The Particle Size of Rubella Virus

By B. RUSSELL, GOLDA SELZER AND HELGA GOETZE

C.S.I.R. and U.C.T. Virus Research Unit, University of Cape Town,
Republic of South Africa

(Accepted 6 March 1967)

SUMMARY

The sedimentation coefficient of rubella virus was found to be 342 S and
the buoyant density of 1.085 g./cm.³. The calculated particle diameter,
850 å, was in good agreement with that found by ultrafiltration, namely
900 å.

INTRODUCTION

In the past 3 years much has been written about the clinical manifestations and
varied complications of rubella infection and much light has been thrown on the
many problems in isolating the virus and establishing the laboratory diagnosis of
infection. Investigations of the physical properties of the virus have given conflicting
results.

Weller & Neva (1962) found that the rubella virus passed through a 3000 å and
not through a 1500 å filter, except for a possible trace. McCarthy, Taylor-Robinson
& Pillinger (1963) in a preliminary investigation found, however, that some virus
passed through a 1000 å filter; while Parkman et al. (1964) found that the virus
passed through a 3000 å filter but not through one of 1000 å average pore diameter.
Norrby et al. (1963) estimated the diameter of the rubella virus from electron micro-
graphs to be approximately 2000 å whilst Phillips et al. (1965), also by electron
microscopy, found evidence for a pleomorphic virus with a cross-section varying
from 500 å to 2500 å. Cusumano (1966) estimated the average isopycnic density of
the rubella virus to be only 1.075 ± 0.005 g./cm.³ in a sucrose gradient. He suggested
the possibility of a high proportion of water or lipid in the particle.

In the present communication sedimentation, density and ultrafiltration results are
presented which indicate that the diameter of the rubella virus is approximately
850 å.

METHODS

Virus. The strain of rubella virus used in these experiments was the Walsh strain
isolated from a human embryo (Selzer, 1963).

Culture of the virus. The virus was grown in primary monolayer cultures of kidney
cells (MK cells) from the Vervet monkey (Ceropithecus aethiops pygerythrus) in 1 oz
bottles and test tubes, at 35°. The nutrient medium used was Hanks balanced salt
solution (a.s.s.) with 0.5% (w/v) lactalbumin hydrolysate and 2% (v/v) fowl serum.
The presence of the virus was demonstrated by challenging the rubella-infected cells
with 10,000 tissue culture doses per ml. of Echo XI virus.

Titration of virus. In some experiments fivefold and in others tenfold dilutions of
the virus were made in the nutrient medium and 1 ml. of the dilution inoculated into
either 4 MK culture tubes or two tubes plus two bottles. Cultures were challenged with Echo XI virus on the 5th day of culture. Infectivity was calculated by the method of Reed & Muench (1938).

_Solutions and media._ Because of its instability in acid solutions (Chagnon & Laflamme, 1964) rubella virus was suspended in 0.06 m-phosphate buffer containing 1% (w/v) bovine plasma albumin (BPA) (Armour Pharmaceutical Co.). The pH value of this solution was adjusted to 7.5 by the addition of N-NaOH. Isopycnic density was determined in both Ficoll (Pharmacia, Uppsala, Sweden) and CsCl gradients. Four g. of Ficoll (mol. wt 400,000) were dissolved in 0.06 m-phosphate buffer containing 1% (w/v) BPA. The pH value was adjusted to 7.5 with N-NaOH and the volume made up to 10 ml. with phosphate buffer containing BPA. This solution was diluted as required in phosphate buffer of the same reaction containing 1% (w/v) BPA. Three g. CsCl (Analar, British Drug Houses) were similarly dissolved in 10 ml. phosphate buffer BPA diluent. For the sedimentation and ultrafiltration experiments the virus was left in the tissue culture fluid in which it was harvested, BPA being added to give a final concentration of 1% (w/v) (pH 7.5). The medium used for pretreating collodion membranes was 1% (w/v) solution of Panmede (Paines and Byrne Ltd., Greenford, England) in phosphate buffer (pH 7.5).

Bacterial contamination was controlled by autoclaving or boiling solutions where appropriate. Protein solutions were sterilized by Seitz filtration. Penicillin (100 units per ml.) and Streptomycin (1 mg./ml.) were added to the solutions before use.

_Ultracentrifugation._ A suspension of virus, clarified by centrifuging for 10 min. at 7000 rev./min. in the No. 40 rotor of a Model L Spinco centrifuge, was layered over a 40% solution of sucrose in phosphate buffer in a specially marked centrifuge tube. This was then centrifuged according to method A of Polson & van Regenmortel (1960) for exactly 30 min. in an SW 39 rotor at approximately 4°C. The top 1 cm. of fluid was then carefully collected and titrated for infectivity. The procedure was repeated at a number of different rotor velocities ranging from 5000 to 30,000 rev./min.

_Density._ The buoyant density of the virus was found by the method of Meselson, Stahl & Vinograd (1957) as adapted by Polson & Levitt (1963). The virus suspension was adjusted to a suitable density by the addition of either Ficoll or CsCl and introduced at a selected position in a preformed density gradient. It was found, in agreement with Parkman _et al._ (1964), that if the virus was suspended in a solution of 60% (w/v) CsCl, all of the infectivity was lost after standing for 3 hr at 5°C. As it appeared that the virus had a low density, a 30% (w/v) solution of CsCl was tested. Caesium chloride at this concentration did not inactivate the virus. It was found inadvisable to mix a suspension of rubella virus with the CsCl solution. A better procedure was to dialyse the virus suspension against a relatively large volume of CsCl solution of the required density. The gradual change of the osmotic pressure so effected, coupled with the presence of 1% (w/v) BPA in the solution, ensured that the virus retained its infectivity.

The virus was brought to its isodensity level after 3 hr centrifuging in an SW 39 rotor at 33,000 rev./min. in the CsCl gradient and after 5 hr at the same rotor velocity in the Ficoll gradient. After centrifuging, the tube was removed from the rotor and placed in a graduated glass tube. The upper half of the gradient column was then sampled using a finely drawn Pasteur pipette with a right-angled tip. Three or four samples were collected in this way. The remainder of the column was then sampled by puncturing the bottom of the centrifuge tube. This procedure reduced the possi-
bility of contaminating one sample with another. After mixing each individual sample, one drop was removed, its refractive index measured and its density estimated from the predetermined linear relation between density and refractive index. Samples collected from CsCl columns were dialysed overnight against phosphate buffer at 5°C. All the samples were made up to 2 ml. with phosphate buffer containing BPA before being titrated in tissue culture. Samples obtained from the Ficoll density columns were titrated without preliminary dialysis.

**Ultracentrifugation.** A series of collodion membranes was prepared and calibrated according to the method of Elford (1931). Membranes of the required average pore diameter (APD) were mounted in filter holders and pretreated with Panmede broth. A suspension of rubella virus was clarified by centrifuging for 10 min. at 7,000 rev./min. and then passing the supernatant fluid through a filter of APD 5360 Å. Five ml. portions of this suspension were then passed through a series of membranes using filtered air at a pressure of 1 atm. The filtrates were titrated.

**RESULTS**

The information from 3 experiments was considered together; 90% of the virus was estimated to have sedimented through a distance of 1 cm. at a rotor velocity of 18,750 rev./min. in 30 min. (Fig. 1). The viscosity of the suspending medium was found to be 1.68 cP at the temperature of centrifugation, 3.8°C. Using the relation

\[ S_{50} = \frac{3.5 \eta_T \log X}{\eta_0 N^2 t}, \]

where

\[ X = \frac{x + l}{x + l \frac{C_i}{C_o}}, \]

\[ \eta_0 = \text{viscosity of water at } 20°C, \]
\[ \eta_T = \text{viscosity of dispersion medium at } 3.8°C, \]
\[ N = \text{rotor velocity in rev./min.}, \]
\[ t = \text{time of centrifuging in min.}, \]
\[ x = \text{distance from upper meniscus to centre of rotation}, \]
\[ l = \text{‘effective column’ length (1 cm.)}, \]
\[ C_i/C_o = \text{ratio of average virus concentration in the ‘effective column’ after and before centrifuging}, \]

the sedimentation coefficient was found to be 342 Svedberg units.

**Density**

Three isopycnic density determinations of rubella virus were made. The buoyant density of the virus was found to vary mainly between 1.075 g./cm.³ and 1.09 g./cm.³ with some scatter above and below these values (Fig. 2). This was more noticeable in the Ficoll density gradients. The scatter may have been due to variations of density of the virus particles or to inaccuracies in sampling, especially in the case of the more viscous polysucrose solutions. The average buoyant density of the majority of the infectious particles was estimated to be 1.085 g./cm.³. This figure is in close agreement with that of Cusumano (1966) who found a density of 1.075 g./cm.³ for rubella virus in a sucrose gradient.
Fig. 1. Ultracentrifugation of rubella virus. The results of three experiments have been combined. The rotor velocity necessary to reduce the virus titre by 90% in 30 min. was found to be 18,750 rev./min. The arrows indicate that the titre is less than that shown.

![Ultracentrifugation](image)

Using the Stoke's relation

\[
r^2 = \frac{9S\eta}{2(d-p)}
\]

where \( r \) = radius of particle in cm., \( S \) = sedimentation coefficient, \( d \) = density of particle, \( p \) = density of suspending medium, \( \eta \) = viscosity of suspending medium, the particle diameter of the virus, calculated from the sedimentation coefficient and density data was found to be 850 Å.

Fig. 2. Buoyant density of rubella virus as determined in two experiments using Ficoll (○ ⋅ ⋅ ⋅, ⋅ ⋅ ⋅, ⋅ ⋅ ⋅) and one using CsCl (□ ⋅ ⋅ ⋅, ⋅ ⋅ ⋅) solutions. The average density as found from these curves was 1.085 g./cm.³. Arrows indicate the position of the virus in the gradient column before centrifuging.
The particle size of rubella virus

Ultrafiltration

No infective material passed through membranes of 1160 \( \text{\AA} \) APD or less; filtration through a membrane of 1640 \( \text{\AA} \) APD reduced the virus titre more than 1000-fold. The curve relating virus titre and APD of the gradocol membranes may be extrapolated to a point indicating a size of approximately 1400 \( \text{\AA} \) (Fig. 3). As stressed originally by Elford (1931) and later by Black (1958) this end point must be multiplied by a factor in order to obtain the actual diameter of the filtered particles. Using Black's factor, 0.64, a diameter of approximately 900 \( \text{\AA} \) was calculated for rubella virus.

![Ultrafiltration of rubella virus. The limiting pore size was found by extrapolation to be approximately 1400 \( \text{\AA} \).](image)

DISCUSSION

The particle size of the rubella virus determined by us differs from previously published figures. Most investigators have stated that the virus passed through a 3000 \( \text{\AA} \) filter but failed to pass a 1000 \( \text{\AA} \) filter. This we have confirmed in the present work. The filtration end point, namely 1400 \( \text{\AA} \), was multiplied by the factor 0.64 to obtain a more accurate estimation of the actual particle diameter. It is not clear in other published work whether this factor was taken into account.

Virus particles containing a large proportion of water or lipid or both may become distorted or partially break down when subjected to high vacuum. Distortion could account for the large particles of variable morphology seen in electron micrographs.

In applying the Stoke's relation a spherical particle was assumed. The agreement between the size of the virus calculated from the sedimentation coefficient and that obtained by ultrafiltration data increases the likelihood of a spherical particle. Although the virus particles may be somewhat pleomorphic they are unlikely to be rod-shaped or filamentous.
The interest of Professor A. Kipps and Dr A. Polson is gratefully acknowledged. This investigation was supported in part by U.S. Public Health Service research grant A1-04044-06 from the National Institutes of Health, Bethesda, Md, U.S.A.

REFERENCES


(Received 30 January 1967)