SPECIAL ARTICLES

AN ESTIMATE OF THE MAXIMUM VALUE FOR THE MOLECULAR WEIGHT OF THE TOBACCO MOSAIC VIRUS PROTEIN

DATA obtained by ultracentrifugal studies reported by Inga-Britta Erikson-Quensel and The Svedberg[1] and by R. W. G. Wyckoff, J. Biscoe and W. M. Stanley[2] indicate that the molecular weight of the infectious unit of tobacco mosaic virus is of the order of 1.7×10^7. Independent values of the particle weight normally may be obtained from diffusion studies, and H. Neurath and A. M. Saum,[3] using a virus preparation supplied by Stanley, found a diffusion constant of 3×10^{-8}. The implied molecular weight is unreasonably large, although by correcting for the shape of the particle,[4] a value of 41 million was obtained.

Curve B, Fig. 1, was obtained from a diffusion study[5]

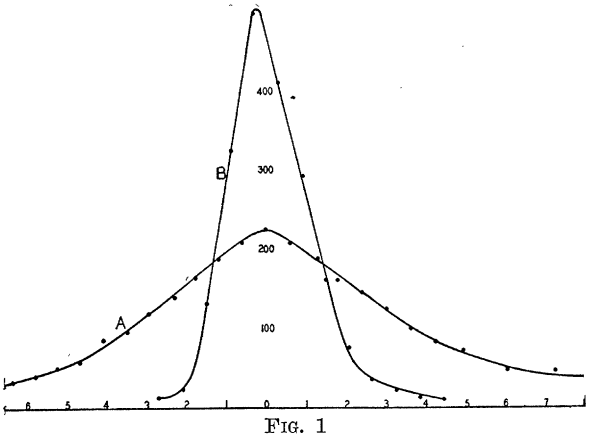

FIG. 1

of a virus preparation obtained by the method used by B. M. Duggar at the University of Wisconsin. It will be noted that the curve is skewed, and it is skewed toward the solvent side. The suggestion is that Fick's law is not followed. On the abscissa are plotted the distances from the boundary in 10^{-1} millimeters, and on the ordinate are plotted the displacements of the scale lines in microns. The time for this particular run was 343,000 seconds. An approximate value for the diffusion constant of 4.5×10^{-9} was obtained from these data following the method suggested by Lamm.[6] As this value is considerably lower than the diffusion constant obtained by Neurath and Saum, one would presume that the difference may be attributed in part to an aggregation of the protein particles induced by the heat treatment called for in the Duggar method. Bawden and Pirie[7] also voice the opinion that the heat treatment induces an aggregation. A diffusion constant of 4.6×10^{-7} was obtained from the data presented in curve A of Fig. 1. These data were obtained from a diffusion study of the same protein preparation, indicated above, dispersed in 6 M. urea + .1 M. phosphate buffer at pH 7. The time for this particular run was 58,080 seconds. The diffusion studies were started after the protein had been in the urea solution for 24 hours.

An increase in diffusion constant from 4.5×10^{-9} to 4.6×10^{-7} is induced by dispersing the virus protein in 6 M. urea. An appreciation of the magnitude of the decrease in particle weight implied in this tremendous increase in diffusion constant is obtained when it is recalled that the relation between the diffusion constant and the radius of a spherical particle given by the Stokes-Einstein equation is

$$kT/6\pi nr = D$$

k = Boltzmann constant
T = Absolute temp.
n = viscosity
r = radius of particle
D = diffusion constant

and that it is assumed that for spherical particles the particle weight varies with the cube of the radius.

A comparison of the biological activity of the virus that had stood in 6 M. urea + .1 M. phosphate buffer and the normal virus in .1 M. phosphate buffer at pH 7 is of particular interest. Data given in Table 1

TABLE 1

Hours in urea	Number of lesions per leaf		
	Concentration of protein in gr/cc		
	3×10^{-5}	3×10^{-6}	3×10^{-7}
0	4.6	1.5	.12
27	5.9	.54	.4
51	7.6	2.2	.12
75	4.1	1.7	.12
96	4.5	2.7	.4
150	1.2		

were obtained by the local lesion method of Holmes.[8] *N. glutinosa* plants were used as test plants; an average of 25 leaves were used for each test. The urea solutions were diluted greatly immediately preceding inoculation.

Inasmuch as the "molar" concentration of the virus in the presence of urea was appreciably greater than in the phosphate buffer alone, it is surprising that there was not a corresponding increase in the number of local lesions produced on the test plants.

Comparative viscosity studies of this same protein

[1] Inga-Britta Erikson-Quensel and The Svedberg, *Jour. Am. Chem. Soc.*, 58: 1863, 1936.

[2] R. W. G. Wyckoff, J. Biscoe and W. M. Stanley, *Jour. Biol. Chem.*, 117: 57, 1937.

[3] In press.

[4] Vernon L. Frampton and H. Neurath, SCIENCE, 87: 468, 1938.

[5] We are greatly indebted to H. Neurath for the privilege of using the Lamm diffusion apparatus he assembled at Cornell University.

[6] O. Lamm, *Zeits. Phys. Chem.*, 143A: 177, 1929.

[7] F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc. London*, 123B: 274, 1937.

[8] F. O. Holmes, *Bot. Gaz.*, 87: 39, 1929.

in .1 M. phosphate buffer at pH 7 (curve A) and in 6.6 M. urea + .1 M. phosphate buffer at pH 7 (curve B) are presented in Fig. 2. The solution yielding data

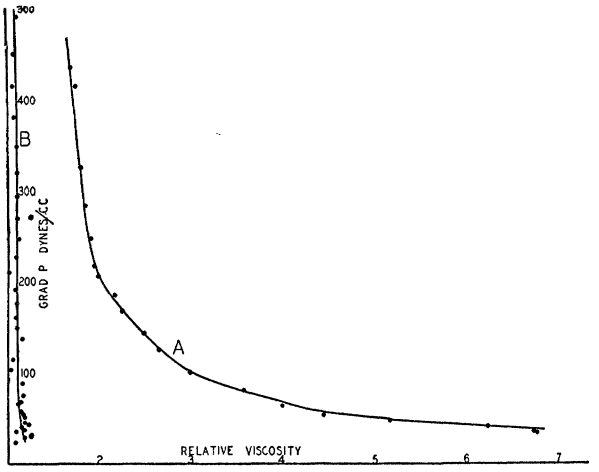

FIG. 2

for curve A contained 4.3 mg protein per cm³, whereas the solution yielding data for curve B contained 6.3 mg protein per cm³. The latter curve was obtained after the virus preparation had stood in the urea for 24 hours. Virus preparations prepared after the method of Stanley[9] show a comparable anomaly when dispersed in either water or in phosphate buffer.

In view of the anomalous viscosity shown by sols of the virus protein in phosphate buffer and the apparent deviation of the diffusion processes from those demanded by Fick's law, it is doubtful that unambiguous values for the particle weight of the chemically prepared virus protein may be obtained by methods involving diffusion in phosphate buffer. A probably less ambiguous value for the particle weight may be obtained from the data for the diffusion in urea solutions. On the basis of the observations presented in this communication, and recalling that a molecule is the smallest particle which can exist as an independent entity, would one not reason that a maximum value for the molecular weight that may be assigned to the virus protein is of the order of 10^5?

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RELATION BETWEEN THE INCREASE IN OPACITY OF YEAST SUSPENSIONS DURING GLUCOSE METABOLISM AND ASSIMILATION[1]

WHEN washed yeast cells are suspended in glucose-phosphate solutions, the opacity of the suspension, as measured by a photoelectric densitometer, increases with time.[2] The rate of change in opacity has been found to vary with the strain of yeast employed, the age of the culture and the experimental conditions. In a typical experiment with Fleischmann's GM strain of bakers' yeast (obtained 2 days after separation at the factory), the optical density (log opacity) changed from 0.170 to 0.417 within 6 hours after the addition of 5 per cent. glucose to cells previously washed and suspended in M/15 KH_2PO_4 (aerobic conditions, 25° C.). The observed change in optical density with time is entirely referable to the suspension and not to the instrument, since variation in the intensity of the light source or in the "constants" characteristic of the phototubes was eliminated by the type of circuit employed and the procedure used in obtaining the measurements.[3] From a consideration of the theory of densitometry as applied to the type of instrument employed,[4] it was apparent that the observed change in optical density resulted from an increase in cell number and from some progressive change in optical characteristics of the cells or from either of these factors operating alone.

We have found no significant increase in cell number under the experimental conditions employed above. This conclusion is based on 11 experiments on GM yeast and 4 experiments on Strain 4360 cultivated in this laboratory. The cell counts were made with a haemacytometer on at least 4 separate samples taken at various intervals during each experiment; 2,000 to 3,000 cells were counted in each sample. In the experiment on GM yeast cited above, the cell counts were as follows: immediately after adding the glucose, 49,640 cells/c.mm.; after 1 hour in glucose, 52,380; after 3 hours, 50,220; after 5 hours, 52,100; and after 6 hours, 52,350. A difference greater than 6 per cent. was considered significant for these suspensions.

Investigations of the changes which the cells of the GM strain undergo with time after suspension in a glucose solution show (a) that the opacity of the individual cells increases with time (evidence from direct microscopic observations and from photomicrographs); (b) that the dry weight per unit number of cells increases progressively with time, very nearly paralleling the increase in optical density of the suspension; (c) that the increase in weight results from the storage within the cells of substances syn-

[9] W. M. Stanley, *Jour. Biol. Chem.*, 115: 673, 1936.

[1] Support from the William F. Milton Fund of Harvard University is gratefully acknowledged.

[2] *Cf.* legend to Fig. 2 in T. J. B. Stier, W. Arnold and J. N. Stannard, *Jour. Gen. Physiol.*, 17: 383–392, 1934, where an increase in opacity under similar experimental conditions was reported.

[3] T. J. B. Stier, W. Arnold and J. N. Stannard, *ibid.*, p. 386.

[4] *Cf.* H. Mestre, *Jour. Bact.*, 30: 335, 1935; and L. G. Longsworth, *Jour. Bact.*, 32: 307–328, 1936.