[22] Rownd, R., J. Marmur, and P. Doty (unpublished results).
[23] Avery, O. T., C. M. MacLeod, and M. McCarty, *J. Exptl. Med.*, **79**, 137 (1944).
[24] Jacob, F., and E. L. Wollman, in *Symposia of the Society for Experimental Biology* (London: Cambridge University Press, 1958), vol. 12, p. 75.
[25] Lavalle, R., and F. Jacob, *C.R. Acad. Sci. (Paris)*, **252**, 1678 (1961).
[26] Schildkraut, C., R. Rownd, and P. Doty (unpublished results).
[27] Sueoka, N., *J. Molec. Biol.*, **3**, 31 (1961).
[28] Schildkraut, C., J. Marmur, and P. Doty (in preparation).
[29] Schildkraut, C., R. Rownd, J. Marmur, D. M. Green, and P. Doty (unpublished results).

*BIOPHYSICAL STUDIES OF BROAD BEAN MOTTLE VIRUS**

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Viruses are useful test objects for studying structural and functional relationships between proteins and nucleic acids. For these purposes it is desirable, at least for the present, to deal with viruses that are as small as possible with the expectation that small size and structural simplicity will go hand in hand. The members of the group of so-called *spherical viruses* perhaps approach this desideratum most closely. Among the most extensively studied are the plant viruses turnip yellow mosaic virus,[1] tomato bushy stunt virus,[2] wild cucumber mosaic virus,[3] southern bean mosaic virus,[4] and squash mosaic virus,[5] the bacterial virus ϕX174,[6] and polio virus.[7] All have molecular weights in the range 5–10 $\times$ 10^6 and nucleic acid content in the range 1.5–2.5 $\times$ 10^6 (in molecular weight units). Recently it has been shown that the molecular weight of bromegrass mosaic virus[8] is even lower —4.6 $\times$ 10^6—and that its content of nucleic acid is only 1 $\times$ 10^6.

The data to follow show that broad bean mottle virus (BBMV) is also quite low in molecular weight—5.2 $\times$ 10^6—and in nucleic acid content—1.1 $\times$ 10^6. This virus could become a useful complement to bromegrass mosaic virus since the two differ markedly in both amino acid and nucleotide composition. Indeed, because its yield from infected tissues is exceedingly high, broad bean mottle virus could be the more favorable subject for structural studies.

Broad bean mottle virus was first isolated and purified by Bawden, Chaudhuri, and Kassanis[9] who investigated some of its biological properties. Serological and some chemical properties have been studied by Wetter, Paul, Brandes, and Quantz.[10] Wittmann and Paul[11] determined its amino acid composition.

Methods.—Isolation and purification procedures: The virus was obtained from a stock culture kept by F. C. Bawden.[12] It was inoculated onto horse bean (*Vicia faba* L. var. broad windsor) and was isolated from infected leaves 3 weeks later.

Infected leaves, frozen at −25°C., were homogenized in a Waring blendor in cold potassium phosphate buffer (0.01 *M* in phosphate, pH 7). In some isolations as much as 0.01 *M* ascorbic acid was added to this solution in order to prevent oxidation of cellular constituents. An equal volume of a 1:1 n-butanol-chloroform mixture was added to the homogenate and the resulting mixture was shaken gently for 15 min. The emulsion was broken by a low speed centrifugation. The aqueous layer was removed and was immediately centrifuged in a No. 30 rotor in a Spinco Model L preparative centrifuge at 15,000 rpm for 15 min. The supernatant was then centrifuged at

28,000 rpm for 150 min. The pellets were resuspended in phosphate buffer. The resulting solution was clarified by low speed centrifugation and then centrifuged in the No. 40 rotor at 38,000 rpm for 90 min. After another similar cycle of differential centrifugation the colorless pellets were resuspended in phosphate buffer at 4° (0.1 *M* in phosphate, pH 7). After a final low speed centrifugation, the material was used immediately or frozen at −25°. The yield is exceedingly high—150 mg per 100 gm tissue.

It will be shown in the next section that the above procedure results in a single macromolecular component. Analyses of zonal centrifugation of the preparations in sucrose density gradients in the manner described by Bockstahler and Kaesberg[8] indicated that infectivity and this component sedimented at the same rate.

Since the virus has some physical similarities to bromegrass mosaic virus, reciprocal serological tests were carried out.[13] The results showed that there was no antigenic relationship.

Analytical procedures: Analytical sedimentation studies were made in the 12 mm cell of a Spinco Model E analytical ultracentrifuge. All runs were made at approximately 20°. The sedimentation coefficients were corrected to conditions obtaining in water at 20°. The buffer was 0.10 ionic strength, pH 7.0 (0.0067 *M* NaH_2PO_4, 0.0133 *M* Na_2HPO_4, 0.0534 *M* NaCl).

Electrophoretic studies were made in a Spinco model H electrophoresis-diffusion apparatus. Sodium acetate and phosphate buffers were used at ionic strengths 0.10.

Diffusion measurements were made in the same apparatus. A sharp boundary was created between the solvent and the virus solution in a standard Tiselius cell and the change in concentration of the solute as a function of distance and of time was followed by means of the Rayleigh interference optical system. The diffusion was allowed to proceed at 2° for a period of 3 days. The buffer was the same as that used for sedimentation experiments. Apparent diffusion coefficients were calculated from the fringe data according to the method of Longworth.[14] The diffusion coefficient, corresponding to infinite time, was obtained from a plot of the apparent diffusion coefficients against the reciprocal of time. This value was corrected to conditions in water at 20° under the assumption that the diffusion rate was linearly dependent upon viscosity and absolute temperature.

The partial specific volume of the virus was obtained from measurements of concentration and of densities of the solution and solvent. Densities of the virus solutions were measured by pycnometry at 25°. The concentration of the solution was obtained by the difference in dry weights of known volumes of solvent and solution.

Ultraviolet absorption measurements were obtained with a Cary Model 11 spectrophotometer, in the same buffer as that used for the sedimentation experiments. The absorption was corrected for the optical density due to light scattering.

For the determination of nucleotide composition, the lyophilized virus, without prior removal of protein, was digested in 0.5 *N* potassium hydroxide at 37° for 20 hours with shaking. The separation of nucleotides from the protein and the chromatographic separation of the four nucleotides were made according to the procedure of Osawa and associates.[15] Chromatography was carried out on Dowex 1 (×2), 200–400 mesh. Continuous elution was used with the formic acid system originally developed by Hurlbert and associates.[16] The phosphorus content of the nucleic acid was calculated from the nucleotide composition.

Phosphorus content of the virus was determined in triplicate by the method of Allen[17] on 3 separately purified samples. The nucleic acid content of the virus was calculated from the phosphorus content in the virus and in the nucleic acid. The nucleic acid content was also determined by means of the optical method of Englander and Epstein.[18] For this procedure the absorbancy index of the nucleic acid *as it exists in the virus* was calculated from the nucleotide composition, the absorbancy indices of the constituent nucleotides,[19] and an assumed hypochromicity of 1.50 for the nucleic acid.[20] The absorbancy index for protein was taken to be $^1/_{40}$th that of RNA. The nucleoprotein concentration was determined refractometrically. The specific refractive increment was taken to be 18×10^{-5} for a 1 mg per ml solution.

Results.—Sedimentation: A single sharp peak is seen in the analytical ultracentrifuge as shown in Figure 1. The sedimentation coefficients have a concentration dependence (Fig. 2) approximately expressed by

$$s_{20,w} = (84.8 - 0.47c)S$$

where c is concentration in mg per ml.

Electrophoresis: When the virus is examined electrophoretically at pH 7.0, only one peak is resolved (Fig. 3) even in runs lasting more than 5 hours. At this pH the virus is negatively charged and the mobility is -6.9×10^{-5} cm^2 per volt per second. Unpublished studies by J. S. Semancik and J. B. Bancroft (personal communication) show that the virus migrates as a single peak over the entire pH range 3.0–8.0.

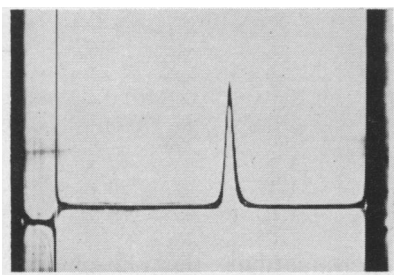

FIG. 1.—Sedimentation pattern of broad bean mottle virus at a concentration of 3.55 mg/ml. Photographed 14 min after the rotor reached speed of 35,600 rpm. Direction of sedimentation is to the right.

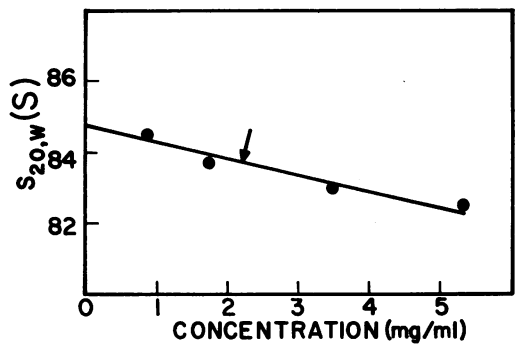

FIG. 2.—Sedimentation coefficients plotted against cencentration of the virus. The arrow shows the concentration at which the diffusion experiment was made.

Diffusion: The diffusion coefficient at 2° in the buffer used is 0.766×10^{-7} cm^2 per second. The diffusion coefficient, $D_{20,w}$, corresponding to a temperature of 20° in a solvent with the viscosity of water is 1.380×10^{-7} cm^2 per second.

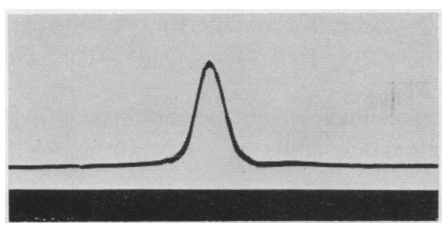

FIG. 3.—Electrophoretic pattern (descending pattern) of broad bean mottle virus in 0.1 ionic strength phosphate buffer (pH 7.0) after 322 min at 1.93 volt/cm.

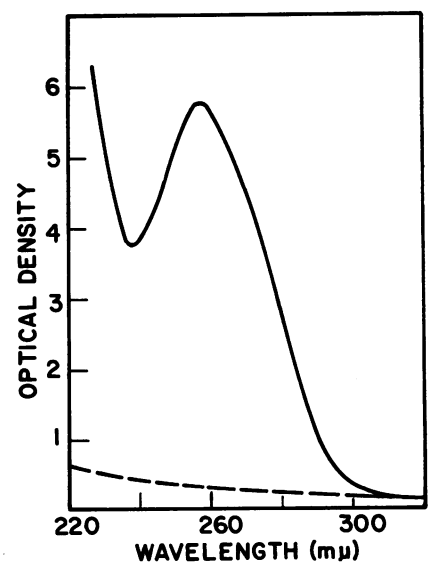

FIG. 4.—Optical density plotted against wavelength for broad bean mottle virus at a concentration of 1 mg/ml.

Partial specific volume: The partial specific volume of the virus is 0.717 ml per gm.

Ultraviolet absorption: Figure 4 shows the ultraviolet absorption curve for a 1 mg per ml virus solution at pH 7.0. The ratio of the absorbancy at 260 mμ to that at 280 mμ, after correction for light scattering, is 2.1. The curve has a maximum at 258 mμ and a minimum at 238 mμ. The absorbancy index at 260 mμ is 5.40 cm^2 per mg.

Nucleotide composition: The molar nucleotide residue composition of the RNA of the virus is given in Table 1. From the nucleotide composition the phosphorus content of the RNA is 9.62 per cent.

TABLE 1

THE MOLAR NUCLEOTIDE RESIDUE COMPOSITION IN THE RNA OF BROAD BEAN MOTTLE VIRUS

Preparation	Cytidylic acid	Adenylic acid	Guanylic acid	Uridylic acid
I	19.9	27.2	25.1	27.8
II	18.9	27.4	24.1	29.6
Average	19.4	27.3	24.6	28.7

Nucleic acid content: From the phosphorus determinations, the phosphorus content of the virus is 2.12 ± 0.10 per cent. Since the nucleic acid contains 9.62 per cent phosphorus, the virus contains 22.0 ± 1.0 per cent RNA.

The absorbancy index of the RNA, calculated from the nucleotide composition and an assumed hypochromicity, is 23.4 cm^2 per mg. The absorbancy index for the virus was found (above) to be 5.40 cm^2 per mg. Thus from Englander and Epstein's equation[18] the virus contains 21.1 ± 1.0 per cent RNA.

Molecular weight of the virus: Molecular weight is calculable from Svedberg's equation

$$M = \frac{sRT}{D(1 - \bar{v}\rho)}$$

where s, R, T, D, $\bar{v}$, and ρ are sedimentation coefficient, gas constant, absolute temperature, diffusion coefficient, and partial specific volume, and density of the solvent respectively. The diffusion coefficient $D_{20,w}$ is 1.38×10^{-7} cm^2 per sec at a concentration of 2.22 mg per ml. The sedimentation coefficient $s_{20,w}$ at this concentration is 83.8×10^{-13} second (see Fig. 2). The partial specific volume, $\bar{v}$, is 0.717 ml per gm. The solvent density is, of course, that of water at 20°. Thus the molecular weight of the virus is 5.20×10^6.

Amount of RNA in the virus: From the molecular weight of the virus and the per cent nucleic acid in the virus, each virus particle contains 1.1×10^6 molecular weight units of RNA.

Discussion.—The small amount of nucleic acid contained in viruses such as bromegrass mosaic virus and broad bean mottle virus is of special significance. Ultimately, the complete structure of nucleic acids (or at least their base sequences) will be required for the interpretation of genetic data in chemical terms. For a given virus, the amount of nucleic acid it contains is, presumably, a measure of the magnitude of this task. By way of comparison, tobacco mosaic virus, which is being studied intensively in several laboratories, contains about 6,000 nucleotide residues per particle, bromegrass mosaic virus contains about 3,000, and broad bean mottle virus contains about 3,400.

The sizes of the protein subunits are not known for bromegrass mosaic and broad bean mottle virus, although preliminary experiments in both cases suggest that they will consist of roughly 190 amino acid residues. Thus the coding ratio would be about 17.

Summary.—Broad bean mottle virus has been obtained as an electrophoretically and ultracentrifugally homogeneous preparation. The molecular weight of the virus was found to be 5.20×10^6, based on a sedimentation coefficient of $(84.8-0.47c)S$ and a diffusion coefficient of 1.38×10^{-7} cm^2 per second and a partial specific volume of 0.717 ml per gm. The virus contains about 1.1×10^6 molecular weight units of ribonucleic acid.

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[1] Markham, R., *Discussions Faraday Soc.*, **11**, 221 (1951).
[2] Williams, R. C., and R. C. Backus, *J. Am. Chem. Soc.*, **71**, 4052 (1949).
[3] Yamazaki, H., and Paul Kaesberg, *Biochim. et Biophys. Acta* (in press).
[4] Miller, G. L., and W. C. Price, *Arch. Biochem.*, **10**, 467 (1946).
[5] Mazzone, H. M., N. L. Incardona, and Paul Kaesberg, *Biochim. et Biophys. Acta* (in press).
[6] Sinsheimer, R. L., *J. Molec. Biol.*, **1**, 37 (1959).
[7] Schwerdt, C. E., *Special pub. N. Y. Acad. Sci.*, **5**, 157 (1957).
[8] Bockstahler, L. E., and Paul Kaesberg, *Nature*, **190**, 192 (1961).
[9] Bawden, F. C., R. P. Chaudhuri, and B. Kassanis, *Ann. Appl. Biol.*, **38**, 774 (1951).
[10] Wetter, C., H. L. Paul, J. Brandes, and L. Quantz, *Z. Naturforschg.*, **15b**, 444 (1960).
[11] Wittmann, H. G., and H. L. Paul, *Phytopathol. Z.*, **41**, 74 (1961).
[12] We are indebted to F. C. Bawden for a gift of the virus inoculum.
[13] We are indebted to E. Moorhead Ball for a gift of the bromegrass mosaic virus antiserum.
[14] Longsworth, L. G., *J. Am. Chem. Soc.*, **74**, 4155 (1952).
[15] Osawa, S., K. Takata, and Y. Hotta, *Biochim. et Biophys. Acta*, **28**, 271 (1958).
[16] Hurlbert, R. B., H. Schmitz, A. F. Brumm, and V. R. Potter, *J. Biol. Chem.*, **209**, 23 (1944).
[17] Allen, R. J. L., *Biochem. J.* (London), **34**, 858 (1940).
[18] Englander, S. W., and H. T. Epstein, *Arch. Biochem. Biophys.*, **68**, 144 (1957).
[19] Beaven, G. H., E. R. Holiday, and E. A. Johnson, in *The Nucleic Acids*, ed. E. Chargaff, and J. N. Davidson (New York: Academic Press, Inc., 1955), vol. 1.
[20] Schlessinger, D., *J. Molec. Biol.*, **2**, 92 (1960).

AN EFFECT OF L-LEUCINE AND OTHER ESSENTIAL AMINO ACIDS ON THE STRUCTURE AND ACTIVITY OF GLUTAMIC DEHYDROGENASE

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Glutamic dehydrogenase (GDH) catalyzes the interconversion of α-ketoglutarate (α-KG), a Krebs cycle component, and L-glutamate. With the crystalline protein from beef liver, it has been shown that the activity of the enzyme, which is composed of subunits, depends on its state of aggregation.[1] Our recent finding that several steroid hormones promote the reversible dissociation of the enzyme into subunits[2] suggested that control of the physical state of this protein, and perhaps of others, may be a means by which enzymic reactions are regulated. This idea