amount, 26 per cent., of phospholipids had been found to be associated with an active tumor fraction purified by entirely different methods.[4] The portion of the purified fraction which is not soluble in ether contains 13.11 per cent. nitrogen and about 0.5 per cent. phosphorus.

Chemical tests for thymonucleic acid were negative, whereas the complete fraction or the ether extracted material gave strongly positive tests for pentoses. The biuret and Millon's tests were positive.

The ultra-violet absorption curve of the purified fraction presents a broad maximum at $\lambda 2575$,[5] a region of absorption which is also typical of nucleic acid.[6] This observation, together with the results of the chemical tests and the presence of at least 0.5 per cent. phosphorus, suggests that an important constituent of the active tumor fraction may be a nucleic acid of the ribose type.

The fact that the purified material was found to possess a tumor-producing activity far greater than that of the original extract suggests that centrifugation separated the active agent from inhibitory elements, an observation which is in agreement with previous work.[7] The enhanced activity of the purified fraction as compared to that of the whole extract was especially marked when the tumor tissue had been frozen at $-80°$ C. prior to extraction. Exposure to this temperature is known to disrupt a large proportion of the cells, thus releasing more of the intracellular elements. Under these conditions, the yield in tumor agent was considerably increased, but the relative inhibition of the original extract was also more pronounced. These results may indicate that the inhibitor involved is of intracellular origin.

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AN ESTIMATE OF THE RELATIVE DIMENSIONS AND DIFFUSION CONSTANT OF THE TOBACCO-MOSAIC VIRUS PROTEIN

THE specific viscosity of solutions of the tobacco-mosaic virus protein is proportional to the concentrations of the virus up to concentrations of 1 per cent. Data are presented in Table 1. Assuming a partial specific volume of .646[1] and neglecting hydration, we obtained a coefficient of 86.9, which deviates considerably from the theoretical value of 2.5 demanded by the Einstein equation for spherical particles. Deviations of this kind are frequently observed in the study of viscosities of colloids, and they are attributed to the influences of the shape and solvation of the particles, among other things. The Kuhn[2] Equation (1) relates the relative volume occupied by the particle, the specific viscosity and the relative dimensions of rigid elongated particles in solution. If we assume the validity of the Kuhn equation and set our value of 86.9 equal to the coefficient $(2.5 + 1/16 \left(\frac{a}{b}\right)^2)$, we obtain the value of 36.8 for the ratio of the long to short axis of the virus particle. Assuming a particle weight of 17,000,000, a density of 1.55, and the effective shape of a prolate spheroid for the virus particle, the semi-minor and semi-major axes of the particles assume the values respectively of 3.4×10^{-7} and 1.98×10^{-5} cm.

TABLE I

SPECIFIC VISCOSITY OF SOLUTIONS OF THE TOBACCO-MOSIAC VIRUS

Protein in phosphate buffer at pH 6.8 (temp. = 25° C.)	
$n/n_o - 1$	Concentration of protein in per cent.
.641	1.0
.385	.631
.282	.5
.325	.465
.098	.20
.055	.10
.025	.063
.012	.042

(1) $n/n_o - 1 = \phi\,(2.5 + 1/16\left(\frac{a}{b}\right))$

$(n/n_o) - 1$ = specific viscosity
(a/b) = ratio of long to short axis of particle
ϕ = fraction of total volume occupied by the particle

In the estimate of the diffusion constant from the viscosity data, use was made of the suggestions by Polson[3]; the essential data being those obtained from viscosity studies, and the particle weight, independently evaluated. We also made use of the Herzog, Illig and Kudar[4] Equation (2), which evaluates the effect the relative dimensions of a free diffusing pro-

(2) $$\frac{D}{D_o} = \tfrac{1}{2}\left[\frac{\left(\frac{b}{a}\right)^{2/3}}{\sqrt{1-\left(\frac{b}{a}\right)^2}}\ \ln\ \frac{1+\sqrt{1-\left(\frac{b}{a}\right)^2}}{1-\sqrt{1-\left(\frac{b}{a}\right)^2}}\right]$$

D = diffusion constant of the particle
D_o = diffusion constant on the assumption of a spherical particle
$\frac{b}{a}$ = ratio of short to long axis of the ellipsoid

[4] A. Claude, *Jour. Exp. Med.*, 61: 41, 1935.
[5] A. Claude and A. Rothen, *Am. Jour. Cancer*, 26: 344, 1936; A. Claude, *Am. Jour. Cancer*, 30: 742, 1937. The study of the ultraviolet absorption power was made in collaboration with Dr. A. Rothen.
[6] T. Caspersson, *Skand. Arch. Physiol.*, 73: supplement No. 8, 1936.
[7] A. Claude, *Jour. Exp. Med.*, 66: 59, 1937; SCIENCE, 85: 294, 1937.
[1] Inga-Britta Erikson-Quensel and The Svedberg, *Jour. Am. Chem. Soc.*, 58: 1863, 1936.
[2] W. Kuhn, *Zeits. Phys. Chem. A*, 161: 1, 1932.
[3] Alfred Polson, *Nature*, 137: 740, 1936.
[4] R. O. Herzog, R. Illig and H. Kudar, *Zeits. Phys. Chem.*, A 167: 329, 1933.

late spheroid will have on its diffusion constant. Substituting our value of 1/36.8 for b/a in Equation 2, and solving for D/D_0 we obtain the ratio of .34. If we assume a spherical particle of weight 17,000,000 and a density of 1.55′, we obtain for the ideal diffusion constant, D_0, the value of 1.33×10^{-7}. The diffusion constant of the virus, then, will have the calculated value of 4.5×10^{-8}.

This value of 4.5×10^{-8} for the diffusion constant of the virus protein is in fair agreement with the value of 3×10^{-8} obtained using a sample of the virus protein in a .1 M phosphate buffer at pH 6.8, which was kindly placed at the disposal of Hans Neurath by Dr. W. M. Stanley.[5] In view of our neglect of the hydration factor in the calculation of the ratio a/b, the close agreement between the observed and calculated diffusion constants does indicate that the protein is relatively hydrophobic.

Inasmuch as the equations used in our calculations were derived on the assumption of rigid and essentially isolated particles, the values we have obtained are at best approximations. It is doubtful that we may regard the particles as isolated, inasmuch as the length of the particles is of the order of the inter-particle distances. In addition, it is not to be presumed that the particles are rigid. And finally, the assumption of a prolate spheroid for the shape of the virus protein particles was for the sake of convenience in calculation.

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THE MOLECULAR WEIGHT AND SHAPE OF TOBACCO MOSAIC VIRUS PROTEIN

SEDIMENTATION studies have been made by Eriksson-Quensel and Svedberg[1] and by Wykoff[2] on the tobacco mosaic virus protein isolated by Stanley.[3] In order to calculate the molecular weight from these studies, it is necessary to know the dissymmetry factor of the protein. This is usually obtained from sedimentation equilibrium measurements, but, because of the extremely high molecular weight of this protein, it was not found possible to obtain satisfactory results by this method. Since the tobacco mosaic virus protein is known to have highly asymmetrical rod-shaped particles in aqueous solution,[4] it seems probable that the molecular weight of 17×10^6, ascribed to the material on the assumption that its dissymmetry factor is 1.3, is too low.

It is possible to obtain an idea of the dissymmetry of rod-shaped particles from studies of the viscosity of solutions or suspensions of these particles. Kuhn[5] has derived the following equation relating viscosity of a suspension or solution of rod-shaped particles to the relative volume and the relative dimensions of the particles of the disperse phase:

$$\frac{\eta}{\eta_0} = 1 + 2.5\,G + \frac{G}{16}\left(\frac{b}{a}\right)^2$$

G is the volume of the dispersed material per cc of solution, $\frac{\eta}{\eta_0}$ is the relative viscosity of the solution, and $\frac{b}{a}$ is the ratio of length to diameter of cylindrical rods of the disperse phase. The specific volume of the protein was taken to be 0.73 cc/gm.[6] Viscosities were determined using a high precision quartz viscometer[7] on very dilute aqueous solutions of tobacco mosaic virus protein isolated by ultracentrifugation repeated 4 and 5 times, without any chemical treatment whatever. The data are given in Table I. The viscosity

TABLE I

RELATIVE VISCOSITIES OF AQUEOUS SOLUTIONS OF TOBACCO MOSAIC VIRUS PROTEIN AT 25° C.

$\frac{\eta}{\eta_0}$	grams protein / 100 cc
1.0059	0.0099
1.0165	0.0296
1.0272	0.0458
1.0278	0.0494
1.0542	0.0920
1.0566	0.0988
1.6009	0.920

is a linear function of concentration up to a concentration of 0.1 per cent., but the linearity does not hold for concentrations as great as 1 per cent. The value of $\frac{b}{a}$ calculated from the limiting slope of the viscosity-concentration curve, using Kuhn's equation and assuming little or no hydration, is 35.0.

Perrin[8] has derived the following expression relating the ratio of minor to major axes of an elongated ellipsoid of revolution to the dissymmetry constant of a particle:

[5] The value of $3. \times 10^{-8}$ is the result of a preliminary study of the virus protein using the refractory method of Lamm (*Z. Phys. Chem.*, A 138: 313, 1928). Detailed studies on diffusion will be presented in a subsequent publication.

[1] I. B. Eriksson-Quensel and T. Svedberg, *Jour. Am. Chem. Soc.*, 58: 1863, 1936.

[2] R. W. G. Wyckoff, *Jour. Biol. Chem.*, 121: 219, 1937.

[3] W. M. Stanley, SCIENCE, 81: 644, 1935; *Ergebn. Physiol.*, 39: 294, 1937.

[4] W. N. Takahashi and T. E. Rawlins, *Proc. Soc. Exp. Biol. and Med.*, 30: 155, 1932; M. A. Lauffer and W. M. Stanley, *Jour. Biol. Chem.*, 123: 507, 1938.

[5] W. Kuhn, *Kolloid Zeit.*, 62: 269, 1933.

[6] F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc.*, B 123: 274, 1937; W. M. Stanley, *Jour. Phys. Chem.*, 42: 55, 1938.

[7] The author wishes to express his gratitude to Drs. D. A. MacInnes and L. G. Longsworth for the use of the quartz viscometer and the facilities of their laboratory.

[8] F. Perrin, *Jour. Phys. et Rad.*, 7: 1, 1936.