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Received February 15th, 1957

DUAL SEDIMENTATION OF T2 BACTERIOPHAGE OF *ESCHERICHIA COLI**, **, ***

IRWIN J. BENDET, LOUIS G. SWABY AND MAX A. LAUFFER

Department of Biophysics, University of Pittsburgh, Pa. (U.S.A.)

INTRODUCTION

Two papers in 1946, concerned with the purification and characterization of the T2 bacteriophage of *Escherichia coli*, introduced a puzzling sedimentation phenomenon. In the first, HOOK *et al.*[1] indicated that the sedimentation coefficient characteristic of this virus particle was dependent upon the suspending medium. A sedimentation rate of approximately 700 Svedberg units was obtained in 0.9% NaCl, while a value of about 1000 *S* resulted upon centrifuging the virus in 0.023*M* $CaCl_2$. In addition, the two rates were found to be completely reversible. The second publication[2] indicated that this dual sedimentation phenomenon also was correlated with the hydrogen ion concentration of the medium; below pH 5.8 the faster rate was demonstrated, while above this pH the slower rate was observed. LESLEY *et al.*[3] reported the higher sedimentation value (1040 *S*) at the somewhat higher pH of 6.05 but made no mention of measurements at other hydrogen ion concentrations. Two values (about 1000 *S* and 700 *S*) also were reported by SINGER AND SIEGEL[4] for T2 bacteriophage in cacodylate buffer, pH 6.9. In 1955 the dual sedimentation of this particular bacterial virus was studied by TAYLOR and co-workers[5] in the authors' laboratory.

* These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Pittsburgh, NR 135-110, Contract No. Nonr-624(03).

** Presented before the Division of Biological Chemistry at the *130th Meeting Am. Chem. Soc. Atlantic City, N.J., September, 1956.*

*** Contribution No. 47 from the Department of Biophysics, University of Pittsburgh.

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The serologically related bacteriophage, T6, possessing similar morphology (*cf.*[6]), also has manifested dual sedimentation; PUTNAM *et al.*[7] reported values of 790 and 1030 Svedbergs, and, as might have been anticipated, these results agreed very well with those found for the T2 bacteriophage. Continued investigation[8,9] substantiated these initial reports with but only very slight modification.

The bacteriophages T2 and T6 have been the only bacterial viruses reported thus far, to the authors' knowledge, to exhibit this dual sedimentation characteristic. T7 bacteriophage, of the same series but differing both serologically and morphologically from the even-numbered members, was shown by KERBY *et al.*[10] to possess an essentially constant sedimentation rate (470 *S*) through the pH range 5 to 9. This has been confirmed[11]. PUTNAM has indicated[12] that only a single sedimentation value (about 550 *S*) has been found for T5 bacteriophage. Measurement in this laboratory of the sedimentation rate of the bacteriophage T3 as a function of hydrogen ion concentration between pH 5.5 and 8.9 has yielded a sedimentation coefficient, corrected for viscosity of the solution, of 463 S[13]. Also, it has been determined that the bacteriophage M-4, having as its host *Bacillus megaterium*, possesses a uniform sedimentation rate (about 315 *S*, uncorrected) over a pH range of 5.1 to 8.8[14].

Many hypotheses have been offered to explain the difference in sedimentation rates. Aggregation was first suggested[1,2] as a means of producing the slower component. A study of the sedimentation of lucite models, which oriented themselves during sedimentation in salt solution, supported this hypothesis. Then again, aggregation also has been proposed[9,15] for producing the faster sedimentation rate. At the same time the pertinency of orientation of the particle during sedimentation has been discussed[9]. For relatively small and symmetrical protein molecules it seems likely that the kinetic energy of the system would keep the particles in Brownian motion even under an applied centrifugal field. However, for a relatively large virus particle, highly asymmetric, and probably geometrically inhomogeneous with respect to density[16], perhaps orientation during sedimentation does exist. PUTNAM[9] tested for evidence of this possibility by ascertaining the sedimentation rate of the particle as a function of applied centrifugal force. His data, for T6 bacteriophage, indicate a constant sedimentation rate in the range from 715 to 18,900 *g*. These results do not preclude the possibility, however, that orientation already has taken place at the lowest speed investigated. Additional attention will be directed toward this aspect of the problem later in this paper.

The possibility that the virus particle may be hydrated to different extents at different pH values has been investigated and discussed[5,17], but precluded as a plausible explanation.

In an attempt to resolve the question of aggregation, TAYLOR, EPSTEIN AND LAUFFER[5] approached the problem by consideration of the molecular weights, calculated from diffusion and sedimentation data, for the two different components. By appropriate substitution of their values for S^{w}_{20} = 1066 and 751 Svedbergs, and D^{w}_{20} = 3.46·10^{-8} cm^2/sec and 2.96·10^{-8} cm^2/sec, in pH 5 and pH 7 buffers, respectively, and a partial specific volume of 0.66[18], in the Svedberg equation,

$$M = \frac{RTs}{D(1 - \bar{V}\varrho)} \qquad (1)$$

where R is the gas constant, T the absolute temperature, and ϱ the density of the

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solution, they obtained molecular weights of $220 \cdot 10^6$ and $181 \cdot 10^6$ atomic weight units. These values indicate approximately a 20% difference in molecular weight, whereas any simple association–dissociation of equal units ought to yield values in the ratio of 1:2.

EXPERIMENTAL

Diffusion

Since experimental error could offer a simple explanation for the 20% discrepancy in molecular weight, and since diffusion measurements are generally less reliable than sedimentation rates, additional diffusion experiments were performed.

The Spinco electrophoresis-diffusion apparatus (Model H) was used for these experiments, all performed at a virus concentration of 0.8 mg/ml in 0.1 ionic strength phosphate buffer. Two diffusion runs at pH 5, and three at pH 7, yielded arithmetic mean values of $3.25 \cdot 10^{-8}$ cm^2/sec and $2.63 \cdot 10^{-8}$ cm^2/sec, respectively, as calculated by the maximum ordinate-area method. These figures, when combined with the sedimentation values indicated above[5], indicate molecular weights of $234 \cdot 10^6$ and $204 \cdot 10^6$, approximately a 14% difference. These diffusion rates, while not identical with those previously reported, nevertheless verify the small calculated difference in molecular weight, and, because of the smallness of the difference, continue to support the assumption that the dual sedimentation phenomenon is not due to one-to-one aggregation.

Sedimentation

The first ultracentrifuge experiment consisted of an attempt to define more strictly the pH range over which the transition in sedimentation rate occurs. In all, nine sedimentation runs were made covering a range of pH between 5.55 and 6.60. All determinations were performed at a virus concentration of 0.2%, in phosphate buffer of 0.1 ionic strength, within the temperature range of 27° C–30° C. Uncorrected sedimentation coefficients and pH values are reported in Table I.

TABLE I

SEDIMENTATION RATES OF T2 BACTERIOPHAGE AT VARIOUS pH VALUES

pH	*Sedimentation rate (in Svedbergs)*
5.55	1062
5.80	1112
6.00	1109
6.02	991, 757
6.10	1124, 1000
6.15	1021, 829
6.30	943
6.55	719
6.60	855

At pH values less than 5.80 and more than 6.55 single peaks were observed. At pH values of 6.00 and 6.30, single peaks also were observed, but these were skewed to the fast and slow sides, respectively. At pH values of 6.02, 6.10, and 6.15 two peaks were revealed. These measurements indicate that the transition limits for the

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sedimentation rate change encompass approximately one-half of a pH unit, with a central value at about pH 6.1.

Other experiments were performed in an attempt to determine the length of time associated with the sedimentation rate transition. The technique involved sedimenting the virus from a medium of one hydrogen ion concentration into a second of greater density and different hydrogen ion concentration. A synthetic boundary cell[19] enabled layering the virus preparation above the solution whose density had been increased with either D_2O or sucrose. Experiments were performed in which the virus sedimented from pH 7 to pH 5, and others were performed in which the virus sedimented from pH 5 to pH 7. Fig. 1 is representative of the results observed.

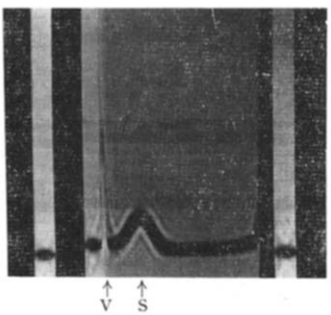

Fig. 1. T2 bacteriophage in phosphate buffer at pH 6.75 sedimenting into buffer of pH 4.8. V = virus peak; S = sucrose boundary.

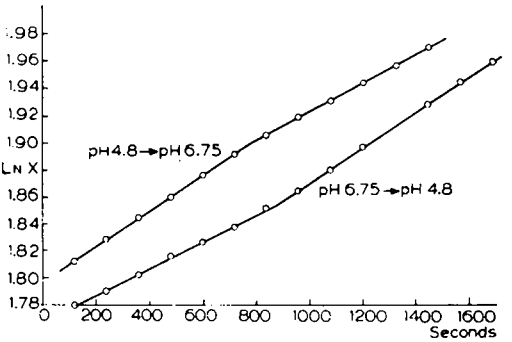

Fig. 2. T2 bacteriophage manifesting an altered sedimentation rate upon passing into buffer of different pH.

In this particular run T2 bacteriophage at a concentration of 1.7 mg/ml, in phosphate buffer at pH 6.75, ionic strength 0.1, was layered over the same ionic strength phosphate buffer having a pH of 4.8. The density of the bottom solution was raised to 1.0076 with sucrose; the virus solution density was 1.0059. In the exposure indicated, sedimentation is proceeding from left to right, with the tall virus peak just approaching the more diffuse sucrose boundary. Substitution of D_2O for sucrose, to raise the density of the bottom solution, yields similar results except that the D_2O boundary is manifested by a negative peak, indicating a reversed refractive index gradient. In either case, plotting the distance of the virus peak from the axis of rotation as a function of time reveals that the sedimentation rate change is quite sharp and complete within a few minutes after passing through the interface and into the medium with the new pH value. Fig. 2 indicates the results of two such experiments. The top graph represents the virus sedimenting from a solution at pH 4.8 into one of pH 6.75, the density of the latter solution having been increased with D_2O. The bottom graph shows sedimentation proceeding, under reversed pH conditions, where the bottom solution has had sucrose added to raise its density. Both centrifugation runs were performed at 10,410 r.p.m. in the Spinco analytical ultracentrifuge (Model E) so that the calculated sedimentation rates demonstrate a change from 1098 to 879 Svedberg units for the top graph, and a transition from 817 to 1100 Svedbergs for the bottom one. Also, the change in slope for both curves occurs, as nearly as can be determined, precisely at the interface of the two solutions, as indicated by the D_2O or sucrose peak.

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Electron microscopy

Fig. 3 is an electron micrograph of air-dried T2 bacteriophage. The virus in 0.01 μ phosphate buffer, pH 7, was diluted ten-fold with distilled water prior to spraying. This picture reveals filaments associated with the tails of many of the bacteriophage, which appear to be about one-fifth of the diameter of the tail, and of approximately the tail's length. In observations on a limited number of pH 5 preparations, no similar filaments were observed.

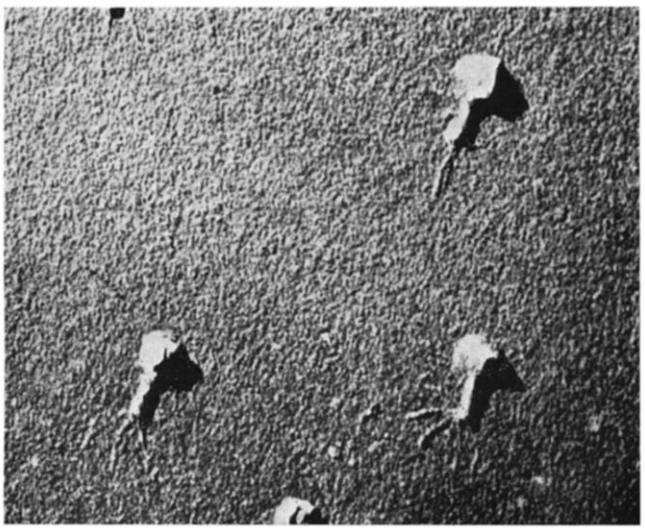

Fig. 3. T2 bacteriophage showing filaments associated with the tails of the characteristic particles. Magnification is approximately 60,000 ×.

WILLIAMS AND FRASER[20], by exposing T2 bacteriophage to cycles of alternate freezing and thawing, have exhibited similar "fibers", estimated at 6 mμ in diameter. And, as the aforementioned authors indicate, sometimes although not commonly, these filaments or fibers have been in evidence in previous pictures of air-dried bacteriophage [*cf.*[6,21]]. Moreover, KELLENBERGER AND ARBER[22] have shown that oxidation of the bacteriophage T2 with H_2O_2-alcohol disrupts the distal portion of the tail and produces "Fäden", or filaments. These authors have demonstrated the same effect with osmium tetroxide, as has one of us[21] with the T4 bacteriophage.

DISCUSSION

The facts established in this study and in the previous one by TAYLOR, EPSTEIN AND LAUFFER[5] eliminate the possibility of one-to-one aggregation and speak against changes in hydration as the cause of the dual-sedimentation phenomenon exhibited by T2 bacteriophage. The only remaining possibility is change in shape. There remains the task of developing a detailed theory.

For this purpose, it is convenient to think in terms of frictional coefficients. The Einstein-Sutherland equation can be used to determine the coefficient of friction possessed by a particle during free diffusion, by evaluating the relationship, RT/ND,

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where R is the gas constant; T is the absolute temperature; N is Avogadro's number, and D is the coefficient of diffusion. If one substitutes the diffusion values obtained in the present study, $D_5 = 3.25 \cdot 10^{-8}$ cm^2/sec and $D_7 = 2.63 \cdot 10^{-8}$ cm^2/sec, into the above equation, where D_5 and D_7 represent the diffusion coefficients in pH 5 and pH 7 buffers, respectively, one finds that during diffusion the particle possesses coefficients of friction f_{D_5} $12.4 \cdot 10^{-7}$ g/sec and $f_{D_7} = 15.4 \cdot 10^{-7}$ g/sec. These calculations demonstrate, then, that T2 bacteriophage possesses a greater coefficient of friction for diffusion in buffer at pH 7 than at pH 5. The ratio, $f_{D_7}/f_{D_5} = 1.24$.

If the assumption is made that the molecular weight and the hydration of the T2 bacteriophage particle are the same at pH 5 and at pH 7, then the ratio of the friction coefficients for sedimentation, f_{s_7}/f_{s_5} equals s_5/s_7. When the appropriate values[5] are substituted, f_{s_7}/f_{s_5} $1066 \cdot 10^{-13}/751 \cdot 10^{-13}$, or 1.42. It seems unlikely that this ratio differs from the ratio for the coefficients of friction in diffusion, 1.24, solely because of experimental error. Consequently, this difference is regarded as real. Any successful theory, then, must account not only for the fact that the coefficient of friction as measured by diffusion is greater at pH 7 than at pH 5, but also for the fact that f_{s_7}/f_{s_5} is greater than f_{D_7}/f_{D_5}.

For several years, the idea has been under discussion in the authors' laboratory that the bacteriophage particle might possess filaments which protrude laterally at pH 7 but not at pH 5. This suggestion was alluded to in a doctoral dissertation[23]. This reversible condition could be explained by hypothesizing, for example, that at the higher pH appropriate acidic groups on the filaments would be dissociated, producing a net negative charge and mutual repulsion of the filaments, whereas at the higher hydrogen ion concentration this effect would be minimized. Certainly, it might be anticipated that these extended filaments would increase the frictional resistance characteristic of the virus particle as it either diffused or sedimented.

As a result of this line of reasoning, evidence for such filaments was sought, and as described earlier, filaments actually were observed with the electron microscope. While other investigators previously obtained electron micrographs showing these filaments, no association with dual sedimentation was proposed.

It is interesting to note that a graph describing the electrophoretic mobility of the bacteriophage T6 as a function of hydrogen ion concentration[7] exhibits a change of slope at about pH 6.2. Quite conceivably this electrophoretic mobility deviation is manifesting the same cause responsible for the dual sedimentation phenomenon.

The assumption, then, of the existence of collapsible filaments, for which electron microscope evidence has been adduced, can readily allow for the higher coefficient of friction at pH 7 than at pH 5. An additional assumption is required, however, to explain the experimentally observed higher friction coefficient ratio for sedimentation, f_{s_7}/f_{s_5}, than for diffusion, f_{D_7}/f_{D_5}. To account for this, it is proposed that during diffusion the bacteriophage particles are randomly oriented but that during sedimentation they are oriented with their long dimension in the direction of sedimentation. To allow for this assumption, the experiment of PUTNAM[9], discussed in the introduction, which showed that the sedimentation rate did not vary substantially with the magnitude of the centrifugal field, must be reinterpreted to mean that the particles are oriented to a considerable degree even in low fields.

If the assumption that the bacteriophage particles are oriented during sedimentation is correct, then, in principle, the coefficient of diffusion applicable in a

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sedimentation experiment should be 5 to 10% greater than that applicable for ordinary diffusion. PUTNAM[9] has shown that the boundary spreading during sedimentation of T6 bacteriophage corresponds with that expected on the basis of diffusion when the usual coefficient is employed in the calculations. While this might seem to be adverse evidence for orientation, in reality this is not the case because the sensitivity of the method is not sufficient to distinguish between coefficients of diffusion which differ by as little as 10%.

If the suggestion of orientation during sedimentation is to be taken seriously, some reason must be adduced for believing that orientation might well occur. Orientation could come about, as the following rather crude theoretical consideration will demonstrate, as a result of the center of gravity of the bacteriophage particle not coinciding with its geometrical center. There is reason to believe that the density of the tail might differ significantly from that of the head. Furthermore, if one were to construct an ellipsoidal envelope for a bacteriophage particle, the bulk of the mass would be concentrated in one end.

Crude calculations of the orienting effect of a gravitational or centrifugal field can be carried out for an ellipsoid of revolution of mass, m, with a center of gravity lying on one of the semi-axes at a distance, l, from the center. The work required to rotate such an ellipse through an angle, θ, about its center in a gravitational or centrifugal field of magnitude, g, is $mgl\,(1-\cos\theta)$ when the angle θ is measured from the line connecting the center and the center of gravity of the particle when it is in the position of minimum potential energy. If one then applies the Boltzman distribution function, one readily obtains the equation, $n/n_0 = e^{-mgl\,(1-\cos\theta)\cdot KT}$, where n represents the probability that a particle will have an orientation between θ and $\theta + d\theta$, and n_0 is the probability that the particle will have an orientation between 0 and $0 + d\theta$.

Consider an ellipsoid with a molecular weight of $234\cdot10^6$ and with a center of gravity displaced along the major semi-axis, a distance of $5\cdot10^{-6}$ cm from the geometric center. Now, one can apply the above formula to evaluate the probability of particles being oriented in fields of various magnitudes. For the case of a temperature of 300° A and an orientation of $\pi/2$ radians, n/n_0 turns out to be virtually 1 in a field equal to the acceleration of gravity. For a field of 10^7 cgs units, which represents the usual condition for carrying out sedimentation studies on reasonably large viruses, $n/n_0 = 0.63$, while for an orientation of π radians, $n/n_0 = 0.39$. For the condition of a field of 10^8 cgs units, which corresponds to an ultracentrifuge operated at approximately half speed, and an orientation of $\pi/2$ radians, n/n_0 is approximately 0.01. These calculations suffice to show that, if the bacteriophage particle behaves at all like the ellipsoid of revolution considered above, it could be oriented to an appreciable extent even at relatively low speeds of the ultracentrifuge, but it would not be oriented in a gravitational field. Thus, our assumption that the bacteriophage particles are randomly oriented during diffusion but preferentially oriented in the direction of the long axis during sedimentation is not unreasonable. It remains, however, to demonstrate just how these assumptions could account for the experimental fact that f_{s_1}/f_{s_2} is greater than f_{D_1}/f_{D_2}.

One way to proceed is to postulate a double ellipsoid of revolution representing an idealized bacteriophage particle at pH 5, and a similar model with two long, thin ellipsoidal protrusions at right angles to the main axis as a model for the bacteriophage

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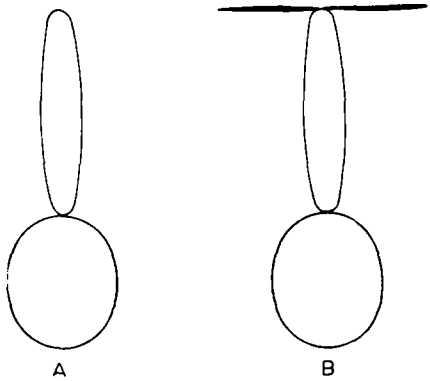

Fig. 4. (a) Model consisting of two prolate ellipsoids of revolution simulating the head and tail, of an idealized bacteriophage particle at pH 5. (b) Similar model with thin ellipsoidal protrusions simulating a filamented bacteriophage particle at pH 7.

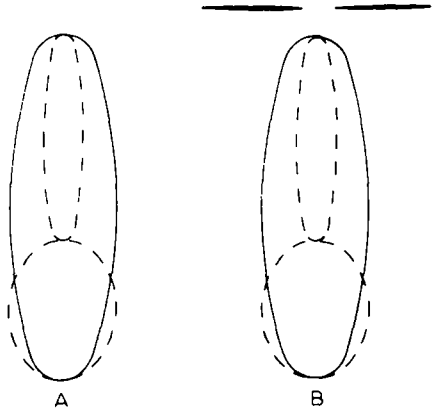

Fig. 5. (a) Model for calculating the coefficient of friction of a chain of elongated ellipsoids of revolution of unequal diameter simulating a bacteriophage particle at pH 5. (b) Similar model with protrusions simulating a filamented bacteriophage particle at pH 7.

particle at pH 7 (Fig. 4, a and b). One would then calculate the coefficients of friction for randomly oriented translational motion and for translational motion in the direction of the principal axis for both models. GANS[24] offers a relationship describing the translational resistance presented to an elongated ellipsoid of revolution as a function of its axial dimensions. When b and a represent the semi-major and semi-minor axes, respectively, and $b > a = c$, the frictional coefficient for the particle, while moving in the direction of its minor axis, can be evaluated from the following equation:

$$f_a = \frac{16\pi\eta b}{\frac{1}{\varepsilon^2} - \frac{1-3\varepsilon^2}{2\varepsilon^3}\ln\left(\frac{1+\varepsilon}{1-\varepsilon}\right)} \qquad (2)$$

where ε is the eccentricity, and $\varepsilon^2 = (b^2 - a^2)/b^2$. When motion occurs in the direction of the major axis, the frictional coefficient is described by the equation:

$$f_b = \frac{16\pi\eta b}{\frac{1-\varepsilon^2}{\varepsilon^3}\ln\left(\frac{1+\varepsilon}{1-\varepsilon}\right) - \frac{2}{\varepsilon^2}} \qquad (3)$$

From f_a and f_b the frictional coefficient for random orientation, f, can be evaluated by solving

$$\frac{1}{f} = \frac{1}{3}\left(\frac{1}{f_a} + \frac{1}{f_b} + \frac{1}{f_c}\right),$$

where $f_a = f_c$. By appropriate algebraic rearrangement it can be shown that

$$f = \frac{12\pi\eta}{\frac{b}{\sqrt{b^2-a^2}}\ln\frac{b+\sqrt{b^2-a^2}}{b-\sqrt{b^2-a^2}}} \qquad (4)$$

As the model for the bacteriophage particle at pH 5 we have chosen two prolate ellipsoids of revolution attached end-to-end to represent the head and tail of the

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characteristic particle (Fig. 4a). For the actual dimensions of the model one can utilize either those offered by electron microscopy (designated Model No. 1), or assume dimensions which will yield the right coefficient of friction for random orientation (designated Model No. 2). WILLIAMS AND FRASER[6] present values of 95 × 65 mμ and 100 × 25 mμ for the head and tail, respectively, of the frozen-dried T2 bacteriophage. These electron microscope dimensions will be used in the following discussion.

The coefficient of friction for the case of random orientation was calculated through the use of equation (4), and the assumption first introduced by LAUFFER AND SZENT-GYÖRGYI[25], namely, that the coefficient of friction of a chain of elongated ellipsoids of revolution of unequal diameter is equal to or less than that of an ellipsoid of revolution with a diameter equal to that of the thickest member of the chain and a length equal to the total length of the chain (Fig. 5a). In this way, the coefficient of friction for random orientation and, therefore, the coefficient of friction for diffusion, f_{D_5}, for this model was calculated to be equal to or less than $9.9 \cdot 10^{-7}$ cgs units. On the basis of the same assumption, the coefficient of friction for translation parallel to the long axis was calculated through the use of equation (3) to be $8.7 \cdot 10^{-7}$ cgs units. This figure is, by assumption, the coefficient of friction for sedimentation at pH 5, f_{s_5}.

For a model corresponding in a general way to the bacteriophage particle at pH 7, two ellipsoidal filaments 75 × 4 mμ are attached at right angles to the end of the tail of the model representing the pH 5 condition (Fig. 5b). The selection of two such filaments to be added and the particular dimensions ascribed to them are not to be interpreted as meaning that a bacteriophage particle at pH 7 has only two filaments and that they are of roughly those dimensions. Although electron micrographs indicate the more usual number of 3 to 5 filaments, with dimensions as reported in a previous section, nevertheless, two filaments were chosen because that represents the smallest integer which can provide bilateral symmetry, and the dimensions were chosen because they give reasonable answers in the subsequent calculations of friction coefficients. To be strictly realistic, the model containing the protruding filaments would have to be somewhat smaller in other respects to preserve the equality of mass and, therefore, of volume. The total volume of the ellipsoidal filaments, however, is such a small fraction of the total volume of the rest of the model as to make this refinement insignificant.

Through the use of equation (4), one can calculate that each one of these filaments possesses a coefficient of friction for translational motion under the condition of random orientation of $1.95 \cdot 10^{-7}$ cgs units. For want of a better method of doing it, the coefficient of friction for translation for the case of random orientation for the entire model was calculated by simply adding to the coefficient of friction for the pH 5 model twice the coefficient of friction for the 75 × 4 mμ filament. This, on the basis of our assumption, gives us f_{D_7}. $f_{D_7} = 9.9 \cdot 10^{-7} + 2 \times 1.95 \cdot 10^{-7} = 13.8 \cdot 10^{-7}$ cgs units. This simple addition would provide the correct answer if the lateral filaments were held perpendicular to the main axis, and at a distance from it, by "hydrodynamically invisible" bonds. For filaments actually attached, there is bound to be some error to this procedure, depending upon the extent to which the stream lines surrounding the main portion of the particle and those surrounding the lateral filaments overlap. It is our judgement that this error is not great. Also, it must be indicated that adoption of filaments oriented perpendicularly to the main axis of our model was done solely for computational expediency. One might more readily

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visualize the filaments trailing at an angle to the major axis during sedimentation; but, then, calculation of the resulting friction coefficient would be a much more difficult problem.

The coefficient of friction for translational motion parallel to the long axis of the principal part of the model is obtained in a similar manner by adding the coefficient of friction for such motion of the principal part to twice the coefficient of friction for motion perpendicular to the long axis of the filament. This latter value equal to $2.29 \cdot 10^{-7}$ was obtained through the use of equation (2). Thus, $f_{s_7} = 8.7 \cdot 10^{-7} + 2 \times 2.29 \cdot 10^{-7} = 13.3 \cdot 10^{-7}$.

For Model No. 2 we have adopted an ellipsoid of revolution with a length of 100 mμ and a diameter of 80 mμ attached linearly to a second ellipsoid of revolution with a length of 150 mμ and a diameter of 30 mμ. This model has approximately the right coefficient of friction for random orientation, while maintaining a gross resemblance to the bacteriophage particle, as described by WILLIAMS AND FRASER[6]. Calculations for this model were performed in the same manner as described above for Model No. 1.

The values of the various friction coefficients of the models and their ratios are presented in Table II and are compared with values obtained experimentally for T2 bacteriophage.

TABLE II

COMPARISON OF ELLIPSOIDAL MODEL AND T2 BACTERIOPHAGE

	Model No. 1	*Model No. 2*	*T2 Bacteriophage*
f_{D_5}	$< 9.9 \cdot 10^{-7}$	$< 12.4 \cdot 10^{-7}$	$12.4 \cdot 10^{-7}$
f_{D_7}	$< 13.8 \cdot 10^{-7}$	$< 16.3 \cdot 10^{-7}$	$15.4 \cdot 10^{-7}$
f_{D_7}/f_{D_5}	*ca.* 1.39	*ca.* 1.31	1.24
f_{s_5}	$< 8.7 \cdot 10^{-7}$	$< 10.8 \cdot 10^{-7}$	--
f_{s_7}	$< 13.3 \cdot 10^{-7}$	$< 15.4 \cdot 10^{-7}$	
f_{s_7}/f_{s_5}	*ca.* 1.53	*ca.* 1.43	1.42 (s_5/s_7)
$\frac{f_{s_7}/f_{s_5}}{f_{D_7}/f_{D_5}}$	*ca.* 1.10	*ca.* 1.09	1.14

It can be concluded that the theoretical considerations presented herein account in a reasonably satisfactory manner for all of the experimentally observed sedimentation and diffusion characteristics of T2 bacteriophage particles in buffers at pH 5 and in buffers at pH 7. Because of the quantitative uncertainties in some aspects of the theoretical treatment, no significance is attached to the degree of quantitative agreement between observation and theory. Rather, it is felt that it is significant that a model has been found which can mimic the T2 bacteriophage particle in possessing the higher coefficient of friction for diffusion at pH 7 than at pH 5 and also in exhibiting a higher value for f_{s_7}/f_{s_5} than for f_{D_7}/f_{D_5}.

An interesting sequel of this theoretical treatment is that it is no longer possible to obtain the correct value for the molecular weight of T2 bacteriophage, using the familiar Svedberg formula, from the sedimentation and diffusion coefficients. This formula presupposes equality of the coefficients of friction for diffusion and for sedi-

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mentation. Calculations based on the model show that, if the particle is, indeed, preferentially oriented during sedimentation but randomly oriented during diffusion, this assumption might be in error by as much as 20%. The direction of the error is such as to result in an overestimation of the molecular weight.

SUMMARY

For T2 bacteriophage, diffusion coefficients of $3.25 \cdot 10^{-8}$ cm²/sec and $2.63 \cdot 10^{-8}$ cm²/sec were obtained in pH 5 and pH 7 buffers, respectively. The transition limits for dual sedimentation encompass approximately one-half pH units, with a central value at about pH 6.1. Sedimenting the virus from a medium of one pH directly into a second medium of greater density and different pH has indicated that the sedimentation rate change is quite sharp and complete within a few minutes after passing through the interface.

s_5/D_5 differs significantly from s_7/D_7 but not enough to represent one-to-one aggregation. It is, therefore, assumed that the molecular weight is the same at pH 7 and at pH 5. It follows from this assumption and the experimental results that f_{s_7}/f_{s_5} is significantly greater than f_{D_7}/f_{D_5}.

A model has been found which can account for all of these facts. It is assumed that at pH 5 the particle is tadpole-shaped but that at pH 7 it projects thin filaments laterally from the tail. Such filaments have been demonstrated with the electron microscope. Calculations of friction coefficients made for complex ellipsoidal models approximating the actual models discussed above yield results consistent with the experimental observations, if it is assumed that the particles are oriented randomly during diffusion but sediment in the direction of their longest axis.

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Received February 21st, 1957