mate for the "middle" falls within half a degree of the peak of the profile derived from the rocket measurements. This is well within the variation in altitude of the layer observed on different rocket flights. However, the estimates of the upper and lower boundaries of the horizon band do not appear entirely consistent with our profile. These judgments were obviously more difficult to make than that of the position of maximum. It seems probable that the presence of the moonlit horizon below approximately 105° zenith angle, with luminance equal to that of the brightest part of the layer, would cause the lower edge of the layer to appear too close to the center.

The discrepancies discussed above are really rather small, and it is gratifying that the rocket measurements and visual observations agree so well on the presence of the night-airglow horizon, its approximate dip angle, and its luminance. The agreement demonstrates the value of simple, careful visual observations in a situation where such observations might have been expected to be unreliable.

It is worth remarking that the observed luminance level, though low, is on the visual threshold after only a few minutes dark adaptation, and is easily perceived thereafter, being in the lower region of mixed rod-cone vision. No color sensation will be evident however, since the total visible airglow is not strongly colored, and would probably appear as a desaturated yellow or yellow-green if it were brighter. Therefore there does not seem to be much possibility of seeing color in the airglow horizon even when it is abnormally intense, and it is significant that no color was reported by Carpenter. However, there may be some possibility of seeing intense equatorial arcs radiating the atomic oxygen doublet 6300-6364 Å (*6*). Whether the intensity of these arcs appears enhanced when viewed from an orbiting altitude is not yet known. In any case the wavelength favors cone vision, and the arcs may appear red. Of course the aurora will often appear strongly colored; it should be a magnificent sight.

In conclusion we point out the obvious fact that the night airglow is only one of many visual phenomena available for study. Observation of the complex and fascinating optical phenomena occurring in the twilight and auroral zones should be strongly encouraged. They are a legitimate part of a manned satellite program.

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Tobacco Mosaic Virus: Purifying and Sorting Associated Particles According to Length

Abstract. *End-to-end aggregation of tobacco mosaic virus and associated particles during extraction and purification may be prevented by transfer of the virus to 0.001M buffer solution of ethylenediaminetetraacetic acid, pH 7.5, as rapidly as practicable. Colored components were removed with charcoal and diatomaceous earth, and salts by rapid passage through a column of granulated 8 percent agar gel. The virus particles were sorted according to length by passage through a 1 percent granulated agar-gel column to isolate the 300- and 200-mμ fractions and through a 5 percent agar gel to isolate the shorter particles.*

Ever since Stanley (*1*) described the first method of purification of tobacco mosaic virus (TMV), attempts have been made to improve the procedure, to determine the minimum length of infectious particles, and to obtain these infectious particles in monodisperse suspensions. Two features characteristic of the virus have interfered with such attempts; (i) the rods formed in plants systematically infected vary in length from 15 to approximately 300 mμ, and (ii) this variation in rod length is accentuated by end-to-end aggregation, which results in lengths up to 600 mμ or more. By using density-gradient centrifugation, Symington *et al.* (*2*) and Commoner *et al.* (*3, 4*) were able to separate fractions on the basis of rod length and to correlate infectivity with rods approximately 300 mμ long. In this report new procedures for purification of TMV and fractionation by agar-gel chromatography of associated particles on the basis of rod length are presented.

The first obstacle to be overcome in preparing the desired TMV suspension is to prevent end-to-end aggregation of rods of various lengths. From published reports (*5*) and from my own experiments, it appeared that three conditions were essential for this purpose: the *p*H of the suspending medium must be held above 7.2, the ionic strength of the suspending medium must be as low as possible, consistent with maintenance of the *p*H at about 7.5, and certain salts, notably phosphates, must be absent even when the *p*H is maintained at 7.5 or higher.

A procedure that prevents aggregation during and after maceration of infected tissue has been developed.

Leaves infected with TMV are dipped in 0.05*M* EDTA adjusted to *p*H 9.5 with NaOH (100 ml of buffer is used for each 100 g of tissue). Buffer soaked tissue is ground in an ordinary food grinder, and excess buffer is added to the mash. Juice is extracted from tissue fragments by squeezing through cheesecloth. The extract is immediately adjusted to *p*H 7.5 by addition of 1*N* NaOH. Activated charcoal (*6*), 5 g/100 ml, is added, and the mixture is shaken for 20 to 30 seconds. Then 5 g of diatomaceous earth (*1*) per 100 ml of extract is added, and shaking is continued for another 20 to 30 seconds. The mixture is filtered through a ¼-inch pad of diatomaceous earth in a Büchner funnel. Additional virus is washed from the filter cake with 10 to 20 ml of 0.05*M* EDTA buffer, *p*H 9. The filtrate, which has a blue color attributable to the Tyndall effect, is desalted by passage through a column of 8 percent granulated agar gel (*7, 8*) equilibrated with 0.001*M* EDTA, *p*H 7.5. The desalted suspension is centrifuged for 20 minutes at 50,000*g*; most of the longer rods are in the pellet and most of the shorter ones are in the supernatant. The colorless pellets are resuspended in 0.001*M* EDTA, *p*H 7.5, the suspension is clarified for 10 minutes at 2000*g*, and recentrifuged for 20 minutes at 50,000*g*. The pellets are resuspended in a small volume of the same buffer. The resultant concentrated suspension (15 to 20 mg/ml) contains rods 90 percent of which are

approximately 300 mμ long. Most of the remaining 10 percent are 200 mμ long, but a few shorter ones are still present.

The 200-mμ and shorter rods are removed by passing the concentrate through a 1 percent granulated agar-gel column 100-cm long and 1.5-cm in diameter (*8*). For this separation, the flow rate must be no greater than 5 ml per hour and the agar gel should be equilibrated with 0.01*M* EDTA, *p*H 7.5. The first fractions to contain virus, which can be detected by light scattering, consist almost entirely of standard-length (approximately 300 mμ) rods as determined by electron microscopy.

To obtain uniform suspensions of shorter rods, succeeding samples from the 1 percent agar-gel column are examined, and those containing a high percentage of the 200-mμ rods are collected, concentrated, and recycled through 1 percent agar-gel columns until the desired degree of uniformity is obtained. For uniform suspensions of still shorter rods, the supernatant liquids from the sedimentation for 20 minutes at 50,000*g* are centrifuged for 4 hours at 105,000*g*; the pellets are suspended in 0.001*M* EDTA, and the various fragments may be sorted on 5 percent granulated agar-gel columns equilibrated with 0.01*M* EDTA, *p*H 7.5. With these rod-shaped particles and the granulated agar-gel columns, there is no clear-cut separation into distinct fractions of rods of the different lengths, so some other method such as electron microscopy must be used to locate the position of desired lengths.

To avoid end-to-end aggregation after the particles are sorted by length, the ionic strength is decreased to 0.001*M* or less by concentrating the particles with high-speed centrifugation and resuspending them in 0.001*M* EDTA, *p*H 7.5, or by dialyzing the suspension against this same buffer.

Electron micrographs which illustrate the degree of homogeneity of fractions obtained by this procedure are shown on the cover of this issue. None of the sorted samples were truly monodisperse. They were obtained by only one or two passages through the appropriate agar-gel column, and the sorting under these conditions would probably require an additional passage or two to remove completely all particles of odd length from any fraction.

When opposite half leaves of susceptible hosts were inoculated with the various fractions, only standard-length (approximately 300 mμ) rods were infective, producing local lesions. No lesions were observed on half leaves inoculated with a suspension of the 200-mμ rods, the optical density of which equalled that of a standard preparation that produced over a hundred lesions on opposite half leaves. If the 200-mμ particles were capable of initiating infection on susceptible hosts, the fraction containing them should have given 50 percent more lesions than the standard preparation. No lesions resulted from inoculation of highly susceptible leaves with the still shorter fragments even at higher concentration. These results are in good agreement with those of Commoner *et al.* (*3*), who reported infectivity only for rods approximately 300 mμ long.

Since micrographs can be easily obtained which show uniform particle lengths, it is obvious that most of the shorter rods are not produced during the processes of preparing the suspensions for electron microscopy as postulated by Williams and Steere (*9*). Breakage of rods during preparation for electron microscopy appears to be restricted to those which overlap or lie across holes in the supporting film (*10*).

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Equilibrium Sedimentation of Uniform Rods of Tobacco Mosaic Virus

Abstract. *Equilibrium was achieved in centrifugal fields with tobacco mosaic virus by the Beams magnetically suspended ultracentrifuge. An attempt was made to account for the earth's gravitational component by using tilted cells in the rotor. The determinations of particle weight on highly uniform rods of tobacco mosaic virus in the apparent absence of nonideal behavior gave a value of (41.6 ± 0.1) 10^6.*

The equation for equilibrium in a centrifugal field relating distribution of solute to its molecular weight (*M*) is

$$M = \frac{2RT \ln (f_2c_2/f_1c_1)}{(1 - \bar{v}\rho)\, 4\pi^2N^2\, (x_2^2 - x_1^2)}$$

where c is the concentration of solute at any distance x from the axis of rotation, f is the activity coefficient, N is the frequency in revolutions per second, $\bar{v}$ is the partial specific volume of solute, ρ is the density of the solution and RT has its usual significance (*1*). The method offers a thermodynamic foundation for the study of the behavior of solutions in a multiphase system. Its usefulness for accurate data, however, has been limited to solutes substantially smaller than $M = 1 \times 10^6$ because of the uncertainty in N, convective disturbances, and the long time necessary to establish equilibrium at the low speeds required for larger solutes.

The Beams magnetically supported ultracentrifuge has overcome the above difficulties because the rotor, when suspended magnetically and coasting freely in a vacuum (10^{-6} to 10^{-7} mm-Hg), is inherently stable and free from hunting or precession at any angular velocity (*2*). Further, the optical system permits a precise knowledge of N and of the solute distribution at all points in the solution. The stability at low speeds also has made possible the application of the speed cutback procedure of Hexner *et al.* (*3*) to reduce the time required for reaching equilibrium by about one order of magnitude. The cutback method loses its advantage at low speeds unless all disturbances are avoided (*4*). Thus, one can use relatively long columns of solution ($\geqq$ 3 mm) to minimize uncertainties in the optical assessment of c as a function of x. Equilibrium with small polyhedral viruses ($M \cong 5 \times 10^6$) has been attained by these techniques (*5*). We have extended the centrifugal method for the study of particles larger by one order of magnitude. For this purpose tobacco mosaic virus (TMV) was chosen as solute. This order of size represents nearly the practical upper