data coincided with the L = 3400 Å. curve down to gradients of 100 sec.$^{-1}$ We attribute this greater homogeneity to prolonged exposure (3 hr.) to 0.01 M Versene at room temperature prior to the third high speed centrifugation. In all other details, the preparative procedure was identical with that outlined above.

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Lengths of Tobacco Mosaic Virus from Electron Microscopy[1]

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A sample of tobacco mosaic virus showing a high degree of uniformity as to length which had previously been studied by physical-chemical techniques was examined and measured with the electron microscope. From a measured population of 200 particles 85% have lengths between 2800 and 3200 Å. and 75% have lengths between 2900 and 3100 Å. The most frequently occurring length is between 2950 and 3050 Å. Methods and precautions taken in making these measurements are described. These results are in excellent agreement with previous electron microscope results.

A sample of tobacco mosaic virus (TMV) prepared by Dr. H. Boedtker who has reported the physical-chemical measurements of this same material[2] has been examined and measured by electron microscopy. Although TMV has been observed on innumerable occasions by electron microscopy, good correlation of results with hydrodynamical data has been lacking. It therefore seems worth while to report the electron microscope results in some detail insofar as they may be of value in the interpretation of the data from indirect methods. It is also interesting to compare our new results with the electron microscope work of Williams and Steere[3] since the two sets of data are in excellent agreement although the methods differ appreciably.

Experimental

A volume of the TMV solution was diluted with double-distilled water containing polystyrene latex to a final concentration of about 0.5 mg./ml. of virus and sprayed from a high pressure gun similar to that described by Backus and Williams[4] onto the surface of freshly cleaved mica. The polystyrene latex (Dow Chemical Company) has an average diameter of 880 Å. and was present in an amount of 0.06 mg./ml. It was employed as an aid on focussing and not for magnification calibration. The surface was shadowcast with 2.4 cm. of 0.1 mm. Pt wire at a shadow-to-height ratio of 10:1, coated normally with 0.5 mg. of SiO evaporated from a distance of 12 cm. to provide rigidity, coated with 0.5% collodion and stripped on water. The stripped specimens were supported on 200-per-inch nickel screens.

Before the specimens were observed with the electron microscope (RCA Type EMU3B, 100 kv.), the microscope was calibrated by recording 5 images of an SiO replica of a 15,000-line-per-inch diffraction grating on a 2 × 10 inch plate which provided a total of 10 spacings (magnification about 12,500 ×). Two plates of TMV showing 10 different fields were then recorded and the calibration procedure repeated. Since the calibration of the instrument drifts as it warms up, the average of the two calibration plates was used for the TMV. Owing to the highly hydrophilic nature of a cleaved mica surface, sprayed droplets spread over a very large area which makes it impossible in general to include an entire droplet in a single field of view. The recorded fields were therefore random samples from droplets covering large areas. Subsequent plates were also recorded in the same manner with a calibration before and after the TMV specimens. Before each plate was recorded, hysteresis effects in the lenses were removed by turning objective and projector lenses to their maximum current values and back to operating value 4 times.

Electron micrographs containing well-dispersed particles were enlarged to a total magnification of 66,000 to 67,000 × as calculated from the calibration micrographs. TMV particles were measured (to the inside of the cap on shadowed ends) with a scale graduated in 0.5 mm. divisions and grouped in intervals of 0.25 mm. All particles whose two ends could be seen without ambiguity were measured. Since magnifications were close together (66,500 ± 500), measurements from all prints were added together to be plotted on the same millimeter scale. It was not necessary to correct for field distortion as was done by Williams and Steere because this effect introduces an error of less than 1% at the magnification employed.

Results

The length-distribution for 201 particles is shown in Fig. 1 where a scale in Ängströms has been superimposed in place of the millimeter scale on the basis of a magnification of 66,500 ×. The graph shows that the sample consists to a large extent of particles whose lengths are very close to 3000 Å. A better visual demonstration of the relative number of particles contained within various length classes is provided by the cumulative plot in Fig. 2 which shows that although there are a few particles present with random lengths above and below the peak value, about 75% of the particles are within the range 2900 to 3100 Å. In view of the fact that random observational errors which might be as high as ±2% in some instances would contribute to broadening of the distribution, the degree of uniformity is remarkable. That the observed breadth around the maximum does not result entirely from calibration error is evident from the fact that observed lengths vary within a given field by about the spread shown on the graphs. Possibly there is some distortion of the particles on drying, but the rigidity of the support might be expected to keep this to a minimum. It would appear that the most frequently occurring length for particles dried in this fashion is very probably within the range 2950 to 3050 Å. There is no evidence for any special tendency to form dimers although a few particles having about double the most frequent length do occur.

(1) This work was supported by a research grant C217I(C5) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) H. Boedtker, THIS JOURNAL, **80**, 2550 (1958).

(3) R. C. Williams and R. L. Steere, *ibid.*, **73**, 2057 (1951).

(4) R. C. Backus and R. C. Williams, *J. Applied Phys.*, **21**, 11 (1950).

The method[5] of depositing macromolecules on mica prior to electron microscope examination was originally developed for the observation of objects very much smaller than virus particles and was not essential to this study of particle lengths. Its advantages for the present work were that it provides (1) a rigid support, (2) a hydrophilic surface undistorted by conventional grid wires resulting in well dispersed population of particles and (3) it eliminates the possibility of substrate topography showing through as apparent surface structure on the virus. The method is also extremely sensitive to the presence of extraneous materials. Substrate backgrounds were exceptionally clean indicating that a high degree of isolation of the virus had been achieved. No regular surface corrugations such as have been reported[6,7] were observed in these studies other than appearances which could be attributed to optical or shadowing artifacts, although experiments have been carried out under conditions such that the reported structures should have been manifest if they were present.

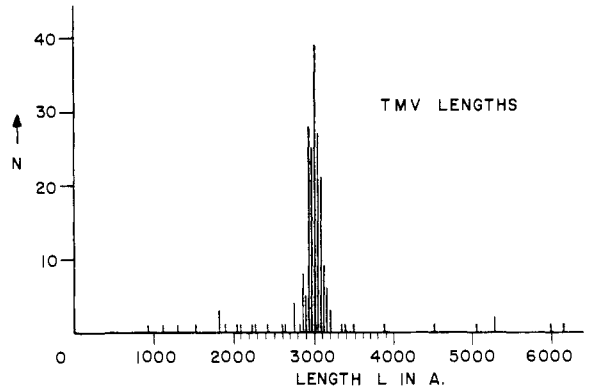

Fig. 1.—Plot showing the distribution of lengths of TMV in a sample of 201 particles.

(5) C. E. Hall, *J. Biophys. Biochem. Cytol.*, **2**, 625 (1956).
(6) R. F. Baker, *Nature*, **178**, 636 (1956).
(7) R. E. F. Matthews, R. W. Horne and E. M. Green, *ibid.*, **178**, 635 (1956).

Discussion

These measurements were made mainly for the purpose of comparing the electron microscope data with the hydrodynamical data which have been discussed by Boedtker. It is interesting, however, also to compare these results with the prior electron microscope observations of Williams and Steere. Although there were differences in methods of extracting the virus, calibration, sampling and specimen supports the two sets of data are in excellent agreement. Their mean unit length of 2980 Å. is well within the limits of 2950 to 3050 Å. which we would set for the most frequently occurring length on the basis of about ±2% possible error in measurements. It is also interesting to note that the breadths of the length–frequency plot as shown in Fig. 1 are very similar to those shown in the earlier work. In both instances the breadth near the base of the peak frequency is about 250 Å. or about 8% of the most probable length. In the present work this variation cannot be ascribed to errors in calibration because of the fact that variations of about this magnitude can be found among particles in the same field. Variations must be due to variations in the lengths of the original particles or to changes produced by the preparative procedures.

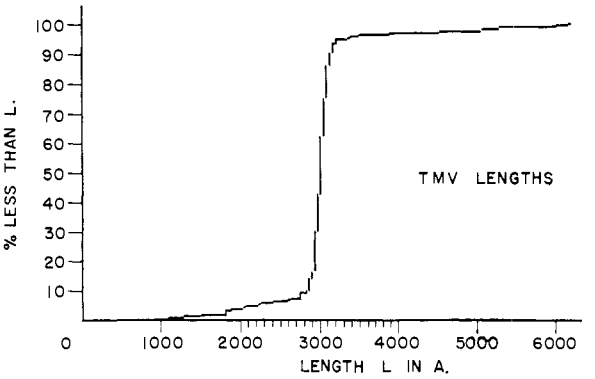

Fig. 2.—A cumulative plot of the data in Fig. 2 showing as ordinates the percentage of particles having lengths less than the corresponding value on the horizontal scale.