Morphology of Type II Poliomyelitis Virus (MEFl) as Determined by Electron Microscopy.* (21082)

C. E. SCHWERDT, R. C. WILLIAMS, W. M. STANLEY, F. L. SCHAFFER, AND M. E. MCCLAIN.

From the Virus Laboratory, University of California, Berkeley.

Previous attempts to determine by electron microscopy the size and shape of particles of human poliomyelitis virus have been equivocal because of uncertainty as to the identity of the virus particle among various objects of similar appearance found in the electron micrographs (1-6). Improved methods of purification applicable to poliomyelitis virus propagated in central nervous system tissue *in vivo*, or in monkey kidney tissue culture, now yield virus concentrates containing at most 2 classes of particles and frequently only one(7,8). Furthermore, the examination of such purified virus concentrates by analytical electron microscopy has resulted in the establishment of a constant ratio of numbers of the particle believed to be the virus to the infectivity titer (8). In a preliminary determination this particle has been found to be approximately spherical and about 28 mμ in diameter, but its successful identification seems to justify at this time a more definitive examination of its shape and size.

Materials and methods. The virus used in this study is a tissue culture MEFl strain received from Dr. Jonas Salk in 1952. It has been passed 3 times in monkey kidney tissue culture in this laboratory, and the third passage has been used as a virus seed pool for the production of liter quantities of tissue culture virus suspension for purification purposes. The method of purification employed was similar to that described by Bachrach and Schwerdt for the purification of Lansing virus from infected cotton rat brains and cords(8). The essential steps of the procedure are: the isoelectric precipitation of virus infectivity from tissue culture fluid at pH 4; the resuspension of the precipitated virus in a one-tenth or one-twentieth volume of 0.94 M NaCl buffered at pH 7.8 with 0.02 M Na_2HPO_4; 2 n-butanol extractions of the molar salt solution extract of the precipitated virus; concentration of the virus from the extracted aqueous phase by one cycle of differential centrifugation in the Spinco, model L, ultracentrifuge with isotonic saline buffered at pH 7.8 as solvent; treatment of the virus concentrate with crystalline ribo- and deoxyribonucleases; and a final concentration and purification of the virus by a second cycle of differential ultracentrifugation. The final preparation usually represents a thousand-fold concentration of the virus from 1 liter of tissue culture fluid. The increase in specific activity of the purified preparation above that of the original tissue culture fluid as estimated by the plaque assay method of McClain and Schwerdt(9) ranges from 200 to 400-fold in 4 purification experiments. Specimens for electron microscopic examination were prepared by normal air-drying and by the freeze-drying technic(10). Prior to preparing an air-dried specimen, an aliquot of the purified virus concentrate was dialyzed against 0.1 M ammonium acetate in order to replace the non-volatile salts of the isotonic saline with a wholly volatile salt solution(11). The virus preparation was then irradiated with ultraviolet light for a sufficient length of time to reduce the infectivity to a level no longer detectable by tissue culture assay. The irradiation reduced the hazard of releasing aerosols of highly infectious material during the manipulations involved in the preparation of specimens, yet did not appear to alter the morphology of the virus particle. Macrodrops of the dialyzed, irradiated virus suspensions were permitted to dry in air at room temperature on Formvar-coated specimen screens. In some instances the virus particles were resuspended on the membrane in a drop of distilled water immediately after the initial drying from the ammonium acetate solution and again allowed to dry. Either before or after the preparation of the virus

* Aided by a grant from the National Foundation for Infantile Paralysis.

specimen, a dilute suspension of polystyrene latex (PSL) particles was sprayed upon the Formvar membrane. The PSL spheres are uniformly 260 mμ in diameter and serve as a yard stick for the measurement of the diameters of the virus particles and for the determination of the local shadow angle. For the preparation of frozen-dried specimens, aliquots of the virus concentrate were dialyzed against 0.1 M ammonium bicarbonate, irradiated as described above, and sprayed from a high-velocity gun. The spray thus formed consists of micro-droplets of volumes of the order of 10^{-9} ml and these freeze upon and adhere in the frozen state to collodion-coated specimen screens which have been pre-sprayed with PSL particles and precooled to about −80°C(10,12). The ice and volatile salt in the frozen droplets were then sublimed in a vacuum at −50°C, well below the eutectic temperature of an ice-ammonium bicarbonate mixture. In order to enhance the volatilization of the ammonium bicarbonate the specimen was finally heated to about 60°C for 10 minutes before air was admitted to the freeze-drying unit. The non-volatile constituents remain behind, undisturbed by the surface tension forces which distort individual virus particles in specimens prepared by air-drying. Both the air-dried and frozen-dried specimens were shadowed with uranium before electron microscopic examination. Measurements of virus particle diameters were made by direct examination of the original photographic negative by means of a microscope equipped with an eyepiece micrometer scale.

Results. A typical micrograph of an air-dried specimen of MEFl virus is illustrated in Fig. 1. The 2-dimensional hexagonal arrangement of virus particles observed demonstrates a high degree of uniformity of particle size. Measurement of linear arrays of 5 or more particles along any of the 3 axes of a closely-packed hexagon yields an average particle diameter of 27 mμ. The diameter of individual air-dried virus particles measured perpendicularly to the direction of the shadow length is 31 mμ. However, measurements of their shadow lengths, combined with a knowledge of the local shadow angle, shows them to be flattened by about 20%. Most of the measured difference in diameter between the individual particles and the ones hexagonally packed may be ascribed to the effects of flattening.

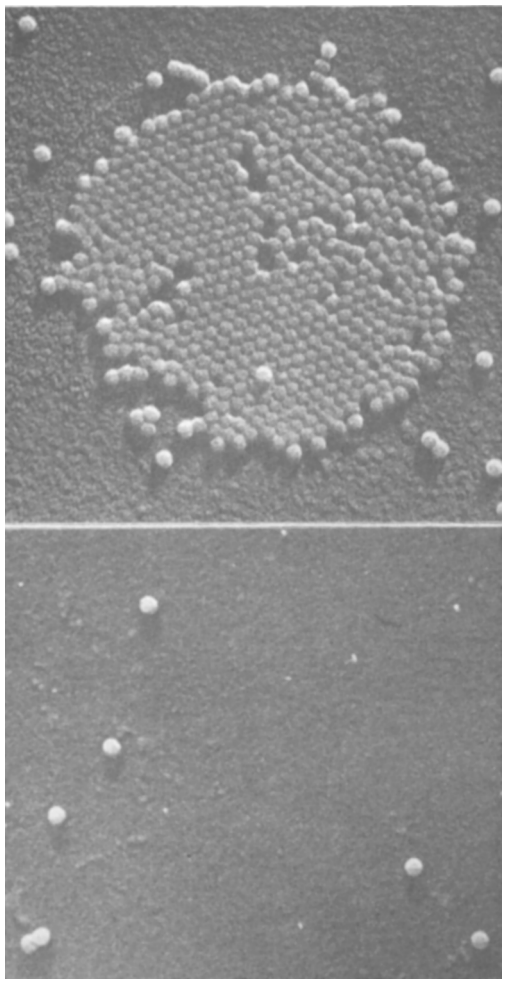

FIG. 1 (top). Poliomyelitis virus (MEFl) air dried from an ammonium acetate suspension. The uniformity of particle size is demonstrated by the existence of regions of close packing. The individual particles are flattened slightly and appear larger than those in the crystalline array. ×74000.

FIG. 2 (bottom). Poliomyelitis virus (MEFl) frozen dried from an ammonium bicarbonate suspension. The particles appear spherical, with no evidence of flattening. They have the same diameter (27 mμ) as those in the packed array in Fig. 1. Shadowed at $\tan^{-1}$ 0.36. ×74000.

Fig. 2 illustrates a frozen-dried specimen of purified virus. The very small, irregularly shaped particles seen over the background are believed to be non-volatile impurities in the ammonium bicarbonate solution, since similar

debris is observed in ammonium bicarbonate blanks. The individual virus particles are spherical in shape, a conclusion based upon the appearance of the particles themselves and upon the similarity in the shape of the shadows cast by virus and by PSL particles. The average diameter of the frozen dried virus is 27 mμ, in agreement with the value obtained for hexagonally packed air-dried virus. Careful examination of the shadowed, frozen-dried virus particles has failed to reveal any which exhibits regular angular contours or casts angular shadows, as has been found for tobacco ring-spot virus(13) and for several bacterial viruses(14).

Discussion. Individual particles of MEFl poliomyelitis virus appear to be spherical in frozen-dried preparations and to have the shape of slightly flattened spheres in air-dried mounts. The high uniformity of particle diameter is demonstrated in Fig. 1 where rows formed by packing are seen to be straight over distances including a dozen or so particles. Diameter measurements of individual frozen-dried particles offer further evidence of uniformity in that the variation of the measured diameters is not unequivocally greater than the estimated uncertainty of measurement. These conclusions are in distinct contrast to the results published by Sabin, Hennessen and Warren(6) for a type II virus where the electron micrograph shows a great variation of particle size.

The absolute value of the diameter of the virus particles is subject to several uncertainties, owing to possible artifacts of preparation, the finite resolving power of the electron microscope, the calibration of magnification, and the limitations of the measurement of the photographic images. If we assume that no appreciable shrinkage of individual particles has occurred during the formation of an array like Fig. 1, the average diameter of the particles measured in a row is least susceptible to errors of image formation and measurement. If individual particles are measured, the most reliable diameter is probably secured from the frozen-dried preparations where no sign of surface-tension distortion is seen. Our estimate of the uncertainty in the value given for the particle diameter is ± 2 mμ.

Summary. The particles of the MEFl poliomyelitis virus, grown in monkey-kidney tissue culture, and subsequently purified by chemical and physical means, are spherical in shape and highly uniform in diameter. The diameter of individual particles, prepared by normal air-drying from a 0.1 M ammonium acetate suspension, is found to be 31 mμ. The diameter of particles packed in a crystalline array, or frozen-dried from a 0.1 M ammonium bicarbonate suspension, is found to be 27 mμ. It is believed that the latter diameter is the more significant and reliable figure.

1. Rhian, M., Lensen, S. G., and Williams, R. C., *J. Immunol.*, 1949, v62, 487.

2. Loring, H. S., Marton, L., and Schwerdt, C. E., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 291.

3. Reagan, R. L., and Brueckner, A. L., *Texas Rep. Biol. and Med.*, 1952, v10, 425.

4. Kausch, G. A., and Bender, A., *Arch. virusforsch.*, 1951, v4, 217.

5. Taylor, A. R., and McCormick, M. J., *J. Applied Physics,* 1953, v24, 1418 (Abstract).

6. Sabin, A. B., Hennessen, W. A., and Warren, J., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 359.

7. Stanley, W. M., Bachrach, H. L., and Schwerdt, C. E., *Science,* 1953, v118, 576 (Abstract).

8. Bachrach, H. L., and Schwerdt, C. E., *J. Immunol.*, 1954, v72, 30.

9. McClain, M. E., and Schwerdt, C. E., *Fed. Proc.*, 1954, v13, 505 (Abstract).

10. Williams, R. C., *Exp. Cell Res.*, 1953, v4, 188.

11. Backus, R. C., and Williams, R. C., *J. Applied Physics,* 1950, v21, 11.

12. Rice, R. V., Kaesberg, P., and Stahmann, M. A., *Biochim. et Biophysica Acta,* 1953, v11, 337.

13. Williams, R. C., *Cold Spr. Harbr. Symp. quant. Biol.*, 1953, v18, 185.

14. Williams, R. C., and Fraser, D., *J. Bacteriol.*, 1953, v66, 458.

Received May 17, 1954. P.S.E.B.M., 1954, v86.