the surface of the tissue would liberate carbon dioxide from the carbonate-incrusted leaves, also apparently increasing the R.Q. According to Gregory and Sen,[3] the rate of liberation of carbon dioxide may indicate the rate at which the protein cycle, involving deamination and protein synthesis, is taking place.

Chemical storage of oxygen is obviously of advantage to the plant, since it is not only subjected to widely varying degrees of oxygen tension in its aquatic environment, but photosynthetic activity in the winter months is apt to be curtailed. In this connection it was interesting to note the change in gaseous metabolism as the rainy season (1940–41) drew to a close. From January to mid-March the oxygen consumption rose about threefold, the carbon dioxide production neither rising nor falling conspicuously. This change was presumably conditioned by an increase in illumination, causing an increase in photosynthetic activity, in turn causing storage and subsequent breakdown of carbohydrates, and in this manner involving a shift to aerobic oxidation. It is conceivable that the oxygen consumption may also have been affected by the cessation of dilution of the pond water by rain and commencement of concentration of ions by evaporation loss. Further pursuit of these questions should throw more light on the physiology and ecology of such forms.

R. R. RONKIN
S. C. BROOKS

UNIVERSITY OF CALIFORNIA

ON THE SIZE AND SHAPE OF THE TOBACCO MOSAIC VIRUS PROTEIN PARTICLE

THE proposition that the tobacco mosaic virus protein is molecularly dispersed in aqueous solution has been staunchly defended by Stanley and his coworkers. In response to a paper by Bawden and Pirie,[1] who originally demonstrated that this virus protein has a marked tendency to aggregate, Loring, Lauffer and Stanley[2] announced that the virus protein "has a molecular weight of at least 46 million, a length of at least 430 millimicrons, and an effective diameter of about 12 millimicrons," and that aggregation, if any does occur in the virus protein solutions, is of little significance. Essentially this same point of view has been maintained consistently by Stanley and his coworkers, although in more recent papers, in particular those relating to the research with the electron microscope,[3,4] they speak of a "marked tendency of the particles to aggregate." These workers have avoided consideration of the various anomalies shown by solutions of this virus protein and they consider the size and shape of the protein particles arrived at from ultracentrifuge and viscosity studies as valid and accurate. The electron microscope photographs are cited as substantiating evidence, and the conclusion is reached from a consideration of these photographs that the length of the protein molecule is 280 millimicrons. This conclusion is reached notwithstanding the fact that there are many particles shown in the electron microscope photographs that are considerably shorter than 280 millimicrons.

One might be expected to suppose that all molecules of a given species would be of the same size. In the case of a protein preparation having a heterogeneous size distribution one would suspect either that the preparation is impure or that association or aggregation has occurred, in which case the size of the various particles would be simple multiples of some basic unit. Fortunately in the case of the electron microscope photographs of this virus protein, the sizes of the various particles may be measured, albeit the accuracy is not of a high order. It is impossible, for example, to be sure that all the particles are lying flat, and measurements made from a half-tone reproduction are not particularly satisfactory. Although the errors in the estimations of the lengths of the virus protein particles from these and similar photographs are large, a length distribution curve for 159 particles occurring in the photographs published by Stanley and Anderson[3] and Anderson and Stanley[4] is presented in Fig. 1.

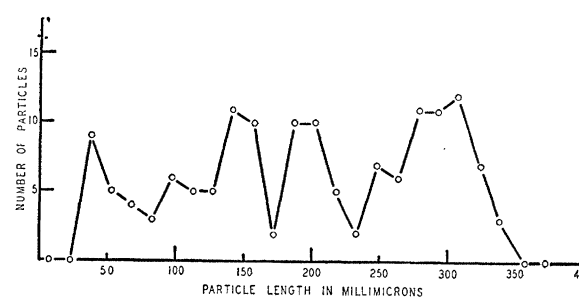

FIG. 1. Length distribution curve for particles of tobacco mosaic virus protein. Data taken from electron microscope photographs.

The class length along the abscissa is 15 millimicrons. Note that a bunching occurs at lengths in the regions of 300, 190, 150, 100 and 37 millimicrons. These lengths are essentially in the ratio of 8 : 5 : 4 : 3 : 1.

The implications of the orderliness indicated above are obvious.

An added item of interest is the observation by Melcher, Schramm, Trurnit and Friedrich-Freksa[5] that the electron microscope photographs of their

[3] F. G. Gregory and P. K. Sen, *Ann. Bot.*, n.s. 1: 521, 1937.
[1] *Proc. Roy. Soc.*, B 123: 274, 1937.
[2] *Nature*, 142: 841, 1938.
[3] *Jour. Biol. Chem.*, 139: 325, 1941.
[4] *Jour. Biol. Chem.*, 139: 339, 1941.
[5] *Biol. Zentr.*, 60: 524, 1940.

preparations showed particles whose lengths were predominantly in the neighborhood of 140 and 190 millimicrons. In keeping with their decision that the length of the virus protein molecule is 280 millimicrons, Stanley and Anderson[3] concluded that Melcher and coworkers must have been dealing with some different strains of the virus. These same particle lengths occur in the electron microscope photographs published by Stanley and Anderson[3] and by Anderson and Stanley.[4]

A further consideration of equal interest is that the size of the suggested basic unit from which the other particles seem to be built is in good agreement with the "diameter" of some 38 millimicrons arrived at by Thornberry[6] from ultrafiltration experiments. One might wonder just what the electron microscope photographs of these ultrafiltrates would show.

VERNON L. FRAMPTON
DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

CHROMATOGRAPHIC SEPARATION OF MIXTURES OF AMINO ACIDS

THE recent report by Tiselius[1] of the adsorption analysis of certain mixtures of amino acids by a modification of the Tswett chromatographic method has led us to publish at this time results of our own work.

We have been able to separate mixtures of *l*-tyrosine and *dl*-leucine, and of *dl*-phenylalanine and *dl*-leucine, in a quantitative, or nearly quantitative manner. Other separations, such as that of *l*-tyrosine from *dl*-phenylalanine or of glycine from *dl*-alanine, have been obtained but are not yet quantitative. A representative experiment is given below. We hope shortly to publish our findings in full.

Materials. A variety of *adsorbents* have been investigated. The experiment described below employed a commercial carbon, Darco G–60.[2] *l*-Tyrosine, partially racemized, analyzed 7.69 and 7.75 per cent. nitrogen and gave no nitroprusside test for cystine. *dl*-Leucine analyzed 10.71 and 10.74 per cent. nitrogen.

Experiment. Two grams of Darco G–60 carbon were mixed with filter paper pulp (a convenient, non-adsorbing bulking agent) and packed in a tube 2.2 cm in diameter to give a column of adsorbent 12 cm long. To this was applied a solution containing 0.5006 g *dl*-leucine and 0.1008 g *l*-tyrosine in 100 cc of water. The column was developed with water in the usual manner and without pressure. The liquid which passed through the column was collected in fractions and analyzed for amino acid. All the leucine was obtained in the first 600 cc of liquid which passed through the column (0.5016 g in 15 fractions), and in no fraction was there any evidence of tyrosine (Folin-Marenzi test). At this point all the tyrosine remained on the column and the percolating liquid gave a negative ninhydrin test and a negative Folin-Marenzi test.

The strongly adsorbed tyrosine could be desorbed by elution with aqueous ethyl acetate: 550 cc of 5 per cent. aqueous ethyl acetate removed about 90 per cent. of the tyrosine (by colorimetric estimation, checked gravimetrically).

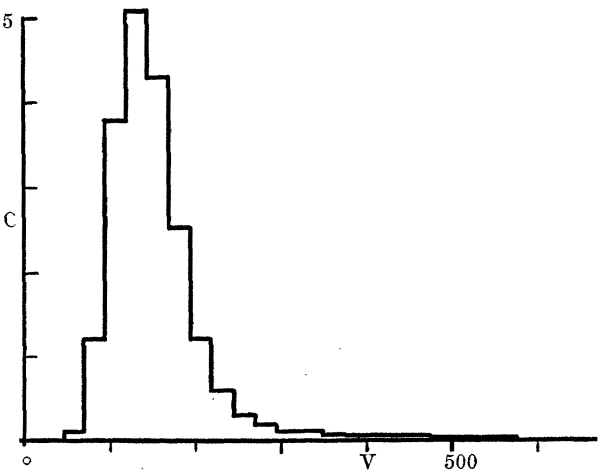

FIG. 1
"Zone" of *dl*-leucine.
V Volume of percolate, in cc.
C Concentration of fractions shown, in mg. per cc.

The concentrations of leucine in the fractions of percolate are plotted (Fig. 1) against the volume of percolate to show how the "zone" passed completely from the column. This method of plotting is useful in dealing with the separation of colorless materials.[3]

This problem is being developed further in this laboratory.

At this time we would like to express our thanks to Dr. Hubert B. Vickery and Dr. Werner Bergmann for many kindnesses, and to the International Silk Guild for funds in support of this work.

JACQUES WACHTEL
HAROLD G. CASSIDY
YALE UNIVERSITY

[1] A. Tiselius, SCIENCE, 94: 145, 1941.
[2] The Darco Corporation, 60 East 42nd Street, New York, N. Y.
[3] H. G. Cassidy, Dissertation, Yale University, 1939; E. Caliri, Thèse, University of Fribourg, 1939; H. G. Cassidy, *Jour. Am. Chem. Soc.*, in press.
[6] *Phytopathology*, 25: 601, 1935.