gently for five minutes. The solution was allowed to cool somewhat and 2 cc. of concentrated hydrochloric acid was added. The mixture was evaporated to dryness *in vacuo* at 60–70°. The solid was extracted twenty times with 5-cc. portions of ether and the ether solution was added gradually to an ethereal solution of diazomethane (prepared from 4.5 g. of nitrosomethylurea). The solution was allowed to evaporate while standing overnight at room temperature. The residual oil was treated with 2 cc. of concentrated ammonia and warmed gently. An additional 2 cc. of ammonia was added and the solution was allowed to remain at room temperature overnight. The reaction mixture was evaporated to dryness *in vacuo* at room temperature and dried over phosphorus pentoxide for forty-eight hours; yield 400 mg., 42% over-all yield, based on the cyanide used.

Malononitrile.[8]—The cyanoacetamide was placed in a sublimation apparatus together with 400 mg. of phosphorus pentachloride. The system was evacuated by means of a water pump and the apparatus was immersed in a boiling water-bath. After the reaction had subsided, chloroform (cooled to −5° by means of a chloroform–Dry Ice-bath) was circulated through the cold finger. With the aid of an oil-bath the reaction mixture was heated to 160° and maintained there until the sublimation was complete (ten minutes).

Phenylazomalononitrile.—The malononitrile was allowed to melt from the cold finger into a 50-cc. glass-stoppered erlenmeyer and rinsed with a few cc. of methanol. Five cc. of 1 *M* phenyldiazonium acetate solution was added and the flask kept in an ice-bath overnight. The product was collected by filtration, washed with cold water and dried in the air; yield, 200 mg., 20% based on the cyanoacetamide used.

Adenine Sulfate.—The phenylazomalononitrile thus prepared was utilized in the preparation of adenine according to the directions described above. The final yield of adenine sulfate was 75[13] mg., which represents an over-all yield of 3.8% based upon the cyanide used. The product possessed an activity of approximately 7000 counts/min./milligram when a film of 1.93 micrograms/cm.^{2} was prepared by evaporation of an aqueous solution on an aluminum planchet and counted with a thin window (1.9 mg./cm.^{2}) Geiger–Müller counter.

Acknowledgment.—The authors wish to acknowledge the advice of Dr. George Bosworth Brown, the coöperation of Dr. Harold Beyer in the isotope determinations, and the assistance of Alice Angelos, Rosco Funk, Jr., and Thelma Kaplan.

Summary

Various syntheses of adenine have been investigated and a satisfactory procedure for the introduction of isotopes of nitrogen and carbon has been developed.

The preparation from isotopic carbon of cyanoacetamide and malononitrile is described.

(13) Of this amount, 32 mg. was obtained from the filtrate by washout dilution with 450 mg. of non-radioactive adenine.

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[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY, THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, NEW JERSEY, AND THE DEPARTMENT OF PHYSICS,[1] UNIVERSITY OF PITTSBURGH, PITTSBURGH, PENNSYLVANIA]

The Hydration, Size and Shape of Tobacco Mosaic Virus[2a,2b]

BY H. K. SCHACHMAN[3] AND MAX A. LAUFFER

Introduction

On the basis of indirect evidence, several investigators have speculated about the degree of hydration of tobacco mosaic virus,[4a,b,c] but there has been no direct experimental attack on this problem. The ultracentrifuge furnishes a possible means for the direct determination of the density of the virus in solution by a study of the effect of varying solvent density on the change of sedimentation rate.[5a,b] The combination of this solution density with the apparent partial specific volume permits an evaluation of the hydration of the virus.

A certain amount of ambiguity arises in a precise evaluation of the size and shape of any molecule because of the difficulty in determining the relative contributions of hydration and anisometry to the physical chemical properties of the material in question. In the case of tobacco mosaic virus, the application of several physical technics, including ultracentrifugation, X-ray diffraction, viscosity, birefringence of flow and diffusion, has led to the evaluations of size and shape which were largely substantiated by direct observation in the electron microscope.[6] These computations, however, were predicated upon the assumption of little or no hydration of the virus particles, an assumption made because X-ray measurements could be interpreted to mean that the particles did not swell when placed in solution.[7]

In view of the apparent success of the centrifugation method of determining the hydration of viruses, it seemed worthwhile to determine the hydration of tobacco mosaic virus and then to re-examine the other physical chemical properties in the light of this result.

(1) Contribution No. 11p-47 of the Department of Physics, University of Pittsburgh.

(2) (a) Paper delivered before the division of Biological Chemistry at the 111th meeting of the American Chemical Society, April, 1947; (b) aided in part by a grant from the National Foundation for Infantile Paralysis.

(3) Junior Research Fellow of the National Institute of Health; present address: University of California, Berkeley California.

(4) (a) R. Markham, K. M. Smith and D. Lea, *Parasitology*, **34**, 315 (1942); (b) F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc. (London)*, **B123**, 274 (1937); (c) N. W. Pirie, *Advances in Enzymol.*, **5**, 1 (1945).

(5) (a) J. E. Smadel, E. G. Pickels and T. Shedlovsky, *J. Exp. Med.*, **68**, 607 (1938); (b) D. G. Sharp, A. R. Taylor, I. W. McLean, Jr., D. Beard and J. W. Beard, *Science*, **100**, 151 (1944).

(6) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).

(7) J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.*, **25**, 111 (1941).

Materials and Methods

Tobacco mosaic virus freshly isolated by differential centrifugation, according to the method of Stanley,[8] was examined in the ultracentrifuge at a concentration of 3.0 mg. per cc. in solvents of varying density. Different concentrations of crystalline bovine serum albumin in *p*H 4.7, 0.2 *M* acetate buffer, were used as solvents. Some of the bovine serum albumin was obtained through the courtesy of Dr. Hans Neurath of Duke University Medical School, and some of it was purchased directly from Armour and Company, Chicago, Illinois. Experiments on two other samples of tobacco mosaic virus, isolated in similar fashion, were conducted at *p*H 7.0 in 0.01 *M* phosphate buffer containing sucrose in varying concentrations. One of these samples, A, was also examined in several concentrations of bovine serum albumin at *p*H 7.0 in 0.01 *M* phosphate buffer. The studies were carried out in an air driven ultracentrifuge of the Bauer–Pickels type, equipped with the Svensson optical system. Specific gravities of the solutions were obtained by the use of a 2-cc. pyknometer, and viscosities were determined in an Ostwald type viscometer.

Experimental Results

Figure 1 shows the relation of sedimentation rate to the density of the suspending medium for the studies on tobacco mosaic virus suspended in serum albumin solutions of various concentrations.

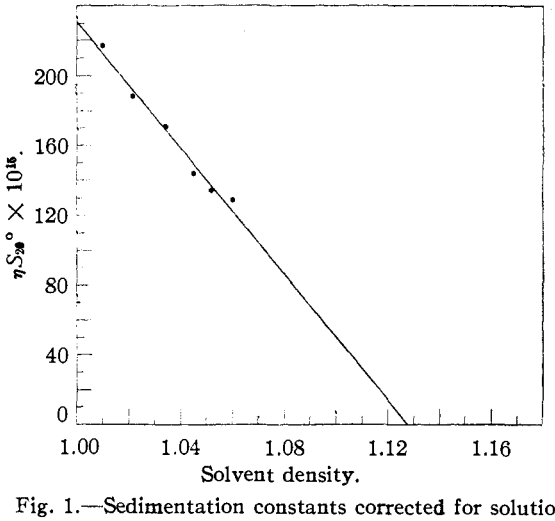

Fig. 1.—Sedimentation constants corrected for solution viscosity of tobacco mosaic virus in serum albumin solutions plotted as a function of solution density. The *p*H was maintained at 4.7 by means of 0.2 *M* acetate buffer. Straight line was fitted by method of least squares.

In Fig. 2 are plotted the results for tobacco mosaic virus in a medium containing sucrose at different concentrations. The ordinate in these graphs is the sedimentation constant corrected for the viscosity to correspond to a value in a solvent with the viscosity of water at 20°. The abscissa is the density of the suspending medium at 20°.

The rate at which particles suspended in a strictly homogeneous medium sediment in a centrifugal field should be directly proportional to the difference between the densities of the particles and of the medium. If the medium causes no alteration in size, shape and density of the particles,

(8) W. M. Stanley, THIS JOURNAL, **64**, 1804 (1942).

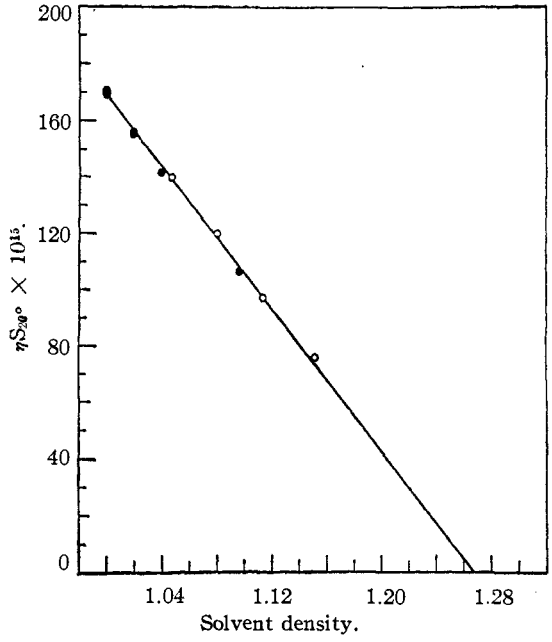

Fig. 2.—Sedimentation constants corrected for solution viscosity of tobacco mosaic virus in sucrose solutions plotted as a function of solution density. The *p*H was maintained at 7.0 with 0.01 *M* phosphate buffer. Straight line was fitted by method of least squares.

then the decrease in sedimentation constant, S, multiplied by viscosity, η, is directly proportional to the increase in density of the solution, ρ_s. In general, it can be shown that eqn. 1

$$\eta S = \frac{M(\rho - \rho_s)}{6\pi N \left(\frac{3VM}{4\pi N}\right)^{1/3} \left(\frac{f}{f_0}\right) \rho} \qquad (1)$$

where M is the anhydrous molecular weight of the sedimenting particles, V is the partial specific volume of the material, ρ is the density of the particles, N is the Avogadro number, and (f/f_0) is the friction ratio, which is a function of the anisometry and hydration of the particles, describes the relationship. Equation 1 can be used to evaluate the hydrated molecular weight, if V and ρ are the specific volume and density of the hydrated particles. In this case the friction ratio is only a function of anisometry. If the sedimenting particles are unaltered by the medium, a plot of ηS *vs.* ρ_s according to eqn. 1 should yield a straight line. The slope of the line is a function of size, shape and the density of the particles, and the intercept at $\eta S = 0$ is dependent only on the density of the particles.

That the curve in Fig. 1 shows this linear dependence of ηS on ρ_s suggests that the medium has little or no effect on the size, shape, and density of the particles. It cannot be said with complete certainty, however, that the physical characteristics of the virus were not altered by the serum albumin. A complex precipitates at *p*H values between about 4 and 5 when the ionic strength is

considerably lower than in the present experiments. Also the virus is precipitated by serum albumin at *p*H values above 5 when the ionic strength is about 0.1.[9]

Similarly, the linearity of the curve in figure 2 suggests that the medium has not altered the virus. Smadel, Pickels and Shedlovsky[4] obtained a non-linear dependence of sedimentation rate on the density of the medium when they studied the sedimentation of the elementary bodies of vaccinia in sucrose solutions. They suggested as one explanation of their results that the sucrose solutions caused an osmotic withdrawal of water from the particles with a consequent increase in particle density. Studies[10,11] on the influenza viruses showed similar behavior. It was this concept of osmotic influence of the medium on the virus particles that prompted Sharp, *et al.*,[5] to use serum albumin rather than sucrose as a means of obtaining solvents of different densities. Because of the higher molecular weight of serum albumin as compared to sucrose, it was expected that such an osmotic withdrawal of water, if real, would be considerably reduced. The resultant straight lines in plots of ηS *vs.* ρ_s for the sedimentation of the influenza viruses in serum albumin solutions[5,11] lend support to the suggestion of Smadel, Pickels and Shedlovsky.

Extrapolation of the curve in Fig. 1 yields a value of $\rho_s = 1.13$ g./cc. for the density of solution in which virus particles do not sediment. If no alteration of the virus has occurred, the density of the virus in aqueous solution can be considered as equal to 1.13 g./cc. Similarly, extrapolation of the results presented in Fig. 2 indicates that the density of the virus in aqueous solution is about 1.27 g./cc. Preparation A, which also appeared to have a density of 1.27 g./cc. in solutions containing sucrose, was studied in serum albumin solutions of low ionic strength at *p*H 7.0, and a value about 1.1 was obtained by extrapolation. This result is in reasonable agreement with that obtained at *p*H 4.7 and presented in Fig. 1.

For the case of non-linear dependence of corrected sedimentation constant on density, Smadel, Pickels and Shedlovsky suggested the construction of a tangent to the curve at the point corresponding to the density of dilute buffer. The value of ρ_s at which this tangent intercepts the abscissa can then be taken as the density of the virus particles in a solvent devoid of sucrose.[12] Sharp, *et al.*, demonstrated a discrepancy between the two methods for influenza virus and concluded that the lower value obtained in serum albumin was more reliable.

The data of Fig. 1 show a value of about 230 S^{14} for the sedimentation constant of tobacco mosaic virus in the absence of serum albumin. In a previous study[6] a value of 185S was found for the sedimentation constant of tobacco mosaic virus in the unaggregated state, and it was shown that boundaries corresponding to higher sedimentation constants could be attributed to dimers and trimers. The high value obtained for the tobacco mosaic virus indicates that the virus particles are in a polymerized state. Inasmuch as the aggregation is predominantly end-to-end, the hydrodynamic density of the polymerized virus probably would not differ from that of the virus in the monomeric state. The data of Fig. 2 indicate that the virus studied at *p*H 7.0 is unaggregated.

If no contraction occurs on the combination of a strictly homogeneous medium and the dry virus, the degree of hydration can be calculated from the partial specific volume of the virus and the density obtained from the sedimentation experiments. Inherent in these calculations is the assumption that the bound water is not unlike the body of water acting as solvent. If a volume, v, of anhydrous virus with density ρ becomes associated with a volume Δv of water with density ρ_w to form a hydrated particle of density ρ_s, the total volume of the hydrated virus particle will be $(v + \Delta v)$ and its mass will be $(v\rho + \Delta v\rho_w)$. The data of sedimentation rate in serum albumin solutions lead to a hydration value of about 1.9 ml. of water per ml. of dry virus or 66% by volume on a wet basis. Similar calculations for the sedimentation in sucrose solutions lead to the value of about 0.37 ml. of water per ml. of dry virus or 27% water by volume on a wet basis.

Discussion

The Hydration of Tobacco Mosaic Virus.—The vastly different hydrodynamic densities obtained in the sucrose and in the serum albumin solutions indicate a marked difference in the apparent hydration of tobacco mosaic virus in the two media. The following considerations, which were proposed in a private communication by Kauzmann,[15] show that this difference can possibly be attributed to a region in the medium surrounding a virus particle which, for purely geo-

(9) M. A. Lauffer, *Arch. Biochem.*, **13**, 145 (1947); *J. Biol. Chem.*, **174**, 481 (1948).

(10) M. A. Lauffer and W. M. Stanley, *J. Exp. Med.*, **80**, 531 (1944).

(11) D. G. Sharp, A. R. Taylor, I. W. McLean, Jr., D. Beard and J. W. Beard, *J. Biol. Chem.*, **159**, 29 (1945).

(12) In the studies in serum albumin solutions a correction was made for the sedimentation of the serum albumin. This correction was made according to the suggestion of Sharp, Taylor and Beard[13] by subtracting the sedimentation constant of serum albumin from the measured sedimentation constant of the virus before multiplying by the viscosity. This correction seems reasonable at high concentrations of serum albumin where the virus is sedimenting very slowly, but such a correction does not seem valid when the virus is sedimenting in a very dilute serum albumin solution. If the correction were not made, the points at high serum albumin concentrations would no longer fall on a straight line, and a tangent to the curve at the density of the buffer would be necessary. In that case, a result not significantly different from the 1.13 g./cc. obtained in Fig. 1 would still be obtained.

(13) D. G. Sharp, A. R. Taylor and J. W. Beard, *J. Biol. Chem.*, **163**, 289 (1946).

(14) The symbol S represents the Svedberg unit defined as 10^{-13} cm. per sec. per cg. unit of centrifugal field.

(15) The authors wish to acknowledge their indebtedness to Dr. W. Kauzmann of Princeton University and to express their appreciation to him for his contributions to this problem

metrical reasons, cannot be penetrated by serum albumin or sucrose molecules. In other words, the assumption that sucrose and serum albumin solutions are homogeneous media may not be appropriate for the present purpose. In that event, computation based upon the assumption of a homogeneous medium would not be valid.

If serum albumin molecules are considered to be rigid, uncharged spheres of radius a, it is clear that the center of a serum albumin molecule will not be able to come closer than a distance a from the surface of a virus particle suspended in a serum albumin solution. This will lead to a region of thickness $2a$ in which the density changes gradually from that of water to that of the serum albumin solution. It can be shown by a statistical procedure that this is equivalent to having each virus particle surrounded by a layer of thickness a of liquid with the density of the medium exclusive of serum albumin. In the case of a virus particle suspended in a sucrose solution, a similar but much thinner equivalent layer of sucrose-free fluid will surround the virus particle. As will be seen in the subsequent more quantitative treatment, this equivalent layer of fluid affects the force operating on a virus particle in a centrifugal field.

If v is defined as the volume of a virus particle, Δv as the volume of a shell of thickness a corresponding to the equivalent layer of albumin-free medium as described above, a as the radius of a serum albumin molecule, ρ as the average density of the virus particle including any water of hydration really part of the virus particle, ρ_s as the density of the serum albumin solution, ρ_w as the density of the medium exclusive of serum albumin, W as the mass of a volume V of serum albumin solution, and W' as the mass of the same volume of solution containing a single virus particle, the following considerations apply. The effective force acting on the virus particle in the centrifuge should be proportional to $(W' - W)$, and, when this difference vanishes, the particle should not sediment. For purposes of these considerations, the excess mass of a serum albumin molecule over that of an equal volume of water can be considered to be concentrated at a point in the center of the molecule. Thus, the shell of volume, Δv, which can contain no centers of serum albumin molecules, must have density ρ_w. On the other hand, the density of the solution outside Δv is ρ_s. Therefore

$$W = V\rho_s \quad W' = v\rho + \Delta v\rho_w + (V - v - \Delta v)\rho_s$$

The particle will not sediment when $W' - W$ is equal to zero, that is, when the effective weight of the virus particle is $W' - W$ or $v\rho + \Delta v\rho_w - (v + \Delta v)\rho_s$. This equation is identical with that used for the calculation of the hydration per unit volume of dry virus. The apparent hydration in this case, however, is entirely a property of the medium in which the virus is suspended.

Physical studies carried out on serum albumin[16] indicate that the molecule is not spherical but is a rod about 150 Å. long and 38 Å. in diameter.[16] This can be approximated by a cylinder capped by hemispheres, with a diameter, $2a$, of 38 Å. and a total length, $2b$, of 150 Å. Obviously, a virus particle will be surrounded by a layer of medium of thickness, a, in which no serum albumin molecule centers can exist and by an additional layer of thickness, $(b - a)$, in which centers of only serum albumin particles oriented in certain ways can be found. It can be shown by a statistical procedure that this amounts to the equivalent of a layer of medium with a thickness of $\frac{1}{2}(a + b)$ from which serum albumin is excluded entirely. These calculations apply only in those cases in which the radius of curvature of the virus particle is substantially greater than the thickness of the surrounding serum albumin-free medium. Calculations carried out by the method of Schultz[17] show that the thickness of this layer surrounding a cylinder with the diameter of a tobacco mosaic virus particle should still be approximately $\frac{1}{2}(a + b)$. Thus, a tobacco mosaic virus particle should be surrounded by the equivalent of a zone of albumin-free medium 47 Å. thick.

Similar effects should also be expected to occur in sucrose solutions. The sucrose molecule is equivalent in size to a sphere of about 4 Å. in radius, but undoubtedly the molecule is somewhat asymmetric, so that perhaps the effective radius is 5 or 6 Å. Thus, in a sucrose solution a tobacco mosaic virus particle should be surrounded by the equivalent of a layer of sucrose-free medium 5 or 6 Å. in thickness because of the inability of sucrose particles to get any closer to the surface.

The apparent water associated with the virus particle in serum albumin solution and in sucrose solution is, of course, a property dependent primarily upon the medium in which the virus finds itself and is not an intrinsic property of the virus particle itself. If there is any intrinsic water of hydration, regardless of the means by which that water is attached to the virus particle, it must be the total water indicated by the sedimentation experiments in sucrose or in serum albumin solution minus that which is to be attributed to the inability of the sucrose or the serum albumin particle centers to approach the virus surface. The apparent hydration of tobacco mosaic virus in serum albumin solution is the equivalent of a layer of water 53 Å. thick surrounding a rod-like particle with an anhydrous diameter of 152 Å. Since a layer approximately 47 Å. thick must be attributed to the inability of the serum albumin molecule centers to touch the surface of a tobacco mosaic virus particle, this leaves only about a 6 Å. shell attributable to the intrinsic hydration of the tobacco mosaic virus particle. Similarly, the results obtained when tobacco mosaic virus was sedimented in a sucrose solution indicate that the virus particle has an apparent hydration equiva-

(16) E. J. Cohn, J. L. Oncley, L. E. Strong and S. Armstrong, Jr., *J. Clin. Invest.*, **23**, 417 (1944).

(17) G. V. Schultz, *Z. Naturforsch.*, **2**, 348 (1947).

lent to a shell 13 Å. thick surrounding the virus rod. However, when the 6 Å. figure corresponding to the probably effective radius of a sucrose molecule is subtracted, a value of only 7 Å. remains as an estimate of the thickness of the layer corresponding to the intrinsic hydration of a tobacco mosaic virus particle. It is apparent that the same order of magnitude is obtained for the intrinsic hydration from the two series of experiments when interpreted in this manner. Thus, the best estimate of the intrinsic hydration of tobacco mosaic virus is about 15% by volume on a wet basis.

X-Ray diffraction measurements have been of value in determining the ultimate virus particle size and the role of associated water. The investigation of tobacco mosaic virus by X-ray scattering has followed two paths. One, the intramolecular structure resulting from large angle scattering and corresponding to small spacings, demonstrated that certain intraparticle spacings were not altered by a change in concentration of the virus from a dry gel to moderately concentrated solutions. Because of the constancy of the internal spacings over a broad concentration region, Bernal and Fankuchen[7] suggested that the particles do not swell appreciably when placed in aqueous solution.

The second problem attacked by Bernal and Fankuchen was a study of the intermolecular pattern as exhibited by small angle scattering. They found that the distance between the particles is inversely proportional to the square root of the concentration by volume. This requires that the particles separate appreciably only in a lateral direction. Despite the large separation distances involved, of the order of 500 Å., there was a regular lattice arrangement in the form of an hexagonal pattern. Thus, the virus particles can exist in solutions and in gels in a regular pattern where the particles are separated by water layers several times the diameter of the particles. The degree of association of the water cannot be determined from X-ray measurements. Centrifuge studies in serum albumin solutions and in sucrose solutions, when interpreted in the manner described above, indicate that the hydrodynamic unit contains an intrinsic hydration equivalent to a shell of water 6 or 7 Å. thick. If one accepts the interpretation of Bernal and Fankuchen of the meaning of the wide angle scattering, most of this water would actually be found in a shell surrounding the virus particle. It is not difficult to conceive of a water layer of such thickness being held to the outside of a virus particle.

Hydrodynamic Complications.—The existence of this shell of diluted albumin solution about the sedimenting virus particles causes complications in connection with the viscosity correction to the sedimentation constant. Evidently the virus moves in an environment of somewhat lower viscosity than that of the bulk solution. Therefore it is not correct to multiply the observed sedimentation constant by the over-all relative viscosity of the solution in order to obtain the equivalent sedimentation constant in water. The difference between the over-all viscosity and this effective viscosity is probably considerable, especially in concentrated albumin solutions, but it does not seem to be easy to compute.[18] This consideration contributes an element of uncertainty to the extrapolated value of the solution density corresponding to zero sedimentation rate, and thus casts some doubt upon the reliability of the extent of hydration indicated above.

Finally, the question might be raised whether such a layer of low density will be able to maintain itself as the virus particle moves in the centrifuge. Thus it might seem reasonable to expect that the virus particle would act as a plow in moving through the albumin solution, with albumin molecules piling up in front of the virus and being swept clear at the back. In the centrifuge, a tobacco mosaic virus particle moves at the rate of about $^1/_5 \times 10^{-3}$ cm./sec. Serum albumin has a diffusion constant of about 6×10^{-7} sq. cm./sec. This corresponds to a root mean squared displacement of serum albumin molecules, due to kinetic energy, of $\sqrt{12 \times 10^{-7}}$ or 1.1×10^{-3} cm. in one second. Thus, in the usual experiment, most of the serum albumin molecules should have time to move out of the path and into the wake of an advancing virus particle.

The Size and Shape of Tobacco Mosaic Virus.—The study of macromolecules has invoked such a varied number of physical tools that it has become possible to validate partially certain of them by their combined use on one substance. Tobacco mosaic virus, because of its characteristic shape and large size, serves as an excellent model for study. It was shown that the predictions of the size and shape of the virus particles from viscosity, sedimentation and diffusion data were in substantial agreement with the results obtained from electron microscopy and X-ray diffraction.[6] The generally accepted value, 15.2 mμ, for the diameter of the rod-like particle resulted from small angle scattering of X-rays. Studies in the electron microscope on purified and unpurified tobacco mosaic virus lend support to the concept of a rod-like particle 280 mμ in length by 15.2 mμ in diameter. On the basis of a partial specific volume of 0.73 cc./g., the molecular weight of the anhydrous particle is 41.9×10^6.

Detailed accounts of the available methods for determining molecular size and shape have been presented elsewhere,[6,19,20] but these are complicated, as mentioned above, by the difficulty in evaluating the relative contributions of hydration and anisometry to the friction ratio. If the value 0.15 ml. of water per ml. of wet virus is used in

(18) J. M. Burgers, *Proc. Acad. Sci., Amsterdam*, **45**, 10 (1942).
(19) M. A. Lauffer and W. M. Stanley, *Chem. Revs.*, **24**, 303 (1939).
(20) J. L. Oncley, *Ann. N. Y. Acad. Sci.*, **41**, 121 (1941).

conjunction with the intrinsic viscosity,[21] (η), 39 ml./ml.,[6] an axial ratio of 18.3 results for the hydrated particle. This value of the axial ratio can be used in the Perrin equation to yield a friction ratio due to asymmetry of 1.92. Allowing for the fact that a hydrated particle has a frictional resistance greater than that of an anhydrated particle produces a value, 2.01, for the over-all friction ratio. Combination of this (f/f_0) value with the sedimentation constant, 185 S, leads to 34×10^6 for the anhydrous molecular weight. This corresponds to a rod about 270 by 14 mμ.[22]

Diffusion and sedimentation data can also be used to predict the size of the anhydrous particles. The calculation of molecular weight by this method is independent of hydration and the value, 31×10^6, previously reported still obtains. This corresponds to a rod-like particle 250 by 14 mμ for the case in which 15% by volume of water on a wet basis are associated with the virus.

The size and shape of the particle can be calculated, also, by a combination of viscosity and diffusion data. Results obtained in this manner correspond to a rod-shaped particle 250 by 13 mμ and a molecular weight of 27×10^6 when 15% of volume hydration is assumed.

(21) Intrinsic viscosity is defined as the ratio, as the volume fraction approaches zero, of specific viscosity to volume fraction.

(22) This calculation was made on the assumption that hydration increases the thickness but not the length of a virus particle.

Summary

The hydrodynamic density of tobacco mosaic virus was determined by centrifugation in bovine serum albumin and sucrose solutions of various densities. In both sets of experiments, straight lines could be used to express the dependence of sedimentation rate upon density of the medium. A hydrodynamic density of 1.13 was obtained for the experiments carried out in serum albumin solutions, and a value of 1.27 was obtained for experiments carried out in sucrose solutions. It was shown that this great discrepancy can be attributed to the effect upon the buoyancy of a virus particle produced by a disturbance of the homogeneous distribution of solute molecules in the immediate neighborhood of a virus particle. This effect depends upon the radius of the solute molecule and is therefore greater for serum albumin than for sucrose. When this effect is taken into account, the data can be interpreted to indicate that tobacco mosaic virus has an intrinsic hydration of approximately 15% by volume on a wet basis. The size and shape of the virus particle were calculated by several methods on the basis of this new hydrated model. Excellent agreement was obtained when the calculations from viscosity and sedimentation data were compared with direct measurements obtained by electron microscopy and X-ray diffraction. Calculations involving the diffusion constant gave less satisfactory agreement.

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[CONTRIBUTION FROM DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

The Separation of the Antibodies, Isoagglutinins, Prothrombin, Plasminogen and β_1-Lipoprotein into Subfractions of Human Plasma[1a,b]

BY J. L. ONCLEY, M. MELIN, D. A. RICHERT,[1c] J. W. CAMERON[1d] AND P. M. GROSS, JR.[1e]

The separation of the protein and lipoprotein components of human plasma into a series of fractions by the use of a five-variable system, as well as the principles involved in this fractionation, have been described in a previous paper.[2] The procedure developed depends upon the use of organic precipitants (ethanol in this case), low temperatures (0 to $-8°$), low ionic strengths of electrolytes (below 0.16 mole per liter), and accurate control of the pH and protein concentration. In this work the albumins were concentrated in Fraction V, fibrinogen in Fraction I, most of the α-globulins in Fraction IV-1 and IV-4. Fraction II + III contained isoagglutinins, prothrombin, plasminogen, certain lipoproteins with properties ascribed to the X-protein of plasma, as well as antibodies.[3]

The aim of this study was to devise methods

(1a) This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. It was aided early in 1941 by grants from the Committee on Medicine of the National Research Council, which included a grant from the American College of Physicians. From August, 1941, to July, 1946, it was carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. Since then it has been aided by a grant recommended by the Panel on Hematology of the National Institute of Health.

(1b) This paper is Number 73 in the series "Studies on the Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross, and Number XIX in the series "Preparation and Properties of Serum and Plasma Proteins" from the same laboratory.

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(2) E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, THIS JOURNAL, **68**, 459 (1946).

(3) The importance of these methods for the concentration of antibodies was pointed out in an earlier paper of this series: E. J. Cohn, J. A. Luetscher, Jr., J. L. Oncley, S. H. Armstrong, Jr., and B. D. Davis, *ibid.*, **62**, 3396 (1940).