Exposures for different lengths of time were taken during analytical ultracentrifugation using u.v. optics and the degree of film blackening, measured with a microdensitometer, plotted as height of recorder deflexion again logarithm of exposure time.

Diffusion coefficients were determined with the same ultracentrifuge, rotor and cells using essentially the procedure of Möller (1964). Virus preparations (about 100 μg./ml. in 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0) were centrifuged at 20,000 rev./min. for 2 to 6 min. to establish a boundary before decelerating to 3397 rev./min. at which speed diffusion occurred. A constant temperature close to 5° was maintained, and u.v. photographs taken a 64 min. intervals.
Photographic conditions. The film density of u.v. plates is proportional to the concentration of material under ultracentrifugal analysis only when exposure times fall within the linear range of the film, which was determined as follows. A virus preparation whose extinction at 260 nm. = 1.0, approximately, was centrifuged in an AnD rotor at 10,000 rev./min. for 4 min. to establish a boundary before decelerating to 3397 rev./min. at which speed a series of photographic exposures for periods up to 640 sec. were made using Kodak Commercial film, CF8 or Royal Blue X-ray film. The blackening of the film, measured with a Joyce–Loebl microdensitometer, was found to be proportional to the logarithm of the exposure time between values of 40 and 320 sec. for Commercial and CF8 film but more conveniently between 4 and more than 100 sec. for X-ray film (Fig. 1). The latter was therefore used with an exposure time of 10 sec. for all ultracentrifugal analyses involving u.v. optics. The densitometer was fitted with a cylindrical condenser to improve the quality of the tracings obtained, since X-ray film had a large grain emulsion. Films were always processed throughout at 20 ° by developing in freshly prepared D 19 B (4 min.), washing with 1 % (v/v) acetic acid (30 sec.), fixing in Amphix (3 min.), washing in filtered running water (30 min.), and after brief immersion in water containing a few drops liquid detergent, rinsing in distilled water and drying in a dust-free atmosphere.

Sedimentation equilibrium studies were made using the technique of short column, meniscus depletion (Yphantis, 1964) with overspeeding (Hexner, Radford & Beams, 1961) in a model E ultracentrifuge fitted with a monochromator and double-beam ultraviolet absorption optical system with photoelectric scanning (Hanlon et al. 1962). Virus preparations for analysis were dialysed at 4 ° overnight against 0.1 M-KCl + 0.2 M-phosphate buffer, pH 8.0 before dilution with diffusate to an extinction at 260 nm. = 0.6, approximately. Equilibrium was achieved using 3 mm. columns in double sector cells in an AnJ rotor at constant temperatures around 5 ° by centrifugation at 6000 rev./min. for 1 hr before decelerating to run at 2000 rev./min for 65 to 90 hr. Photoelectric scans were made at 265 and 280 nm. Sedimentation velocity runs before and after equilibrium runs confirmed the stability of virus samples at the temperature used for ultracentrifugation.

Partial specific volume determinations involved differential sedimentation studies of the virus in D2O to H2O mixtures containing 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0, as described by Martin, Cook & Winkler (1956), in a model E ultracentrifuge fitted with a photoelectric scanner and using double sector cells in an AnF rotor at 25 °. Virus for analysis was either dialysed against the appropriate solvent or collected by two cycles of centrifugation at 65,000 rev./min. for 30 min. in a Spinco 65 rotor and resuspended each time in the appropriate D2O to H2O containing solvent.

Densities of the various solvents were measured with a 25 ml. pycnometer in a water bath at 25 ° ± 0.05.

Viscosities were measured under the same conditions in an Ostwald viscometer with an outflow time of about 600 sec. for water.

Symbols, units and constants used in the calculations were as follows: M = molecular, or particle weight; S = sedimentation coefficient in sec. or in Svedbergs (s); S0,0 = sedimentation coefficient at infinite dilution corrected to a liquid with the density and viscosity of that of water at 20°; D = diffusion coefficient in cm.2/sec.; D0,0 = D corrected in a similar way to the sedimentation coefficient; Ω = angular velocity in rad./sec.; r = distance in cm. from centre of rotation; t = time in sec. from start of experiment; a is the distance in cm. from the mean (50 %) point in the diffusing boundary at time t at which the concentration is 20(80) % of that in the uniform part of the solution below the boundary; y = a constant (0.5951 for 20(80) % conditions); ρv = partial specific volume of virus in ml./g.; ρh =
hydrated partial specific volume of virus; \( v_o \) = partial specific volume of solvent which has a value of 1.000 ml./g.; \( \rho \) = density of solvent in g./ml. at 20°; \( R \) = gas constant (8.314 \times 10^7 erg/degree/mole); \( T \) = 293°K; \( c \) = concentration in mg./ml.; \( d \) = hydrated particle diameter in nm.; \( \pi = 3.142; \eta \) = viscosity of water at 20° (0.01007 poise); \( N \) = Avogadro's number (6.023 \times 10^{23}); \( f/f_0 \) = frictional ratio; \( w \) = water of hydration in g./g. dry virus.

The data obtained were processed in an Olivetti Programma 101 desk top computer using programmes which were written by Trautman (1969) and which not only gave a result but also gave an estimate of the standard error of the result (S.E.) or of the mean (S.E.M.).

Materials

Deuterium oxide (99.9 % D_2O) was obtained from Bio-Rad Laboratories, Richmond, California, the photographic material from Eastman Kodak, Ltd., West Kirby, Liverpool, and the remaining common laboratory reagents either from B.D.H. Ltd., Poole, Dorset, England, or from Fisher Scientific Co., New York.

RESULTS

Sedimentation velocity studies

Ultracentrifugal analysis of purified EMC virus preparations showed a single, sharp, symmetrical peak when examined using schlieren optics (Fig. 2). All the material absorbing at 260 nm. ran as a single boundary with minimum spreading using the ultraviolet optical system (Fig. 3). Both observations suggested that the virus preparations used were homogeneous. Sedimentation coefficients corrected for solvent viscosity and density and for partial specific volume of the virus (equation 63, Svedberg & Pedersen, 1940) were inversely proportional to concentration in the range 0.1 to 5 mg. virus/ml. in 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0 (Fig. 4). Extrapolation to zero concentration using the method of linear least squares gave an \( S_0,0 \) = 162.3 ± 0.77 s (S.E. of intercept, 13 determinations); the sedimentation coefficient \( S_{0,0} \) of the virus at a particular concentration \( c \) mg./ml. is given by the relationship \( S_{0,0} = (162.3 - 1.586 c) \) s. This sedimentation coefficient was similar to those of 161.0 ± 3.3 s and 163.9 ± 4.5 s obtained previously for EMC virus infectivity and haemagglutinin, respectively (Burness, Vizoso & Clothier, 1963), thus establishing that the physical entity described in this report was the infective virion. Faulkner et al. (1961) quoted values for \( S_{0,0} \) of 156 and 160 s for single components present in two preparations of crystalline EMC virus but no concentrations were given.

Diffusion coefficient determinations

Boundary patterns at different times during low-speed centrifugation (Fig. 5) were analysed as described by Svedberg & Pedersen (p. 285, 1940) and by Möller (1964), except that in order to make use of all experimental points and thus increase the accuracy, all values of \( u \) between 10 and 90 % were transferred to probability paper (Chartwell no. 5271, Heffer & Co., Cambridge, England) from which \( u \) at 20(80) % was interpolated as described by Markham (1962).

Plots relating boundary spreading to time from the beginning of the acceleration of the rotor were linear (Fig. 6), indicating boundary stability and homogeneity of material and from the slope of which the diffusion coefficient was calculated from the relationship,

\[
D = \frac{\bar{u}^2 (1 - S_0 \varphi t)}{4 y^2 t}.
\]
Particle weight of EMC virus

Fig. 2. Sedimentation patterns of 2.5 mg. purified EMC virus/ml. in 0.1 M-KCl+0.02 M-phosphate buffer, pH 8.0. Successive photographs were taken at 2 min. intervals at a speed of 29,500 rev./min.; bar angles were (a) 65°, (b) and (c), 70° and (d) to (f), 60°. Sedimentation was from left to right.
The sedimentation coefficient required was that determined at low speed centrifugation during the diffusion run, under which conditions the value obtained was lower than that measured at high speed with negligible diffusion. After correction for temperature and viscosity (equation 176, Svedberg & Pedersen, 1940), a mean value of $D_{0.0, w} = 1.44 \pm 0.04 \times$

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Fig. 3. Microdensitometer tracings of u.v. absorption patterns of 100 μg. purified EMC virus/ml. 0.1 M-KCl + 0.2 M-phosphate buffer, pH 8.0 during sedimentation velocity run at 29,500 rev./min. Successive boundaries were from photographs taken at 2 min. intervals as indicated; direction of sedimentation was from left to right.

Fig. 4. Dependence of $S_{20, w}$ on the concentration of EMC virus in 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0 at 29,500 rev./min.
Particle weight of EMC virus

$10^{-7}$ cm$^2$/sec. (S.E.M., 9 determinations) was obtained by combining results from several preparations containing between 100 and 150 μg virus/ml., at which concentrations the diffusion coefficient was virtually equivalent to that at infinite dilution, $D_{20,w}^o$.

Fig. 5. Microdensitometer tracings of u.v. absorption patterns of EMC virus during a diffusion run at 3360 rev./min. Successive boundaries A, B, C, D, E and F were from photographs taken at 0, 64, 128, 192, 256 and 320 min. from beginning of ultracentrifugation.

Fig. 6. Determination of diffusion coefficient. Boundary spreading, corrected for sedimentation, $(t - So - r)$ was obtained from microdensitometer tracings of photographs of boundaries at different times, $t$, from the beginning of ultracentrifugation at 3300 rev./min. at 5°; virus concentration 100 μg./ml. in 0.1 M-KCl+0.02 M-phosphate buffer, pH 8.0.
Partial specific volume

Sedimentation coefficients were determined for two virus preparations of concentration about 80 μg./ml. in 0.1 M-KCl + 0.02 M-phosphate buffer, pH 8.0, the same solvent used for determination of $S$ and $D$, but containing different proportions of D$_2$O to H$_2$O. As expected, the sedimentation coefficients, temperature corrected for viscosity of the solvent, decreased as the proportion of D$_2$O in the solvent increased (Table 2).

Table 1. Differential sedimentation studies on EMC virus in various D$_2$O to H$_2$O mixtures

<table>
<thead>
<tr>
<th>Sample</th>
<th>D$_2$O %</th>
<th>$K^*$</th>
<th>solvent viscosity (centipoise)</th>
<th>Solvent density (g./ml.)</th>
<th>$S_{20}$ (in Svedbergs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>890</td>
<td>96.4</td>
<td>1.013</td>
<td>1.08264</td>
<td>1.10631</td>
<td>108.0</td>
</tr>
<tr>
<td>4660</td>
<td>96.4</td>
<td>1.013</td>
<td>1.08264</td>
<td>1.10631</td>
<td>111.6</td>
</tr>
<tr>
<td>4659</td>
<td>96.4</td>
<td>1.013</td>
<td>1.08264</td>
<td>1.10631</td>
<td>111.9</td>
</tr>
<tr>
<td>7684</td>
<td>71.4</td>
<td>1.010</td>
<td>1.02745</td>
<td>1.08222</td>
<td>126.0</td>
</tr>
<tr>
<td>890</td>
<td>47.6</td>
<td>1.006</td>
<td>0.98353</td>
<td>1.05646</td>
<td>130.8</td>
</tr>
<tr>
<td>891</td>
<td>47.6</td>
<td>1.006</td>
<td>0.98353</td>
<td>1.05646</td>
<td>130.8</td>
</tr>
<tr>
<td>892</td>
<td>47.6</td>
<td>1.006</td>
<td>0.98353</td>
<td>1.05646</td>
<td>129.7</td>
</tr>
</tbody>
</table>

* $K$ is correction factor for deuterium exchange (see text for estimation of its value).

A partial specific volume of $0.678 \pm 0.004$ ml./g. (S.E.M., 7 determinations) was obtained for EMC virus from these results using the procedure of Martin et al. (1956). For this calculation, the ratio ($K$) of the particle weight of the virus in 100% deuterated to non-deuterated solvent was estimated to be 1.013 based on a virus composed of 31.7% RNA and 68.3% protein (Burness, 1970) and assuming eight replaceable hydrogens/1000 daltons RNA and sixteen replaceable hydrogens/1000 daltons protein (Page, Engelder & Simpson, 1967). For ratios of heavy water to total water of less than 100% $K$ was reduced proportionately (Table 1).

Particle weight from sedimentation and diffusion data

Combination of a sedimentation coefficient of $162.3 \pm 0.77$ s, a diffusion coefficient of $1.44 \pm 0.04 \times 10^{-7}$ cm$^2$/sec. and a partial specific volume of $0.678 \pm 0.004$ ml./g. gave a particle weight of $8.50 \pm 0.30 \times 10^6$ using the equation (equation 3a, Svedberg & Pedersen, 1940):

$$ M = \frac{RTS}{D(1-\bar{e}p)}. $$

Sedimentation and diffusion coefficients and partial specific volume referred to should ideally be determined in the same solvent and at the same virus concentration. Apart from the presence of D$_2$O in the determination of partial specific volume, the same solvent was used throughout. The concentrations of virus (100 to 200 μg./ml.) in the determinations of both $D$ and $\bar{e}$ were very low and virtually corresponded to infinite dilution. Essentially the same particle weight was obtained whether the sedimentation coefficient employed was $S_{20,w}$ or $S_{20,w}$ determined through the relationship $S_{20,w} = (162.3 - 1.586 c)$ s deduced earlier and where $c$ was the concentration of virus used in the diffusion runs.

Particle weight from sedimentation equilibrium studies

Measurements made on photoelectric scans taken after 65 hr and after 90 hr centrifugation gave identical results (Fig. 7) showing that equilibrium conditions has been attained. Plots of the logarithm of concentration (obtained from recorder deflexion) against the
Particle weight of EMC virus

square of the distance from the axis of rotation were linear (Fig. 7), confirming once again the homogeneity of the purified virus; the particle weight was calculated from the slope by a modified equation 11 of Svedberg & Pedersen (1940):

\[ M = \frac{2RT}{(1 - \bar{v}\rho)\omega^2} \frac{d \ln c}{dr^2}. \]

The partial specific volume of 0.678 ml/g. determined in the same solvent was used in the calculation. Combining all results for scans at 265 and 280 nm. for a variety of preparations of concentration between 60 and 80 μg. virus/ml. gave a particle weight = 8.52 ± 0.17 × 10^6 (S.E.M., 9 determinations). This figure is in excellent agreement with that obtained by measuring \( S \) and \( D \) separately.

![Graph](image)

**Fig. 7.** Sedimentation equilibrium centrifugation of EMC virus at 2000 rev./min. for 65 hr (●), and 90 hr (○).

Size, shape and hydration of EMC virus

The main purpose of this work was to measure the particle weight of EMC virus. However, the data obtained from sedimentation and diffusion runs gave supplementary information about the size, shape and degree of hydration of the virion.

The diffusion coefficient was used to calculate the diameter of the hydrated virus particle since \( d = \frac{RT}{3\eta N} \) cm. = \( 4.258 \times 10^{-6}/D_{20} \) nm. (equation 17, Svedberg & Pedersen, 1940) and the value of 29.8 ± 0.079 nm. (S.E.M., 9 determinations) obtained was extremely close to the dry diameter of 30 ± 1.0 nm. determined using the electron microscope (Faulkner et al. 1961), suggesting EMC virus was a rigid particle undergoing minimal alteration in shape on drying.

The diffusion and sedimentation coefficients are related to the frictional ratio by the expression (equation 70b, Svedberg & Pedersen, 1940):

\[ \frac{f}{f_0} = 10^{-6} \left( \frac{1 - \bar{v}\rho}{D_{20} S_{20} \omega} \right)^{1/2}, \]
which gave for EMC virus an $f/fo$ of $1.131 \pm 0.020$ (S.E.M., 9 determinations). The frictional ratio may also be calculated either from diffusion or from sedimentation coefficients combined with particle weight determined by sedimentation equilibrium since

$$f/fo = \frac{2.89 \times 10^{-5}}{D_{20} (Me)^{3/2}} = 1.19 \times 10^{-18} M^{3/2} \frac{(1 - \psi_0)}{S_{20} \psi_0},$$

(equations 70, 70a, respectively, Svedberg & Pedersen, 1940). The frictional ratio for EMC virus using this relationship was $1.129 \pm 0.021$ (S.E.M., 9 determinations). EMC virus was known to be essentially spherical in shape as determined by electron microscopy (Faulkner et al. 1961; Burness, 1969); the deviation of $f/fo$ from a value of 1.0 can thus be attributed to hydration, the extent being 0.296 g. water/g. dry EMC virus as determined by the equation of Kraemer (equation 125, Svedberg & Pedersen, 1940):

$$f/fo = \left(\frac{w\psi_0 + \psi}{\psi}\right)^{1/2}.$$

A hydrated partial specific volume for EMC virus, $\bar{\psi}_h$, of 0.753 ml./g. was calculated from sedimentation coefficient and hydrated particle diameter by the relationship (equation 7, Markham, Smith & Lea, 1942):

$$d = 0.426 \left(\frac{\bar{\psi}_h S_{20}}{1 - \bar{\psi}_h \rho}\right)^{1/2}.$$

The reciprocal of $\bar{\psi}_h$ was 1.33 g./ml., the same as the buoyant density of EMC virus determined by isopycnic centrifugation in CsCl (Goodheart, 1965) suggesting that EMC virus does not appreciably bind the heavy Cs$^+$ ion.

A degree of hydration of 0.288 g. water/g. dry virus was calculated from $\bar{\psi}_h$ using the expression (equation 13, Markham et al. 1942):

$$w = \frac{\bar{\psi}_h - \bar{v}}{1/\rho - \bar{\psi}_h}.$$  

This value from sedimentation velocity analysis was very similar to that obtained above from the frictional ratio based on sedimentation equilibrium studies suggesting the data to be consistent internally.

**DISCUSSION**

A complete list of the biophysical properties of EMC virus based on the present work is given in Table 2. All the data used were obtained using purified EMC virus and no values were assumed from analogous studies with other viruses.

The least reliable determinations were those of partial specific volume since the difference in density between deuterium oxide and water was not sufficient to produce changes in sedimentation coefficient large enough to measure accurately. A fairly good estimate of partial specific volume can be made knowing that the virus contained 31.7% RNA and 68.3% protein (Burness, 1970). These were assumed to have partial specific volumes 0.55 and 0.74 ml./g., respectively (Markham, 1967), giving a partial specific volume for the virus $= (0.317 \times 0.55) + (0.683 \times 0.74) = 0.679$ ml./g., which confirmed the figure obtained by differential centrifugation. However, it is hoped that a more reliable figure can be obtained without assumptions by a more accurate method.

While this work was in progress, Scraba, Kay & Colter (1967) reported that combination of sedimentation and diffusion coefficients, determined in the same way as we have, gave a particle weight of $8.32 \pm 0.7 \times 10^6$ for Mengo virus; like EMC virus this is a member of the Columbia SK and picornavirus groups. Several other biophysical properties for Mengo
and EMC viruses were similar. Their values for particle weight together with the additional information obtained by sedimentation equilibrium studies in the present report suggest that the particle weight of the members of the Columbia SK group is in excess of 8 million.

<table>
<thead>
<tr>
<th>Biophysical property</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle weight ( \times 10^{-6} )</td>
<td>8.51</td>
</tr>
<tr>
<td>( S_{20,w} \times 10^{12} ) sec.</td>
<td>162.3</td>
</tr>
<tr>
<td>( D_{20,w} \times 10^{7} ) cm.(^2)/sec.</td>
<td>1.44</td>
</tr>
<tr>
<td>( \nu ) in ml./g.</td>
<td>0.678</td>
</tr>
<tr>
<td>( \nu_h ) in ml./g.</td>
<td>0.753</td>
</tr>
<tr>
<td>Hydrated diameter in nm.</td>
<td>29.8</td>
</tr>
<tr>
<td>Hydration in g. water/g. dry virus</td>
<td>0.290</td>
</tr>
<tr>
<td>Frictional ratio</td>
<td>1.130</td>
</tr>
</tbody>
</table>

The particle weight of poliovirus is usually given as \( 6.8 \times 10^6 \). This figure is based on the work of Schaffer & Schwerdt (1959) who, themselves, drew attention to the inconsistency between their values for density (taken as reciprocal of partial specific volume) and the RNA content of the virus. Poliovirus has a sedimentation coefficient \( S_{20,w} \) of 157 to 160 s in dilute solutions, a particle diameter of 27 ± 2 nm. determined by electron microscopy and a buoyant density of 1.34 g./ml. in CsCl (Schaffer & Schwerdt, 1959; Mattern, 1962). Since all these values are similar to those found for EMC virus it is likely that both viruses have similar particle weights. It is thus suggested that the particle weight generally assumed for poliovirus is too low and must remain open to doubt until re-examined.

RNA extracted both from poliovirus (Holland et al. 1960) and from EMC virus (Burness et al. 1963; Montagnier & Sanders, 1963) had a sedimentation coefficient of 37 s which was consistent with a molecular weight close to \( 3 \times 10^6 \) based on the formula derived from studies on tobacco mosaic virus RNA (Gierer, 1958). In spite of these findings, the molecular weight of picornavirus RNA is invariably accepted as \( 2 \times 10^6 \), a figure which was calculated from the questionable particle weight of \( 6.8 \times 10^6 \) for poliovirus of 30% RNA content (Schaffer & Schwerdt, 1959). This value has already been incorporated into the definition of a picornavirus (Gibbs et al. 1966; Fenner, 1968), which is unfortunate since evidence is accumulating that the picornavirus EMC contains a piece of RNA of molecular weight greater than \( 2 \times 10^6 \). For instance, Fenwick (1968) has recently shown that the sedimentation behaviour of EMC virus RNA in the presence of formaldehyde, under conditions where hydrogen bond interactions are reduced to a minimum, was consistent with a molecular weight of \( 2.4 \times 10^6 \). In addition, Dr Nicole Granboulan (quoted by Montagnier, 1968) has found RNA from bacteriophage R 17 and from EMC virus to have contour lengths of 1.06 and 2.60 \( \mu m. \), respectively, when examined in the electron microscope. Since bacteriophage R 17 RNA has a molecular weight of \( 1.1 \times 10^6 \) (Gesteland & Boedtker, 1964), EMC virus RNA can be calculated to have a molecular weight of \( 2.7 \times 10^6 \). Finally, the mean particle weight of \( 8.51 \times 10^{6} \) obtained in the present report together with an RNA content of 31.7% (Burness, 1970) suggests that EMC virus contains RNA of molecular weight equivalent to \( 2.7 \times 10^6 \); this figure was derived with no assumptions concerning, for instance, the similarity of sedimentation behaviour of RNA from different sources.

Such findings are not only relevant to a complete description of virus structure but are also important in understanding the details of viral replication since an RNA genome of molecular weight \( 2.7 \times 10^6 \) could possibly code for an extra three polypeptides containing more than 200 amino acids when compared with a genome of molecular weight \( 2.0 \times 10^6 \).
The size of EMC virus RNA is now under detailed study using techniques similar to those described in this report.

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