Molecular Kinetic and Electrophoretic Properties of Bacteriophages[1]

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THE BACTERIOPHAGES (or bacterial viruses) are a group of filterable infectious agents of complex morphological and genetic structure which are capable of reproduction only in specific bacterial hosts. They possess the fundamental attributes of viruses but are named for their dramatic property of producing lysis of the infected host cell. In recent years the bacteriophages have been selected as models for study of the nature of virus reproduction (*11, 18, 19*) because of their nonpathogenicity, the ease and economy of their production, the availability of a rapid, simple, accurate method of assay, and their capacity for growth on bacteria cultured in a synthetic medium. The same characteristics and a relatively large size expedite purification, yet only a few bacteriophages have thus far been purified and studied by physicochemical methods.

Study of the molecular kinetic and electrophoretic properties of the bacteriophages may aid in clarifying the question about the molecular nature of viruses and also affords an opportunity for comparing the validity of the direct and indirect methods of estimating the size and shape of particles, especially for the case of nonellipsoidal particles.

Experimental methods. The molecular kinetic and electrophoretic methods are dynamic methods which measure the translational velocity of an artificially created boundary (concentration gradient between solvent and solution) as the result of imposition of an external field or of normal Brownian motion. The methods are familiar and are described elsewhere (*13, 21*). In the sedimentation velocity ultracentrifuge, the external field is centrifugal force, which causes the sedimentation and separation of constituents on the basis of molecular size and shape. In electrophoresis, the translational motion is produced by application of an electrical field, which results in the migration and separation of constituents at characteristic rates determined by the net charge density. Free diffusion, however, is effected only by Brownian motion (random thermal motion). Since no external force is applied in free diffusion, no separation of constituents occurs, and the mean position of the boundary is unaltered.

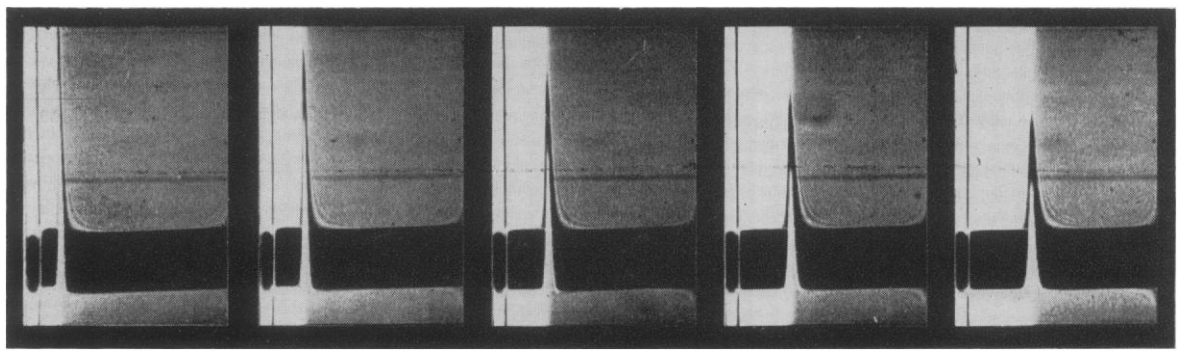

FIG. 1. Sedimentation velocity diagram of T_6 bacteriophage in 0.15 ionic strength sodium chloride-sodium acetate buffer pH 4.95. Photographs taken at 16-min intervals at a rotor speed of 3150 rpm. The sharp vertical line is the meniscus. Note that the light-absorbing region due to opalescence of the phage sediments with the refractive index gradient. A single component is present.

Although each of the three methods yields characteristic molecular constants, only sedimentation velocity and electrophoresis give visible evidence of purity. In the ultracentrifuge each molecular component, and in electrophoresis each electrical component, gives rise to a separate boundary. The velocity of the boundary, as well as its rate of spreading, may be measured precisely by optical techniques which may be identical for the three physical methods. An illustration is given in Fig. 1, showing the sedimentation of an apparently homogeneous preparation of bacteriophage T_6.

Molecular constants. The bacteriophages reportedly range in size from 10 mμ up to 200 mμ. Most of the early estimates of size are based on ultrafiltration or

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TABLE 1

SIZE, SHAPE, AND MOLECULAR CONSTANTS OF SOME PURIFIED BACTERIOPHAGES*

Type	Electron microscope		Ultra-centrifuge		Diffusion		Specific volume	Infectivity		Particle weight	
	head diam	tail	S_{20}	d_{eq}†	D_{20}	d_{eq}†			d_{eq}†	Ms,D	Biol.
	mμ	*mμ*	*10^{-13} sec*	*mμ*	*10^{-7} cm² sec⁻¹*	*mμ*	*ml/g*	*g N/phage*	*mμ*	*10^6*	*10^6*
Coliphages‡											
T_1	50	120 × 10									
T_5	90	170 × 15									
T_2	60 × 80	100 × 20	1000	59			0.66	1.3×10^{-16}	107		584
			700	50							
T_4	"	"			0.80	53					
T_6	"	"	1050	61	0.45	94	(0.66)	1×10^{-16}	98	167	460
			825	54							
T_3	45	none			1.19	36				31	
T_7	45	none	480	43			0.68	0.5×10^{-16}	80		246
Staph. phage			650	77	0.18	235	0.83	1×10^{-16}	93	530	300

* For references, see the text.

† d_{eq}—The apparent spherical diameter, i.e., the equivalent diameter of an unhydrated sphere of the same mass and volume.

‡ Scale: 1 cm = 150 mμ.

radiation inactivation studies, which yield only the order of magnitude (*5*). Later values rely on direct measurements of electron micrograph images of the individual particles (*1*). Although several dozen strains or varieties of phage have been examined by both these methods, only the staphylococcus phage of Northrop (*15*) and some of the "T" strains of *Escherichia coli* bacteriophage have been prepared in relatively pure form and studied by molecular kinetic methods such as sedimentation in the ultracentrifuge (*8, 10, 20*) or diffusion (*7, 9, 16, 17*). Electrophoretic data have thus far been published only for *E. coli* bacteriophage T_6 (*19*). The molecular constants and the apparent sizes and shapes of some well-purified bacteriophages are summarized in Table 1.

In Table 1 the brackets in the "T" group of the first column refer to the serological classification as established by Delbrück (*3*), which also fits with the morphological findings in the second column. The electron micrograph dimensions are those given by Anderson (*1*), and in some instances are smaller than more recent values obtained by the metal shadow method (*8, 10*). The sedimentation constants (S_{20}) refer to average values in the concentration range above 0.5 mg phage per ml.[2] For T_2 and T_6 bacteriophages S_{20} is given for both the slow and the fast forms, each of which can be obtained as a single sharp sedimenting boundary (*8, 19, 20*). The specific volumes were determined by the pycnometric method for T_2 (*22*) and T_7 (*10*), thus referring to the dry density. The specific volume of staphylococcus phage was determined by the sedimentation buoyancy method and is unusually high (*23*); figures in parentheses are assumed. The diffusion constants are corrected to the water basis at 20° C (D_{20}) and were determined by the porous disk method (*15*) for staphylococcus phage (at a concentration greater than 2×10^{11} particles per ml), by the multichambered analytical method (*16*) for T_3 and T_4 phage (at concentrations of 10^8 to 10^{10} phage per ml) and by the refractive index method (*6*) for T_6 phage (concentration of 10^{12} per ml). The infectivities give the grams of nitrogen per plaque (phage particle) for T_2 (*8*), T_6 (*19*), and T_7 (*10*) and refer to grams of protein nitrogen per phage for staphylococcus (*15*).

Apparent diameters from indirect methods. The

[2] All sedimentation constants are given in Svedberg units (10^{-13} sec) and are corrected to the water basis at 20° C.

apparent or equivalent spherical diameters have been calculated from sedimentation, diffusion, and infectivity data and are given for comparison with the dimensions obtained directly from electron micrographs. The apparent diameters were calculated from S_{20}, assuming spherical particles and no hydration, from a formula based on Stokes' Law

$$r^2 = \frac{9\,\eta\,S_{20}}{2\,(\rho_v - \rho_s)}$$

in which r is the radius, η the viscosity of water at 20° C, and ρ_v, and ρ_s the density of the protein and the solvent respectively (*21*). Particle sizes were also computed from the well-known Einstein equation for the diffusion of a spherical particle

$$D = \frac{RT}{N} \cdot \frac{1}{6\,\pi\,r\,\eta}$$

where R is the gas constant, T the absolute temperature, and N the Avogadro number (*21*). In addition, particle sizes have been calculated from infectivity measurements and the nitrogen content from the relation:

$$\text{Weight of particle in grams} = 4/3\,\pi\,r^3\,\rho_v.$$

This procedure is especially appropriate for the phages for which the infectious unit is a single particle. The molecular weights, or more strictly speaking the particle weights, were obtained from S_{20} and D_{20}, using the familiar Svedberg equation (*21*)

$$M = \frac{RT\,S}{D(1 - V\rho_s)}$$

which is derived without making any assumptions about the shape, structure, or hydration of the particle. Particle weights computed from the infectivities by multiplying the weight of the single infectious unit by N may be considered maximum values, since they are dependent on the efficiency of plating factor in the assay as well as on the purity of the virus.

Electron micrograph characteristics. Of all viruses the bacteriophages present the most unusual and complex morphology in electron micrographs. As indicated in Table 1, the coliphages are of two types: the round phages, and the tailed phages. The latter are characteristic sperm-shaped or tadpole-shaped particles with large heads and elongated tailpieces, and give some evidence of differentiated internal structure. In this group T_2, T_4, and T_6 represent a family of serologically related phages which are indistinguishable in the electron microscope and have similar molecular kinetic constants. The tailed structure is not a peculiarity of the coliphages, but is found in many other types, some of which have rather bizarre shapes. Several recent reviews have described the various forms of phages and have discussed the significance of the electron microscope evidence for internal structure and the possible presence of an external membrane on some of these particles (*1*). The function of the tail is uncertain, but some authors have suggested that the bacteriophages possess a special kind of motility (*17*). Other authors have concluded that even the tailless phages dissociate on dilution to form smaller particles (*9, 15*). These hypotheses will now be examined by comparison of the size of the infectious unit as revealed by direct electron micrograph measurements, the indirect physical methods, and biological infectivities.

Comparison of electron microscope and molecular kinetic data. From Table 1 it is evident that reasonable agreement exists on the apparent diameters of the various phages, provided allowance is made for the fact that several of the particles are not spherical and that some assumptions have been made with regard to the hydration and density of the phages. It should be emphasized that the dimensions obtained from electron micrographs represent the vacuum-dried particles and are not necessarily identical with those of the virus particles in aqueous solution. Even for the most favorable case, that of spherical particles, only qualitative agreement may be expected between the calculated sizes and those obtained directly from electron micrographs, for the latter values depend upon the type of contrast employed. For example, the spherical phage T_7 is of uniform size and has a diameter of 45 mμ (*1*) or 51 mμ (*10*) in unshadowed micrographs but of 73 mμ if metal shadowing is employed (*10*). The tailed phage, T_6, has an apparent equivalent spherical diameter of 98 mμ, as calculated from the unit infectivity, of 94 mμ, according to diffusion measurements, and of about 60 mμ from the sedimentation constant. From electron micrographs the head of this phage is 60 mμ × 80 mμ or 80 mμ × 100 mμ, according to the type of contrast employed. It should be noted that the equivalent spherical diameter calculated from S_{20} or D_{20} is the diameter of a sphere of the same mass and volume as the actual nonspherical particle, and therefore does not correspond to the over-all dimensions of the particle.

Recent molecular kinetic studies have shown that caution must be exercised in the interpretation of the data given in Table 1. Two phenomena require explanation: 1) the diffusion constant of several of the bacteriophages is reputed to vary with concentration in such a manner that dissociation or motility of the phages must be predicated; and 2) the tailed phages, T_2 and T_6, appear to exist reversibly in two states with different sedimentation rates in the ultracentrifuge. However, only a single characteristic type particle of uniform dimensions is seen for each phage in electron micrographs without any evidence of dissociation or specific aggregation.

Diffusion constants. Investigation of the rate of diffusion of bacteriophages using biological assay has led to much confusion. Bronfenbrenner and associates (*9*), using the porous disk method, first reported that the particles of T_2 (then called PC) bacteriophage varied in size over the range of about 1 mμ to 10 mμ radius and that particles with molecular weights below 100,000 had all the properties of the original active agent. However, recently, on reinvestigation of the diffusion behavior of this phage, Hershey, Kimura, and Bronfenbrenner (*7*) withdrew this claim and concluded that the earlier experiments were in error because of circulation of fluid through the pores of the disk and other anomalies. Using the same method in the study of the diffusion of a staphylococcus phage, Northrop (*15*) also found that D appeared to vary with the concentration of phage and concluded that the virus existed in solution as particles of varying size over the range of 10 mμ to 100 mμ. More recently, Polson measured the diffusion constants of T_3 and T_4 phage, using a multichambered analytical method with biological assay. In a preliminary article (*16*) he reported fair agreement between the particle sizes computed for diffusion of a spherical particle and the diameters of the actual particles as revealed by electron micrographs (see Table 1) and concluded that the tail of T_4 bacteriophage does not contribute to its motion. However, in later work at lower phage concentrations Polson and Shepard (*17*) claimed that the diffusion constants of these coliphages depended strongly on concentration. Since no evidence for heterogeneity of size was found from the diffusion experiments, the unexpectedly high diffusion constants at low concentrations could not be attributed to dissociation of the particles as in Northrop's experiments. Alternatively, it was suggested that the phage particles "possess an independent specific motility in addition to normal diffusion." It is noteworthy first that the latter conclusion was adduced principally from data for the tailless phage T_3, and secondly that the variation in D in all experiments cited here was encountered only at low virus concentrations.

In our laboratory Mr. E. Goldwasser has recently made several determinations of the diffusion constant of T_6 bacteriophage by the refractive index method of Longsworth (*13*). The phage was purified by differential centrifugation as previously described (*18, 19*) and was apparently homogeneous in electrophoresis and ultracentrifugation. Although the optical method necessitates the use of high concentrations of virus (above 10^{12} particles per ml), the precision and accuracy of the method should be superior to methods based on biological assay of the rate of diffusion in dilute solutions. The rate of diffusion of T_6 bacteriophage at a titer of 3.2×10^{12} particles per ml was measured at 2° C in 0.2 ionic strength sodium acetate buffer, pH 5.5, for a period of from 5 to 7 days. In this buffer the phage sediments in the ultracentrifuge with a single sharp boundary similar to that shown in Fig. 1, with S_{20} = about 1050. The average diffusion constant in several experiments was $0.45 \pm 0.06 \times 10^{-7}$ cm^2 sec^{-1}. The details will be published separately.

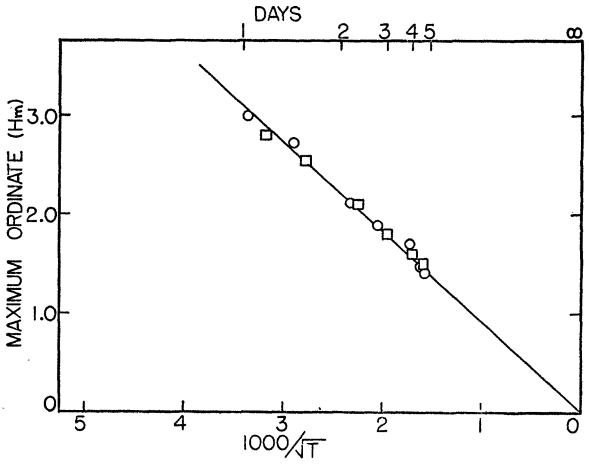

FIG. 2. Normal diffusion of T_6 bacteriophage in 0.2 M acetate buffer pH 5.5. The time, t, is expressed in sec on the lower abscissa, and the corresponding time in days is given on the upper abscissa. The two sets of points are for the same experiment and represent the separate values obtained in each limb of the diffusion cell. H_m is given in cm.

It has not yet been possible to obtain sufficient pure phage with $S_{20} = 800$ to make diffusion measurements on the more slowly sedimenting form. However, the low value of the diffusion constant found for the $S_{20} = 1050$ component and the good agreement between the apparent diameter from diffusion and the electron microscope dimensions appear to exclude motility by this tailed bacteriophage. This conclusion is perhaps to be anticipated, since no respiration or other independent metabolic activity has yet been detected in bacteriophages.

Ideal diffusion. In the case of ideal diffusion when a sharp boundary is formed between a solution and a solvent, the maximum height of the refractive index gradient (H_m) is inversely proportional to the square root of the time (t), since $D = A^2/4\pi t H_m^2$, where A is the area under the gradient curve and is proportional to the solute concentration and to apparatus constants (*13*). Hence, as a test for normal diffusion a linear relationship should obtain between H_m and $1/\sqrt{t}$. On extrapolation to infinite time, H_m should be zero, since the concentration of solute must be uniform throughout the cell. This relationship has been found to hold in all our experiments (see Fig. 2), indicating that this tailed bacteriophage obeys the laws of normal diffusion.

Of all molecular kinetic methods, measurement of the diffusion constant has proved to be the least reliable for ascertaining the shape or size of virus par-

ticles. The sources of error of the nonoptical methods have been summarized elsewhere (*5, 7*). They derive chiefly from the instability of boundaries formed between solvents and extremely dilute phage solutions, and in the inherent errors of the biological assay. The errors in technique, such as convection disturbances, are generally in the direction of too rapid diffusion and would be most frequently experienced with decreasing virus concentrations. In some instances the errors in assay are magnified because D is related to the ratio of the square of the phage concentration of the diffusate and the original solution (*16*).

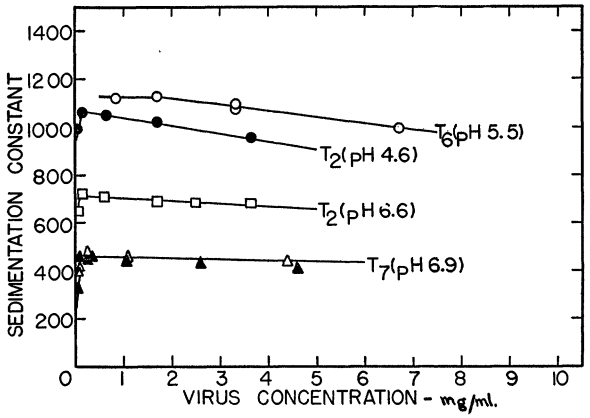

FIG. 3. Effect of virus concentration on the sedimentation constant of the *E. coli* bacteriophages. Data for T_2 and T_7 phages taken from (*10*) and (*20*).

Sedimentation velocity analysis. The bacteriophages are most readily purified by differential centrifugation in a high speed centrifuge under conditions which cause the selection of particles of about 50 mμ diameter or greater. Up to 99 percent of the larger tailed phages such as T_2 or T_6 are sedimented by centrifuging at 20,000 G for one hour, but smaller amounts of T_1. To date only four phages have been studied in the analytical ultracentrifuge. Of these, three (T_2 [*8*], T_7 [*10*], and the staphylococcus phage of Northrop [*23*]) were investigated using the ultraviolet light absorption method, and only one, (T_6), has been examined by a schlieren optical method which permits quantitative estimation of the homogeneity of the virus (*19*).

All phages investigated in the analytical ultracentrifuge have been obtained in a condition yielding a single sharp sedimenting boundary indicating a high degree of molecular homogeneity. However, two anomalies have been encountered: (1) Within the pH stability range both of the tailed phages, T_2 and T_6, exhibit two different rates of sedimentation, representing a "slow" and a "fast" form. (2) At high dilutions S_{20} for T_2 and T_7 falls unaccountably. This behavior is illustrated in Fig. 3. In the figure the two sedimentation rates of T_2 are indicated but only that for the "fast" form of T_6, for data on the concentration dependence of the slow component of T_6 (S_{20} = about 800) have not yet been accumulated.

The sudden drop in S_{20} reported by Sharp *et al.* (*10, 20*) for T_2 and T_7 phages occurs at concentrations ordinarily below the limits of resolution of the ultracentrifuge. The observation was made possible only by the extraordinarily high ultraviolet light absorption of these phages, and ultracentrifuge studies at similar concentrations cannot be made for most other viruses or protein molecules. No explanation of this unique phenomena has been offered, but it is of significance, since S_{20} is ordinarily determined by extrapolation to infinite dilution.

Dual sedimentation behavior of T_2 and T_6 bacteriophages. The occurrence of two different rates of sedimentation for the same substance is rare, but has been observed previously in several instances. For the hemocyanins, the phenomenon has been attributed to dissociation equilibria with lengthwise splitting of the molecules to form halves or eighths (*21*); with tobacco mosaic virus the more rapidly sedimenting component has been shown to result from end-to-end aggregation of two rodlike unit particles (*12*). With T_2 phage a single boundary with S_{20} = about 1000 is always found below pH 5.8, and a single boundary with S_{20} = about 700 is obtained above pH 5.8. An immediate transition from the slow form to the fast can be effected by pH adjustment or by the addition of $CaCl_2$. A similar effect of pH is observed with T_6 phage, except that both forms (S_{20} = 1050 and S_{20} = about 800) have frequently been found together over a wide range of pH. With T_6 the proportion of slow component increases with the age of the preparation as well as with pH. Though a single component with S_{20} = 1050 may invariably be obtained by adjustment of T_6 solutions to pH 4.9, the slowly sedimenting component with S_{20} = about 800 is rarely observed singly.

Abnormal sedimentation in the ultracentrifuge may result from: (1) temperature-induced convection, (2) retardation, (3) flotation, and (4) particle orientation, interaction, or aggregation. In our experiments the first three types of disturbances may be eliminated for the following reasons: (1) the rise in rotor temperature is negligible because of the slow speed and short duration of the run, (2) retardation due to the presence of viscous impurities is apparently absent, since ultracentrifuge study of T_6 phage over the entire practical velocity range (3,150 to 59,780 rpm) has demonstrated the absence of impurities in fresh preparations, and (3) the flotation anomaly (sedimentation towards the axis instead of the periphery), depends on the solvent density, whereas the double boundary phenomenon with bacteriophage is dependent on pH. For the fourth possibility, special tests to be described have been made to distinguish among orientation, interac-

tion, and aggregation or dissociation of the sedimenting particles.

Are bacteriophage particles oriented during sedimentation? With proteins of ordinary molecular weight the Brownian motion is so strong that the molecules are not oriented in the high centrifugal fields of the ultracentrifuge. Since T_6 phage is one of the largest particles investigated in the ultracentrifuge

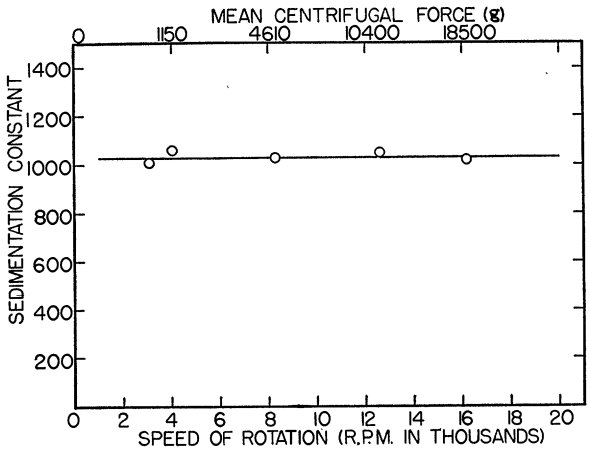

FIG. 4. Effect of centrifugal force on the sedimentation constant of *E. coli* bacteriophage T6. The constancy of S_{20} indicates lack of orientation of the phage particles during sedimentation in the ultracentrifuge.

and has a tadpole shape with a dense head, it may be asked whether the phage is oriented during sedimentation. Orientation is easy to detect since it must be proportional to the centrifugal force. In a test for orientation the homogeneity and sedimentation constant of T_6 phage were studied in 0.15 ionic strength sodium chloride-sodium acetate buffer, pH 4.95 over the entire practical range (157 to 18,900 *G*). The results, plotted in Fig. 4, show that S_{20} is independent of the centrifugal force, indicating the absence of orientation. Under these conditions the phage sedimented in all cases with a single sharp boundary.

The interaction of particles may evoke ultracentrifugation anomalies due to the mutual interference of sedimenting particles and thus should be dependent on the concentration of solute. In the concentration range usually suitable for ultracentrifuge study (above 0.125 mg protein per ml) the sedimentation constants of the coliphages are only slightly dependent on concentration. Since the slopes are low and are rather similar for the spherical phage T_7 and the slow and fast components of the tailed phages, the dual sedimentation behavior of the latter cannot be attributed to particle interaction or interference.

One explanation of the dual sedimentation phenomenon that has been offered by Sharp *et al.* (*20*) is that the form of T_2 phage with S_{20} = about 1000 represents the virus dispersed as unit particles and that S_{20} = about 700 indicates a state of heterogeneous aggregation. This hypothesis was based on study of the sedimentation of lucite models which were oriented during fall under the influence of the earth's gravitational field. However, our results demonstrate that the bacteriophage particles rotate randomly during sedimentation in the ultracentrifuge. If the diffusion constant of the $S_{20} = 800$ form of T_6 phage were known, it would be possible to calculate the frictional ratio and the particle weight. Even in the absence of this constant some deductions can be made from the following relationship (*21*)

$$S_{20} = \frac{K M^{2/3}}{(f/f_0)}$$

in which K is a constant, and f/f_0 is the frictional ratio, the latter quantity being an indirect measure of both molecular shape and hydration. Calculations based on this equation will be presented elsewhere (*6*). The results indicate that a change in mass must accompany the transition from $S_{20} = 1050$ to $S_{20} = 800$. By neglecting hydration of the virus particles and assuming that they can be represented by elongated ellipsoids, one can calculate the expected S_{20}, assuming that either the slow or the fast form is the dimer and that the alignment is either parallel or end-to-end. On this basis all possibilities have been excluded but one. If it is assumed that the fast form ($S_{20} = 1050$) is the dimer, and that end-to-end aggregation takes place, splitting at the mid-axis would give rise to a slow monomeric form with a sedimentation constant of 790. Although the latter value is in good agreement with S_{20} observed for the slow form, it is possible that dimerization occurs without preferential alignment, for hydration was neglected in this calculation. In electron micrographs particle association is observed but not with specific alignment. However, this may be due to the manner of preparation of the specimens. It is noteworthy that the dual sedimentation phenomenon is characteristic only of the tailed phages. The round phage T_7 gives only a single sedimenting boundary throughout the stability range (*10*).

Electrophoretic properties of bacteriophage T_6. Electrophoretic analyses of bacterial viruses have been reported only for *E. coli* bacteriophage T_6 (*11, 19*). Over the entire pH stability range the phage migrates toward the anode, the mobility decreasing from about -7.3×10^{-5} cm^2 volt^{-1} sec^{-1} at pH 8.6 to about -3.6×10^{-5} cm^2 volt^{-1} sec^{-1} at pH 5.1. Since the electrophoresis could be carried out only above the acid region of precipitation and within the pH stability zone, the isoelectric point of the phage could not be determined by this method. It seems probable that the isoelectric point is below pH 4.6, where precipitation first takes place. Referring to unpublished re-

ports, Sharp *et al.* (*20*) mention that the electrophoretic behavior of T_2 phage indicates an isoelectric point at about pH 4.2 corresponding to the inception of acid precipitation of this virus. It should be noted that the isoelectric point could be determined by the microelectrophoretic method. The relatively low mobility of T_6 bacteriophage is surprising in view of its extraordinarily high content of desoxyribonucleic acid (DNA) (approaching 40 percent [*11*]).

The pH mobility curves for T_6 phage from broth and from synthetic medium lysates are nearly identical, although differences in the size and chemical composition have been reported for the corresponding phage T_2 when harvested from nutrient and synthetic media (*8, 22*). A rapidly migrating impurity found in some preparations of bacteriophage T_6 has been isolated by electrophoretic separation and identified as desoxyribonucleic acid (*11*). DNA is one degradation product of the phage, and some DNA may perhaps be adsorbed to the phage. Its presence is best established by electrophoretic analysis and may go undetected in ultracentrifugation. The presence of free or adsorbed DNA may profoundly influence the properties of the bacteriophages, for example, causing a retardation effect in sedimentation because of the high intrinsic viscosity of the more slowly sedimenting nucleic acid. However, by careful purification, preparations of bacteriophage T_6 have been obtained which are apparently free of nonconstituent DNA or other impurities, according to electrophoretic analysis.

E. coli bacteriophage T_6 is apparently the largest particle yet successfully studied in the Tiselius electrophoresis apparatus without the addition of stabilizing proteins. The influence of particle shape on electrical mobility is usually negligible with proteins, but the possibility of preferred orientation due to particle morphology or polarity must be considered with the coliphages. It would be worth while to test for a disturbing effect of orientation on electrophoretic mobility by variation of the field strength in analogy to the test for orientation in sedimentation by changing the gravitational field. Although single boundaries were obtained on electrophoresis of T_6 bacteriophage throughout the stability range, the boundaries were markedly diffuse in some instances. Since the diffusion experiments already cited have excluded the possibility of phage motility, the boundary blurring is indicative of electrical heterogeneity. The boundary sharpness, however, was not restored by reversal of the current, as would be expected in the event of electrical heterogeneity. This behavior might be due to the influence of particle morphology or polarity on the electrical behavior of the bacteriophages. The problem could be investigated by a comparative electrophoretic study of the round and the tailed phages.

Homogeneity of the bacteriophages. It is customary to speak of a substance which sediments in the ultracentrifuge with a single boundary as an "apparently molecularly homogeneous" substance and of one which migrates in electrophoresis with a single boundary as an "apparently electrically homogeneous" material. With large particles, such as the bacteriophages for which diffusion can be neglected under the conditions of study, the purity can be judged both qualitatively and quantitatively by the sharpness or degree of spreading of the boundary. Thus, inspection of the sedimentation diagrams obtained by the ultraviolet light absorption method suggests that T_2 bacteriophage has been obtained in a rather high degree of purity and T_7 phage and the staphylococcus phage in a somewhat less homogeneous state. The refractive index diagrams are amenable to exact analysis, but simple inspection reveals that the boundary spreading is as great in one hour in the ultracentrifuge as in about a week in free diffusion. Since the spreading is not due to phage motility, it may be attributed to separation of a population of particles varying progressively in size or shape. The phenomenon of boundary spreading may also result from slight thermal convection at the low phage concentrations investigated, or possibly from vibration of the ultracentrifuge rotor. The latter, however, is apparently ruled out by studies we have made, over a range of centrifugal fields, indicating that the sharpness of a boundary is directly proportional to the distance of separation from the meniscus, not to the time or velocity of centrifugation. Electron micrographs suggest that the coliphages consist of particles of uniform size and shape, but size distribution analyses have not yet been published. Moreover, the many damaged and tailless particles seen in the electron microscope pictures may account for some of the apparent heterogeneity observed in ultracentrifugation.

Summary of physicochemical studies. In summary of the physical evidence for the size and shape of the bacteriophages, it may be said that all indirect methods, with the exception of certain types of diffusion measurements, indicate that bacteriophage activity is identified with and indissociable from the large characteristic particles observed in the electron microscope. Anderson (*1*) has previously reviewed other arguments relating to this question—for example, the invariable occurrence of the particles in active phage suspensions and their absence in controls, the adsorption of the characteristic particles by host bacteria and their apparent liberation upon lysis, and the relation betwen morphology and strain of phage. In addition, it has been shown that a single phage particle may produce a plaque and approximate quantitative agreement (*14*) has been found between the

plaque count titer and the number of particles visible in the electron microscope pictures. The plaque count assays are done at extreme dilution (about 10^3 phage per ml), the electron microscope measurements at moderate concentrations of about 10^{10} phage per ml, and the sedimentation studies at high concentrations (about 10^{12} phage per ml). Yet all these methods give particle sizes in approximate agreement and similar to those found from diffusion measurements at phage concentrations of about 10^{10} or higher. It has already been pointed out (*21*) that if dilution gives rise to dissociation, as proposed by Northrop (*15*), the plaque count assays done at extreme dilution should correspond to the number of small virus particles. Yet plaque counts and electron microscope counts are in good agreement and particle size calculations from infectivity measurements give values similar to those found by both direct and indirect physical methods.

Biological implications. The molecular kinetic properties discussed refer to the purified virus in the relatively inert state of extracellular existence for which no respiration or enzymatic activity has yet been detected. However, in adsorption and intracellular reproduction—other stages of the life cycle—the bacteriophages manifest specific biological activity. The rate of adsorption is greatly influenced by the physiological state of the bacteria and by the presence of electrolytes (*4*). With some coliphages there is a requirement for adsorption cofactors such as L-tryptophane (*2*). It can be shown that the low rate of diffusion of bacteriophages, as indicated by the constants given in Table 1, is not necessarily a rate-limiting factor for adsorption of the virus. If we assume that diffusion alone determines the number of collisions between the virus and the host cell, and that each hit leads to adsorption,

$$k_{max} = 4\pi Da,$$

where k_{max} is the upper limit of the adsorption rate constant and a is the effective radius of the bacterium (*4*). Diffusion constants calculated from this relationship and the measured adsorption constants are either lower than the values given in Table 1, for staphylococcus phage and the coliphages, or are compatible with these values.[3] The latter result is the more surprising in view of the receptor theory of bacteriophage adsorption, which should introduce an adsorption-rate steric factor dependent on the number and distribution of receptor spots. That is, the number of fruitful collisions would be much less than the actual number of hits unless the bacterial surface consists of a mosaic of receptor spots (*2*).

It should be observed that the intracellular concentration of bacteriophage just prior to lysis of an infected cell is greater even than the concentration of purified solutions submitted to molecular kinetic studies. The volume of a single *E. coli* cell is about 2 μ^3 (assuming an average length of 2.5 μ, and a uniform diameter of 1 μ). If the burst size is 200 phage per bacterium, the concentration within the cell is of the order of 10^{14} phage per ml. Solutions of phage *in vitro* settle out spontaneously at concentrations above about 4×10^{12} per ml. Ultracentrifuge analysis of a suspension of T_6 containing 10^{13} phage per ml indicates that most of the virus is in an aggregated form having a wide range of sedimentation constants above 1000. Prior to lysis the phage may occupy from 1 to 10 percent of the total volume of the host cell. After lysis the particles penetrate the matrix of the burst bacterium, presumably by diffusion, and then undergo a dilution of some 3 to 10 orders of magnitude on liberation into the medium.

[3] Delbrück (*4*) concluded that the low value of D obtained by Northrop (*15*) for staphylococcus phage was incompatible with the measured maximum adsorption rate constant. However, there is an arithmetical error in his conversion of Northrop's D in units of $cm^2\ day^{-1}$ to $cm^2\ min^{-1}$.

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Another article on bacteriophage, by S. E. Luria, will be published next week.