Equilibrium centrifugation of two strains of tobacco mosaic virus in density gradients

We have examined two strains of tobacco mosaic virus by equilibrium centrifugation in salt density gradients according to the method of MESELSON, STAHL AND VINOGRAD[1].

A volume of 0.65 ml of a salt solution containing 30 μg virus was placed in a 12-mm cell and centrifuged at a speed of 44,700 rev./min for 16 h at 20° in a model E Spinco analytical centrifuge. The density of the salt-virus solution was determined with the aid of a refractometer which had been calibrated with a series of salt solutions of known density. The density gradient in the cell at 16 h was determined by the method of JOHNSON *et al.*[2]. The virus formed a sharp band early in the run. The position of the band within the cell was carefully determined at 16 h. Conditions were so arranged that the band was near the midpoint of the liquid column. The density of the solution at the position of the virus band (effective density) was calculated assuming a linear density gradient and a density at the midpoint of the liquid column equal to the density of the solution before centrifugation. The two strains of virus which were examined, U1 and U2, have been described[3].

Data for the effective densities of the two strains determined in a series of salts are presented in Table I. The two strains differ in effective density by 0.003 g/ml in all of the salts tested. In each salt a mixture of the two strains yielded two distinct bands. Furthermore, it was observed that the apparent density determinations were unaffected by the pH of the salt solutions over the range pH 2 to 8. These facts taken together suggest that the observed difference between the strains represents a true difference in density and is not the result of a differential interaction of the two strains with the salt solutions.

TABLE I

THE EFFECTIVE DENSITIES OF TWO STRAINS OF TOBACCO MOSAIC VIRUS DETERMINED IN SEVERAL SALT SOLUTIONS

The salts were used without further purification and were obtained from the following sources: NaBr, J. T. Baker Chemical Co., C. P. grade; KBr, Baker and Adamson, Reagent Grade; CsCl, Fisher Scientific Co., purified; RbCl and RbBr, K and K Chemical Lab., Long Island City, N.Y.

Salt	*Virus strain*	
	U1	*U2*
KBr	1.306*	1.303
NaBr	1.316	1.313
RbBr	1.307	1.304
RbCl	1.325	1.322
CsCl	1.325	1.322

* The standard error is 0.001 or less.

The values obtained with the different salts do not fall into an obvious pattern. It may be that the disparate values result from possibly erroneous assumptions as to the linearity of the density gradient and the position in the gradient where the density is equal to the starting density of the solution.

During the course of the experiments, it was noted that 3 *M* CsCl had no effect either on the infectivity or the ultraviolet-light sensitivity of the two strains.

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The microestimation of succinate and the extinction coefficient of cytochrome *c*

Phenazine methosulphate has been shown to be a very good electron acceptor in the oxidation of succinate by succinic dehydrogenase[1], whereas cytochrome *c* is an extremely poor acceptor in this system. However, cytochrome *c* is reduced rapidly in a non-enzymic reaction with reduced phenazine methosulphate[2]. Hence the oxidation of succinate by succinic dehydrogenase can be coupled to the reduction of cytochrome *c* in the presence of trace amounts of phenazine methosulphate to give an extremely sensitive method for the estimation of succinate.

$$\text{succinate} + \text{phenazine methosulphate} \xleftarrow[\text{dehydrogenase}]{\text{succinic}} \text{fumarate} + \text{reduced phenazine methosulphate} \qquad (1)$$

$$\text{reduced phenazine methosulphate} + 2\ \text{cytochrome } c \longrightarrow \text{phenazine methosulphate} + 2\ \text{reduced cytochrome } c \qquad (2)$$

The optimal conditions for this estimation have not been explored fully, but the following conditions have been found satisfactory. Spectrophotometer cells are filled with the following reagents to a total volume of 2.95 ml; phosphate, pH 7.6, 150 μmoles; cytochrome *c*, 0.125 μmole; succinate, 0–0.05 μmole; succinic dehydrogenase prepared according to the method of SINGER *et al.*[1], 0.2 mg. The cells are incubated at 25° and the reaction begun by the addition of 0.05 ml of 0.01 % phenazine methosulphate (freshly diluted from a 1 % stock solution).

A blank without any succinate but containing all the other reagents must be run, since a red colour develops on the incubation of phenazine methosulphate with most proteins, due to a complex with sulphydryl groups[2]. The absorbance at 550 mμ is recorded until the difference between sample and blank becomes constant. Fig. 1 shows the results of a number of estimations carried out in this way. It will be noted that the amount of cytochrome *c* reduced is the same in the presence or absence of oxygen. As reduced phenazine methosulphate is very auto-oxidizable (in fact its reaction with O_2 is the basis of the usual assay for succinic dehydrogenase[1]) it is evident that the rate of reaction of reduced phenazine methosulphate with cytochrome *c* must be very much greater than that of its re-oxidation with O_2. As cyto-