# SEDIMENTATION CHARACTERISTICS OF THE T3 BACTERIOPHAGE OF ESCHERICHIA COLI B

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W. N. Mack
Major professor

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# SEDIMENTATION CHARACTERISTICS OF THE T3 BACTERIOPHAGE OF ESCHERICHIA COLI B

By

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A THESIS

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# INTRODUCTION

Characterization of viruses, both plant and animal, has shown considerable individual differences in physical, chemical and morphological properties. Among the bacterial viruses, very little physical and chemical information characterizing this group has been obtained. Most work on bacteriophages has been performed with the familiar "T" strains of Escherichia coli B. Even members of this system have been investigated only sparsely in relation to their particle constants.

A search of the literature reveals work on purification and concentration of bacteriophages for comparatively few strains. Thus, physical and chemical analyses have been executed for relatively few bacterial viruses.

For example, sedimentation characteristics have been collected for only T2, T6 and T7 of the "T" system and for the staphylococcus bacteriophage of Northrop (Sharp et al. 1946, Kerby et al. 1949, Northrop 1937 and Putnam et al. 1949). Diffusion data have been compiled for the T4, T6, T3 and the staphylococcus bacteriophage

(Northrop 1937, Polson and Shepard 1949, Goldwasser and Putnam 1951). The staphylococcus virus and T2, T6 and T7 have been analyzed for nitrogen content (Northrop 1937, Hook et al. 1946, Putnam et al. 1949, and Kerby et al. 1949). Specific volumes have been determined for T2, T6, T7 and Northrop's staphylococcus bacteriophage. The most popular approach to investigating the bacteriophages has been electron microscopy. In this respect, all the T-phages have been photographed by various workers (Williams and Fraser 1953, Anderson 1952, and Fraser and Williams 1953).

The work accomplished by various investigators on purified bacteriophages has been compiled by Evans (1952) in tabular form (figure 1).

Responsible, in part at least, for an aversion to physical and chemical inquiry of these entities, is the difficulty of purification and concentration.

In the past, most workers have labored with procedures for purification and concentration which are inherently time-consuming and laborious. Any method which would yield a high degree of purity and concentration, yet, which could be simply and quickly performed, would lend an impetus not only to bacteriophage research but also to the utilization of bacterial viruses as research tools.

| Type | Ultracentrifuge | | Diffusion | | Specific Volume | Infectivity | | Particle Weight | |
|---|---|---|---|---|---|---|---|---|---|
| | $S_{w20}$ $10^{-13}$ sec. | $d_{eq}$ mu | $D_{w20}$ $10^{-7}$ $cm^2$ $sec^{-1}$ | $d_{eq}$ mu | ml/g | gN per phage | $d_{eq}$ mu | $S_{w20}$ $10^6$ | Biol. $10^6$ |
| T1 | | | | | | | | | |
| T5 | | | | | | | | | |
| T2 | 1000<br>700 | 59<br>50 | | | 0.66 | 1.3 x $10^{-16}$ | 107 | | 584 |
| T4 | | | 0.80 | 53 | | | | | |
| T6 | 1050<br>825 | 61<br>54 | 0.45 | 94 | 0.66 | 1.0 x $10^{-16}$ | 98 | 167 | 460 |
| T3 | | | 1.19 | 36 | | | | | |
| T7 | 480 | 43 | | | 0.68 | 0.5 x $10^{-16}$ | 80 | 31 | 246 |
| Staph | 650 | 77 | 0.18 | 235 | 0.83 | 1.0 x $10^{-16}$ | 93 | 530 | 300 |

Figure 1. Particle constants of some purified bacteriophages. (after Evans, 1952).

The determination of the particle constants of bacteriophages allows not only bases for comparison between these viruses but also permits these constants to be used as criteria in other experiments with each particular strain of bacteriophage. For example, if the sedimentation constant of a particular bacteriophage is known at neutral pH, then this constant can be used as a basis in showing the effect of pH on its sedimentation properties. Experiments of this type, of course, can be extended to include the effect of any physical, chemical or biological agent on the sedimentation characteristics.

Of the T group of bacterial viruses, one of the least investigated has been the T3 strain. With the exception of electron microscopic studies (Williams and Fraser 1953, Fraser and Williams 1953) and diffusion results (Polson and Shepard 1949), no other chemical or physical information has been revealed for this bacteriophage.

Although T3 is more difficult to concentrate than the even-numbered bacteriophages, it should be more ideal for the study of physical behavior because of its more spherical shape.

It has been the purpose of the present study to

augment the characterization of the T3 bacteriophage by securing data on some of its physical and chemical properties and, especially, on its sedimentation characteristics.

# MATERIALS AND METHODS

## Medium

F medium was used for cultivation of E. coli B. Its components were:

| | |
|---|---|
| $NH_4Cl$ | 1 gram |
| $MgSO_4$ | 0.1 gram |
| $KH_2PO_4$ | 1.5 grams |
| $Na_2HPO_4$ | 3.5 grams |
| Lactic acid | 9 grams |
| Distilled $H_2O$ | 1 liter |
| Agar | 20 grams |
| pH adjusted to 6.9 | |

## Buffer Systems

The buffer systems and their respective pH values used in these experiments were as follows:

| pH | Buffer System |
|---|---|
| 5 | M $CH_3COOH$ + M $NaC_2H_3O_2$ |
| 6 | $\frac{M}{5}$ $Na_2HPO_4$ + $\frac{M}{5}$ $NaH_2PO_4$ |
| 7 | $\frac{M}{5}$ $KH_2PO_4$ + $\frac{M}{5}$ $Na_2HPO_4$ |
| 8 | $\frac{M}{5}$ $Na_2HPO_4$ + $\frac{M}{5}$ $NaH_2PO_4$ |
| 9 | M $NH_3$ + M $NH_4Cl$ |
| 10 | M $NH_3$ + M $NH_4Cl$ |
| 11 | $\frac{M}{5}$ $Na_2HPO_4$ + $\frac{M}{5}$ $Na_3PO_4$ |

## Preparation of T3 Bacteriophage Stock Suspensions

Throughout the course of these experiments, care was exercised in manipulating the T3 bacteriophage so that the least amount of inactivation of the bacteriophage might occur.

Petri dishes, containing standard "F" agar medium adjusted to pH 6.9, were inoculated with one drop of a suspension from an 18 - 24 hour slant culture of E. coli B. The drops were spread evenly over the entire surface of the agar, using a glass spreader. These plates were incubated for approximately 18 hours at 37° C. At this time, 0.5 ml. of T3 bacteriophage was added to the agar surface of each plate and thoroughly mixed with the bacteria by means of a glass spreader. The plates were again incubated at 37° C. for about 18 hours. Upon the completion of this incubation period, 5 ml. of buffer (pH 7) was added to each plate. Once more, the mixture of bacteria and bacteriophage was agitated with a glass spreader and the suspension from each plate was decanted into an Erlenmeyer flask. Four to six batches of crude lysate were prepared in this manner and pooled.

The pooled lysate, obtained from the agar plates, was

centrifuged at 3,000 rpm. for 10 minutes in an International centrifuge (Model PR-1). The supernatant fluid was decanted and filtered through a bacterial-sterilizing, Seitz filter. The filtrate was placed in plastic tubes, transferred to rotor (B) and centrifuged at 42,040 rpm. (114,610 g) for one hour in a Spinco model E ultracentrifuge.

The supernatant fluid was withdrawn by means of a syringe and needle and the sediment was redispersed in buffer (pH 7) in an amount to accomplish about a ten-fold increase in bacteriophage concentration. The redispersed bacteriophage was centrifuged at 6,000 rpm. for 10 minutes in an International centrifuge (Model PR-1). The supernatant fluid was withdrawn carefully with a flat-tipped needle and a syringe, placed in plastic tubes and transferred to rotor (J). This suspension was centrifuged again at 42,040 rpm. (110,660 g) for 30 minutes in the ultracentrifuge. After this second cycle of ultracentrifugation, the supernatant fluid was withdrawn and the sediment was redispersed in buffer (pH 7) in a volume about 1/5 that of the immediately preceding suspension. Hence, an approximately fifty-fold concentration of bacteriophage was achieved by volume reduction.

The concentrated bacteriophage suspension was

centrifuged once more in an International centrifuge (Model PR-1) at 6,000 rpm. for 10 minutes and the supernatant fluid was withdrawn very carefully. This supernatant fluid was used as stock material and the sediment was discarded.

The volumes of crude lysate, at the beginning of the concentration procedure, usually amounted to about 400-500 ml. The final volumes, after concentration, were usually about 6-10 ml. The mean infective titer of the T3 bacteriophage increased from $7.7 \times 10^{10}$ plaque-forming particles per ml. in the Seitz filtrates to $3.4 \times 10^{12}$ plaque-forming particles per ml. in the concentrated stock suspension.

Concentrations of the T3 bacteriophage were best expressed as weights per unit of volume when physical constants were being determined. In order to obtain concentrations expressed in these units and, yet, prevent the bacteriophage from desiccation, the following procedure was utilized. A clean, tared pycnometer was filled with the buffer (pH 7) used to suspend the T3 bacteriophage. This was transferred to an analytical balance and weighed. The pycnometer was then thoroughly cleaned and dried. Using this pycnometer, the same volume of buffer (pH 7) plus T3 bacteriophage suspended in it was weighed. The difference in the two weights

was considered the weight of the T3 bacteriophage. The result of the weight of the bacteriophage divided by the volume used, gave the concentration as weight per unit of volume. The pycnometer and its contents were always allowed to reach the temperature of the weighing room before weights were determined.

An important factor in preparing the stock bacteriophage suspensions for this study was the preparation of a material without loss of biological activity. In order to determine the loss of activity resulting from the process of preparation, the volume from the Seitz filtrate was measured before processing, after which an assay of the infective activity of the T3 bacteriophage was performed by the standard agar plate method (Adams 1950). At the end of the processing, the preparation's volume was measured and another assay for infectivity was made.

From this information, an estimation of the active T3 particles that should be present in the final stock suspension, assuming no loss of activity whatsoever, could be computed. For instance, the volume of the filtrate from preparation B was 240 ml. and its infective titer was $8.7 \times 10^{10}$ plaque-forming units per ml. The final volume of preparation B was 6 ml. The reduction in volume of the suspending medium was forty-fold. On

multiplying the infective titer of the filtrate by 40, the calculated titer for active T3 particles in the concentrated stock was $3.5 \times 10^{12}$ plaque-forming units per ml. The actual, infective titer of the concentrated preparation was $3.6 \times 10^{12}$ plaque-forming units per ml. Similarly, the calculated titer of preparation A demanded $3.3 \times 10^{12}$ plaque-forming units per ml. and the actual titer was $3.1 \times 10^{12}$ plaque-forming units per ml. Preparation C was not tested for infective titer.

Such close agreement between the calculated and observed titers of the concentrated preparations indicates very little loss in activity of the T3 particles when processed by the method employed in the present study.

Biological Assay of Infective Bacteriophage Particles

A 0.5 ml. amount from a sample to be assayed was pipetted into 4.5 ml. of nutrient broth and diluted serially. The dilutions selected for assay (usually three successive dilutions) were transferred in 0.1 ml. amounts to tubes containing 2.5 ml. of 0.2 per cent agar and 0.5 ml. of a bacterial suspension, which was washed from a 12-18 hour slant culture of E. coli B. The soft agar had been melted previously and cooled to 45° C. The bacterium-bacteriophage mixture was poured onto the

surface of nutrient agar plates and spread evenly over the entire surface of the nutrient agar. After hardening of the soft agar layer, the plates were incubated for 18 hours at 37° C. and the plaques which formed on the plates were counted. Each dilution that was assayed had been plated in triplicate and the average number of plaques that had been formed on the three plates was used as the plaque-count for a particular dilution. The plaque-count from the dilution which yielded the statistically best value was utilized to calculate the concentration of bacteriophage in the original sample. The concentration was expressed as "plaque-forming units per ml."

Titer computations were performed in the following manner:

plaque-forming units per ml. = plaque-count x reciprocal of dilution x 10

Nitrogen Analyses

Nitrogen determinations were performed by a micro-Kjeldahl method. Because of the small amounts of nitrogen expected, a colorimetric method was adopted. Digestion and nesslerization were accomplished by the method of Folin and Denis (1916). Nessler's solution was made according to that prescribed by Koch and

McMeekin (1924). The transmittancy readings were performed on a Bausch and Lomb Monochromatic Colorimeter at a wave length of 505 mu.

Electron Microscopy

Electron micrographs of the T3 particles were prepared in order to have another means of determining the purity of the preparations and to measure the particle sizes directly.

Samples of the final stock suspension were prepared. The samples were placed on collodion membranes. One set of the samples was shadowed with palladium and photographed, while the other set was photographed unshadowed. The electron microscope's magnification was calibrated from a ruled diffraction grating.

Density Determinations

The densities of the T3 particle and the medium in which it was suspended were determined in an identical manner. A tared pycnometer, which was calibrated to contain 5 ml. of distilled water at 20° C., was employed for both determinations of density. The pycnometer was made of Pyrex glass, having a low coefficient of thermal expansion. Before filling, the empty pycnometer was

placed in a constant temperature water bath at 24.0° C. ($\pm$ 0.1° C.) for 5 minutes. It was then filled and reimmersed in the water bath for another 5 minutes. If any change in volume occurred, it was corrected to volume in the bath. The pycnometer's surface, after removal from the water bath, was thoroughly dried with an absorbent, lintless paper and immediately weighed on an analytical balance. The balance weights employed in all of the weighings conducted in the present study were unlacquered metal. Weights of specimens were measured accurately to $10^{-4}$ place. The calibrating liquid was distilled water.

The temperature of the pycnometer and its contents was equilibrated to the average temperature of the ultracentrifugal experiments.

Three weighings were performed for each sample and the values obtained were averaged.

Calculations of density were made using the formula given by Bauer (1949) which yields results accurate within about 0.1 per cent.

The formula prescribed by Bauer is:

$$d = \left(\frac{W}{W_o}\right) d_o \qquad (1)$$

where,

$W$ = uncorrected weight of sample

$W_o$ = uncorrected weight of calibrating liquid

$d$ = density of sample at experimental temperature

$d_o$ = density of calibrating liquid at experimental temperature

This equation does not correct for the effect of air buoyancy of the samples on the balance pans.

Viscosity Determinations

An Ostwald capillary viscometer, within the range of pure water, was used to determine viscosities. All viscosities were conducted in a constant temperature water bath at 24.0° C. ($\pm$ 0.1° C.). The timing mechanism was a mechanical stop watch ($\pm$ 0.2 seconds).

Apparatus was arranged, by means of clamps, in such a manner, that the viscometer was always in the same vertical position within the constant temperature bath.

After filling the viscometer, it was placed in the constant temperature bath and the temperature was allowed to equilibrate by waiting for a period of 5 minutes before starting any determinations.

Viscosities were performed in the usual manner. That is, distilled water's known viscosity and density

were used to determine a constant C in relation to the viscometer used. Then, knowing C, the sample's viscosity was determined. The relation may be expressed mathematically as follows:

$$n_k = C\, p_k\, t_k \qquad (2)$$

where,

$n_k$ = viscosity of water

C = a constant

$p_k$ = density of water

$t_k$ = time of flow of water

then,

$$C = \frac{n_k}{p_k\, t_k} \qquad (3)$$

and,

$$n_u = C\, p_u\, t_u \qquad (4)$$

where,

$n_u$ = viscosity of sample

$p_u$ = density of sample

$t_u$ = time of flow of sample

Sedimentation Constant Analyses

It is well known, that in the determination of sedimentation constants, the sedimentation rate is

dependent upon concentration of the solute. Therefore, it is customary to measure the sedimentation rate at different concentrations and obtain the sedimentation constant of a freely sedimenting particle at infinite dilution by extrapolating to zero concentration.

The same procedure was employed for the sedimentation determinations reported within the present study.

Varying concentrations of the T3 particles were made by taking a measured volume (usually 2 ml.) of the stock material and placing it in an open glass vessel. The open glass vessel was transferred to a desiccator containing anhydrous $CaSO_4$. The desiccator was placed in a 37° C. incubator and the liquid was allowed to evaporate. It was found, by trial, that about 0.03 ml. of liquid evaporated per hour under the conditions described. When enough evaporation had occurred to give the desired concentration (determined by accurately measuring the remaining volume) the material was removed and centrifuged at 2,000 rpm. for 5 minutes in an International centrifuge (No. 2). The supernatant fluid was carefully withdrawn and transferred to the analytical cell of the ultracentrifuge. Two cells of 3 mm. and 12 mm. light-path diameters were used according to the concentration of the solute being centrifuged.

Calibration of the free thermocouple against the reference thermocouple was performed before the analytical sedimentation experiments were started.

All analytical experiments were performed at 12,590 rpm. (11,518 g) and photographs were taken at eight minute intervals after the centrifugal velocity had become constant.

The temperature of the analytical rotor was determined immediately preceding and following an experiment. This was accomplished with the free thermocouple which measures the temperature of the rotor. The mean temperature rise of all the sedimentation rate experiments was 0.3° C. The range of variance of the temperature was 0.0° C. - 0.9° C.

The measurements of the boundary displacements were made on the photographic images after the photographic plates were developed. At first, measurements were made only after the photographic images were enlarged, but it was decided to test the necessity of this extra manipulation. Both direct measurements and enlarged measurements were conducted on the same photographic image. The mean deviation between the two methods amounted to 0.006 cm. Since sedimentation rates, even under the best conditions, were reported reproducible to within

only about 1 per cent (Pickels 1952, Svedberg and Pedersen 1940), it appeared that the enlargement procedure could be obviated because the difference found between the two sets of measurements would mean a difference in the sedimentation rate of only about 0.5 per cent. A perusal of the literature revealed that the direct method of measurement is commonly employed (Svedberg and Pedersen 1940, Pickels 1944).

For each sedimentation experiment, measurements were made of all the boundary positions, except the last, and these values were averaged. Five boundary positions in each experiment were photographed. The first photograph was made immediately after the ultracentrifugal velocity had become constant and the four succeeding photographs were exposed at 8 minute intervals thereafter. The migration of the boundaries of the first four photographs was measured. Each migration of the boundary from the first four photographs was calculated for the sedimentation constant and these three values were averaged, yielding the sedimentation constant for a particular experiment.

Calculations of the sedimentation rates were made according to the equation of Svedberg.

The Svedberg expression states that by measuring

the position of the boundary which demarcates the supernatant fluid and sedimenting solute, at known intervals, the mean value for the sedimentation constant is obtained. Svedberg's equation is,

$$s = \frac{\ln x_2 - \ln x_1}{w^2 (t_2 - t_1)} \qquad (5)$$

where,

s = sedimentation constant

x = distance of boundary from axis of rotation

w = angular velocity

t = time

Sedimentation experiments are conducted at different temperatures in various laboratories and so, for the sake of comparison, it is customary to correct measured values of the sedimentation constant to some standard condition. Svedberg has standardized conditions not only in relation to temperature but also to the density and viscosity of the suspending medium. This sedimentation constant, symbolized $s_{w20}$, is the rate a material would have in a medium having the viscosity and density of water at 20° C. Svedberg's equation for $s_{w20}$ is:

$$s_{w20} = \frac{s\ n\ (o - p_{w20})}{n_{w20}\ (o - p)} \qquad (6)$$

where,

$n$ = viscosity of medium at experimental temperature

$o$ = density of solute

$p$ = density of medium

$n_{w20}$ = viscosity of $H_2O$ at 20° C.

$p_{w20}$ = density of $H_2O$ at 20° C.

Units of sedimentation are usually given in Svedberg units, symbolized S, which may be defined as centimeters per second x $10^{13}$. In other words, one Svedberg is $10^{-13}$ centimeter per second.

From the sedimentation and diffusion data, the particle weight may be computed. Svedberg has derived an expression utilizing these data. This formula is as follows:

$$M = \frac{RTs_{w20}}{D\left(1 - \frac{p}{o}\right)} \qquad (7)$$

where,

$M$ = particle weight

$R$ = gas constant

$T$ = absolute temperature

$D$ = diffusion constant

$s_{w20}$ = sedimentation constant

$o$ = density of particle

$p$ = density of medium

When calculating the T3 particle weight, the value for D in this equation was taken from the data of Polson and Shepard (1949). These authors reported the value for D as $1.19 \times 10^{-7}$ cm.$^2$ per second. The diffusion constant given by these authors was measured at various concentrations and extrapolated to zero concentration.

Not only particle weight can be estimated from $s_{w20}$ but also particle size. The apparent or equivalent diameter of a particle may be defined as the diameter of an unhydrated sphere of the same mass and volume as the actual particle under scrutiny and, therefore, does not correspond necessarily to the over-all dimensions of the particle.

Apparent diameters were estimated, assuming spherical particles and no hydration, from a formula based on Stokes' Law,

$$r^2 = \frac{9ns_{w20}}{2(p_v - p_s)} \qquad (8)$$

Here,

$r$ = radius of particle

$n$ = viscosity of $H_2O$ at 20° C.

$p_v$ = density of particle

$p_s$ = density of solvent

It is possible to estimate the particle size from infectivity and nitrogen data also. If the infectivity is determined, assuming that each plaque formed is the result of one phage particle (Luria, Backus and Williams 1951), and the nitrogen measured for a purified preparation (assuming here that no extraneous nitrogen is present), it becomes possible to approximate apparent particle sizes.

A relation expressed by Putnam (1950) is:

$$\text{weight of particle in grams} = \frac{4}{3}\pi r^3 p_v \qquad (9)$$

hence,

$$r = \left[ \frac{w}{\frac{4}{3}\pi p_v} \right]^{1/3} \qquad (10)$$

where,

$w$ = weight of a single infectious unit in grams

$r$ = radius of particle

$p_v$ = density of particle

$w$ may be obtained from the following relation:

$$w = \frac{N}{cP} \qquad (11)$$

where,

$N$ = grams of nitrogen

$c$ = concentration of phage from infectivity measurements

$P$ = nitrogen ratio to concentration weight

In these equations it is assumed that the bacteriophage is composed of all nitrogen-containing compounds. Such an assumption is, perhaps, reasonably justified since most bacteriophages, which have been chemically analyzed, are composed of approximately 100 per cent protein and nucleic acids (Taylor 1946, Kozloff and Putnam 1950, Hook et al. 1946 and Evans 1952).

In addition, particle weights may be computed from infectivity measurements. Using Avogadros' number,

$$M = wN \qquad (12)$$

in which,

M = particle weight

w = weight of a single infectious unit

N = Avogadros' number

All the assumptions made for the particle size expression from infectivity measurements must be considered also for this relation. Values obtained from it may be regarded as maximum values, since they depend on the efficiency of the biological assay and the purity of the bacteriophage preparation.

pH and Sedimentation Rate

Samples of stock bacteriophage of known concentration

(mg. per ml.) were sedimented for 30 minutes in the ultracentrifuge using rotor J at 42,040 rpm. (110,660 g). The pellets were resuspended in distilled water and centrifuged again for 30 minutes at the same velocity. The supernatant fluid was removed and the pellets were redispersed in buffer solutions at different pH values. These samples were analyzed for sedimentation properties. Each sample possessed the same concentration (mg. per ml.) of bacteriophage.

At pH 5 and pH 11, after ultracentrifuging at the operating velocity employed for the T3 particles in neutral pH, the rotor was accelerated to 54,000 rpm. (211,906 g) to determine the presence or absence of any other boundaries.

Precision of Techniques

It is important to know the precision of a set of measurements or of a laboratory procedure in an experiment. A statistical measure of dispersion or variance is often useful as an index of precision. Two of the most familiar parameters of variance are the standard deviation and the coefficient of variation. The standard deviation is expressed as:

$$\sigma = \left[ \frac{\sum_{i=1}^{N} (x_i - u)^2}{N} \right]^{\frac{1}{2}} \qquad (13)$$

$\sigma$ = standard deviation

$x$ = a measurement

$u$ = arithmetic mean

$N$ = number of measurements

The coefficient of variation is defined as:

$$C.V. = \frac{100\ \sigma}{u} \qquad (14)$$

This parameter is expressed in per cent while the standard deviation is given in the same units as those measured.

The coefficient of variation for viscosity determinations was 0.6 per cent. The same parameter for density measurements of the T3 particle was 15 per cent while that of the medium was 0.5 per cent. Nitrogen analyses gave a value of 23 per cent for the coefficient of variation. The sedimentation constants resulted in a value of 1.3 per cent for the same parameter.

In the determinations of viscosity and density of the suspending medium, the coefficient of variation applies to the method employed, since portions of the

same sample were used. For the remaining, probably most of the variance is due to differences between sample materials and not to the method or technique, since different samples of the same type of material were analyzed. It is known, for example, from repeated nitrogen determinations on preparations of bacteriophage produced in this laboratory, that analyses performed on portions of the same preparation are precise within less than 1 per cent.

The coefficient of variation for particle densities, nitrogen analyses and sedimentation constants gives an estimate of the degree of dispersion that may be encountered between different preparations of the T3 bacteriophage when measured under the conditions described in the present study.

## RESULTS

All values reported in this study are average values of all experiments completed for each particular item. For example, the density values are the average values of three determinations, viscosity results of five, nitrogen content of three and sedimentation constant of three.

The density of the suspending medium was 1.01 grams per ml. at 24.0° C. The T3 particle density was 1.43 grams per ml. at the same temperature. Its specific volume, which is the reciprocal of the density, was 0.70 ml. per gram.

The nitrogen content of the T3 bacteriophage was found to be 9.3 per cent. On a gram weight basis, the nitrogen content was $0.3 \times 10^{-16}$ gram of nitrogen per infective bacteriophage particle.

The sedimentation of T3, at neutrality, is depicted in figure 2. A single, sharp, symmetrical boundary migrating at a constant rate is observed. It is noted that the light-absorbing region, due to opalescence of the bacteriophage, sediments with this boundary.

Sedimentation constants of three preparations of T3 suspended in buffer (pH 7) are given in figures 3, 4 and 5. Figure 6 is a plot of mean values for these

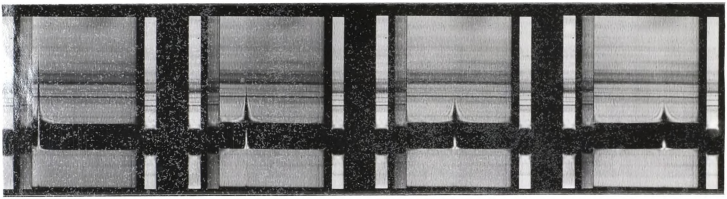

Figure 1. A single, sedimenting boundary of T3 bacteriophage suspended in buffer (pH 7) at a concentration of 3.9 mg. per ml. Centrifugal force = 12,590 rpm (11,510g). Time interval between exposures = 8 minutes.

PREPARATION A

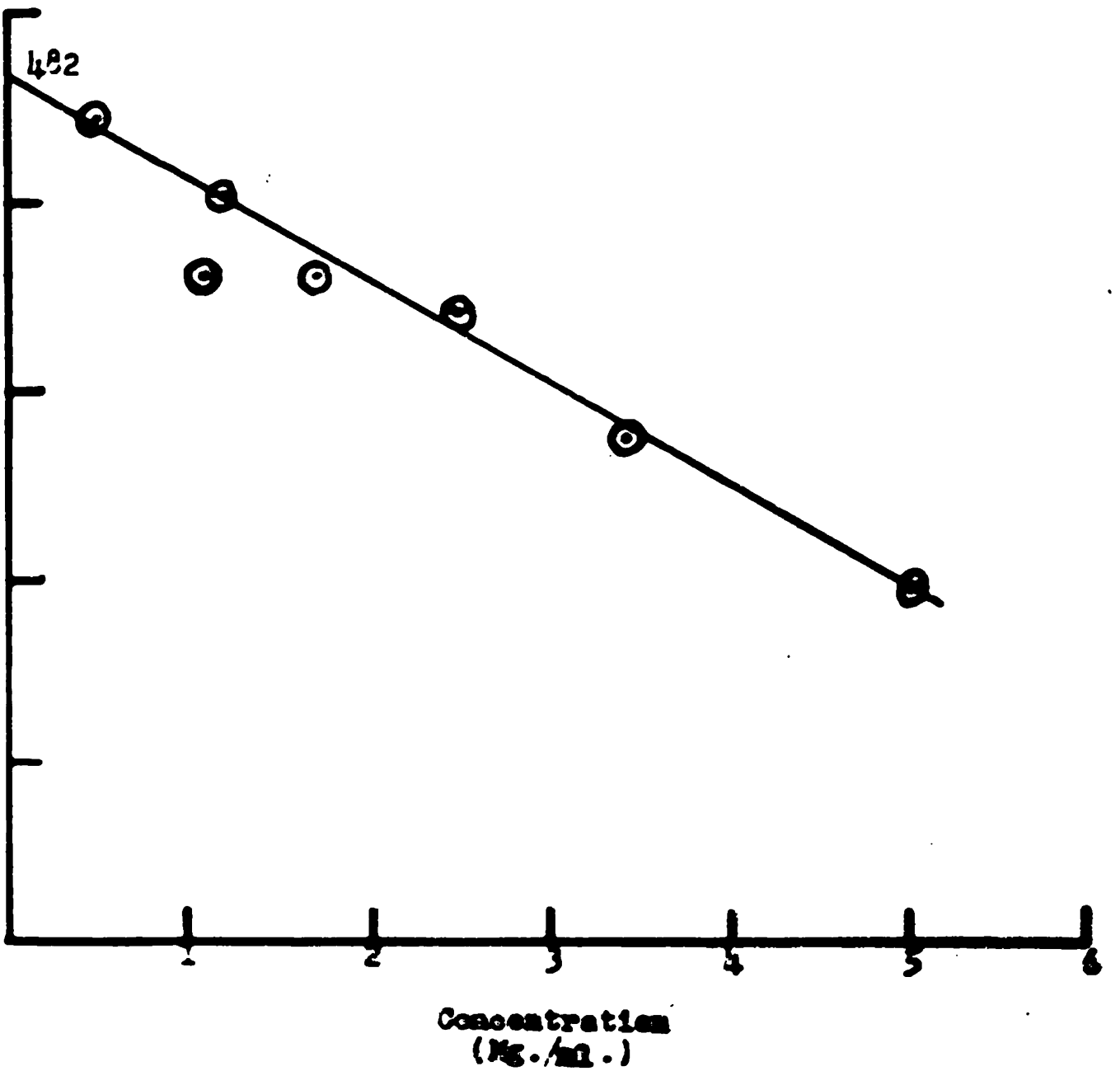


Figure 3. Sedimen[illegible] experiments performed at an average temperature of 2[illegible] pH 7 buffer as the suspending medium.

PREPARATION B

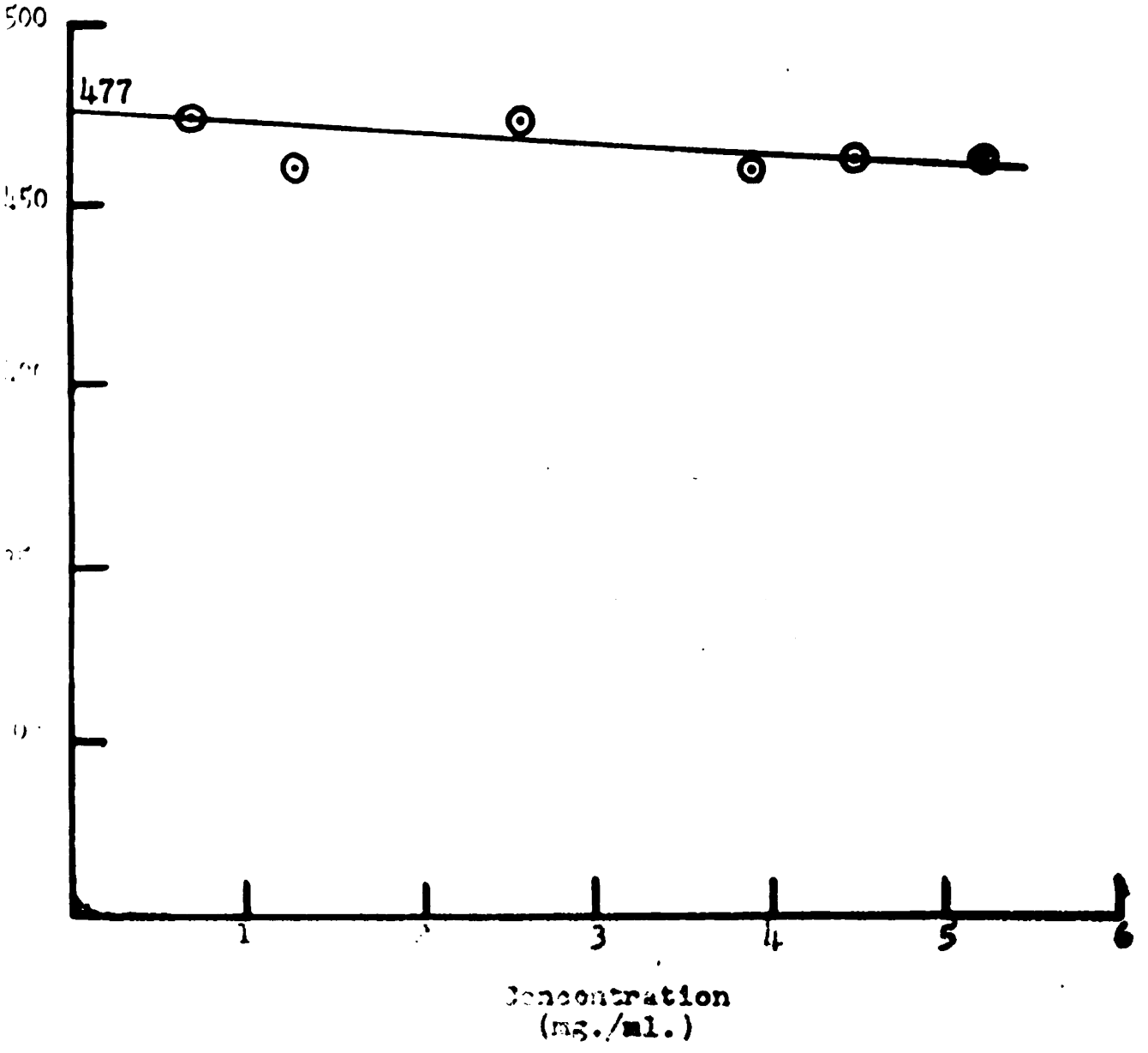


Figure 4. Sedimentation [illegible] performed at an average temperature of [illegible] the suspending medium.

PREPARATION C

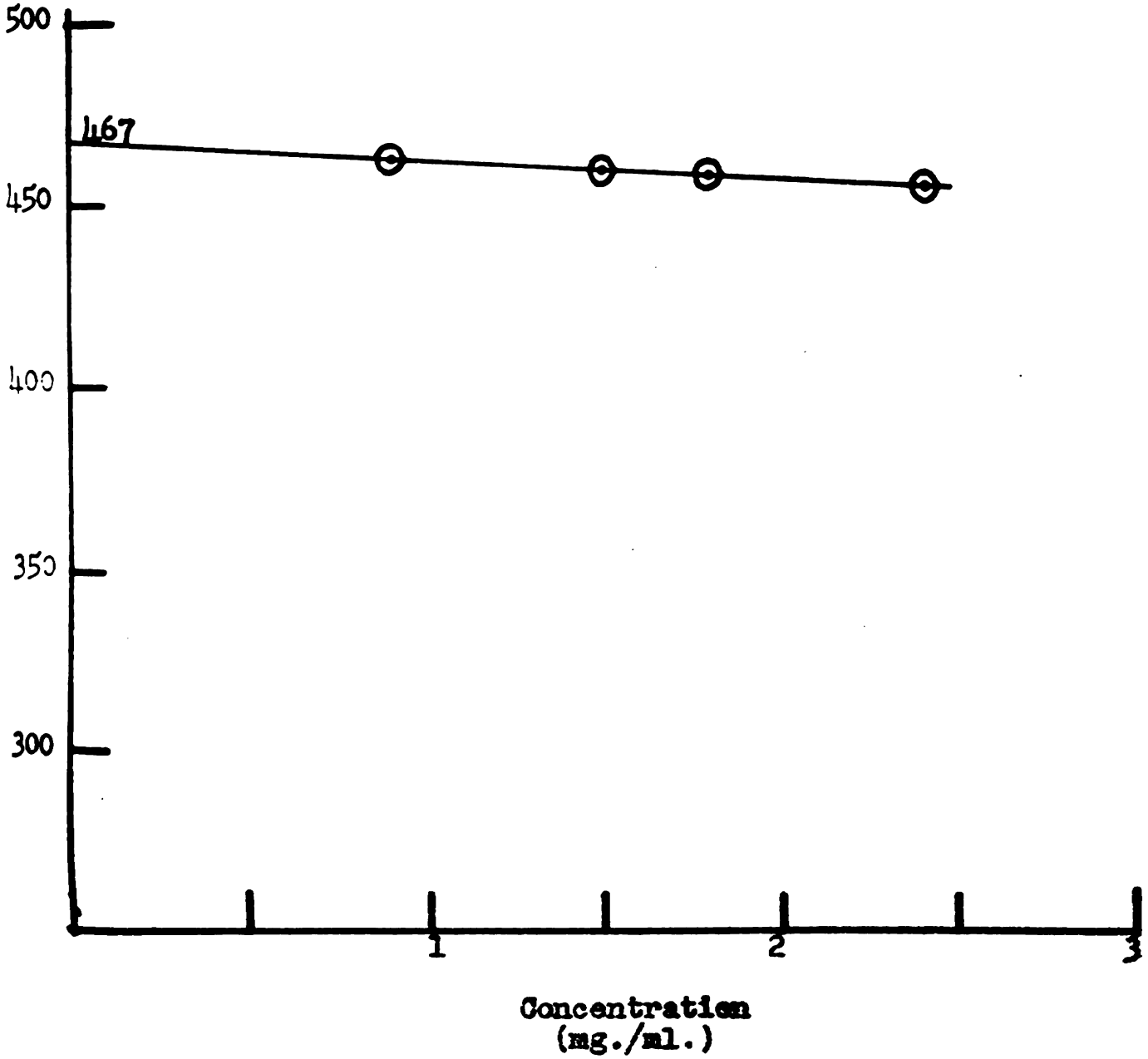


Figure 5. Sedimentation [illegible]periments performed at an average temperature of 25.1°C. [illegible]7 buffer as the suspending medium.

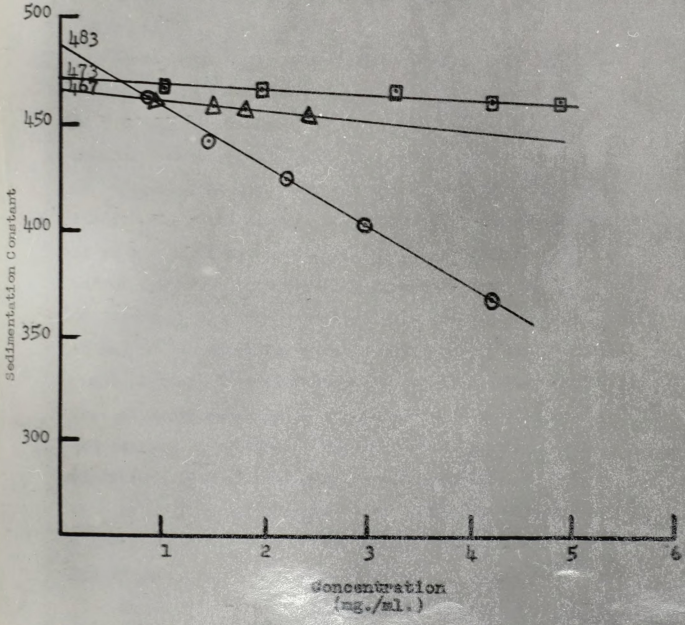


Figure 6. Mean value plots of preparations A, B and C.

constants, derived from the graphic method of Askovitz (1955). It is seen that the extrapolated values from the mean plots (483S, 473S and 467S) do not change significantly from the direct extrapolations (482S, 477S and 467S).

From these three preparations the $s_{w20}$ was computed, yielding a value of 469S.

Within the range of pH 6 - pH 10 only a single boundary was observed.

Figure 7 is a plot of pH versus sedimentation constant. The small differences observed within the range of pH 6 - pH 10 may be due to the use of different buffer systems. This same phenomenon is reported for T7 (Kerby et al. 1949) and these authors suggest that it may be due to differences in hydration of the bacteriophage particle in different buffer systems. They state that although corrections for differences in viscosity and density of the buffer systems were made the slight variations in the sedimentation rate remained.

At pH 11 a multiple boundary, which was rather diffuse, appeared (figure 8), indicating degradation of the T3 particle.

At pH 5 the sedimentation rate increased to such an extent that the lowest, automatically-controlled, centrifugal

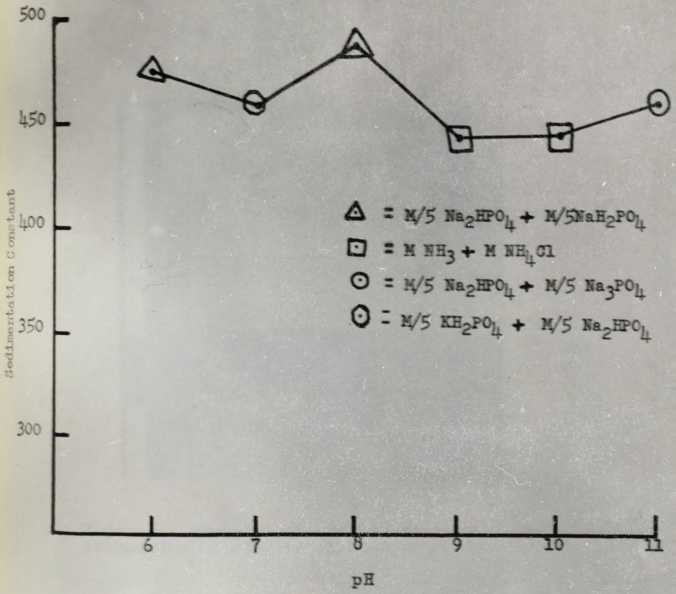


Figure 7. Sedimentation experiments performed at a concentration of 1.5 mg. per ml. and an average temperature of 24.0°C.

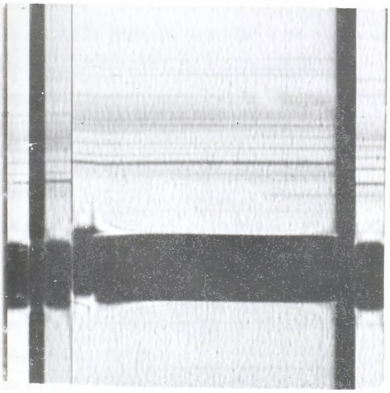

**Figure 8.** Asymmetrical, diffuse boundary of T3 sedimenting in a buffer adjusted to pH 11.

velocity on the ultracentrifuge proved to be too great to obtain a sedimentation pattern. A boundary accumulated at the meniscus at about 5,000 rpm. which, on further application of centrifugal force, became very diffuse and in a short time disappeared completely.

A possible explanation for such sedimentation behavior at pH 5 might be the occurrence of heterogeneous aggregation of the particles. That is, at this pH the T3 population might be composed of aggregates containing different numbers of particles.

At pH 5 and pH 11, after termination of the usual observation period, the centrifugal force was increased to 54,000 rpm. (211,906 g) to determine if boundaries other than those observed at the centrifugal force employed for sedimenting T3 particles were present. None were observed.

Figure 9 demonstrates the absence of boundaries at 54,000 rpm.

Using the sedimentation data, the apparent diameter of the T3 particle was calculated from equation (8), resulting in a value of 45 mu, while that calculated from equation (10), utilizing biological data, was 76 mu. The particle weight of T3 was computed from equations (7) and (12). Equation (7), employing sedimentation information,

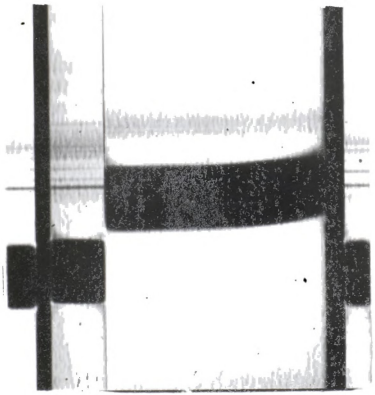

Figure 9. Photograph showing the absence of any boundaries in buffer (pH 11) at a centrifugal force of 54,000 rpm (211,906 g).

gave a value of 28 x $10^6$ and equation (12), which utilizes biological data, gave a value of 199 x $10^6$.

Figure 10 is an electron micrograph of a preparation of the T3 bacteriophage. The preparation is quite homogeneous in relation to size and morphology of the particles and little extraneous material is present. The T3 particle displays the shape of an almost spherical structure which, upon close observation, is seen to have somewhat of a hexagonally-shaped periphery. Also irregularities appear on the surfaces of many of the particles. These irregularities are illustrated better in figure 11.

In figure 12 it can be observed that some of the particles display definite, short processes extending from their main structures.

Unshadowed preparations had been expected to reveal some internal structure of the T3 particle. This was not demonstrated (figure 13) because of the low absorbing power for electrons by the T3 particle.

Measurements of the particles were made from shadowed and unshadowed air-dried specimens. The average diameter of the particle in the shadowed photographs measured 50 mu, while in the unshadowed, the average diameter measured 38 mu. These two values differed by 24 per cent.

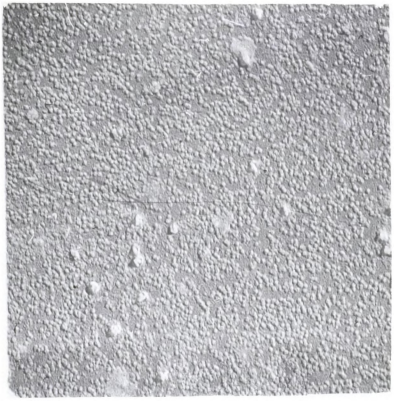

Figure 10. An air-dried specimen of the T3 bacteriophage stock suspension. Magnification (25,500 ×). Palladium shadowed.

Figure 11. Photograph of T3 particles displaying irregularities on the surfaces of the particles. Magnification (58,500 X). Palladium shadowed.

Figure 12. Photograph showing "tails" of the T3 particles (circles). Magnification (47,500 X). Palladium shadowed.

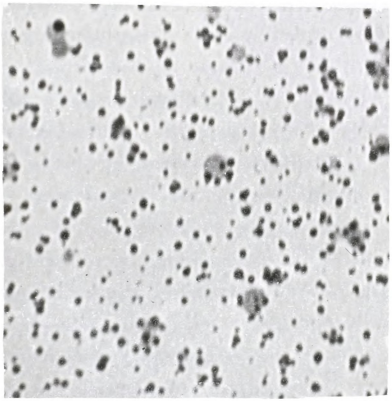

Figure 13. An unshadowed, air-dried specimen of the T3 bacteriophage stock suspension. Magnification (48,960 X).

## DISCUSSION

A substance which sediments with a single, sharp, symmetrical boundary is customarily designated an "apparently molecularly homogeneous" substance. This designation is meant to convey the understanding that a substance is purified within the limits of an ultracentrifugal method. It is entirely possible that impurities may be present in concentrations below that of the sensitivity of the ultracentrifugal instrument. Moreover, an "apparently molecularly homogeneous" substance need not be homogeneous in other respects; for example, it may not be homogeneous in relation to its electrophoretic properties. Thus, semantically, it would be more correct, perhaps, to label such a substance "apparently centrifugally homogeneous". Nevertheless, a single sedimenting boundary of the T3 bacteriophage may be obtained.

The purity of a substance, as defined above, can be judged qualitatively by inspection of the sharpness and symmetry of the boundary. The sharper the boundary, the more homogeneous is the substance.

Sedimentation diagrams that have been observed in the present investigation appear to be as sharp and

symmetrical as any that have been reported in the literature for any of the bacteriophages (Putnam et al. 1949, Jesaitis and Goebel 1955, and Putnam et al. 1952).

In order to make a comparison of the measured particle constants of T3 with those of other purified bacteriophages more facile, figure 1 has been inserted once more on page 46 but this time with the values of T3 tabulated. The strains, which are related morphologically and serologically, have been grouped together.

Since there is nothing in the literature on the sedimentation properties of T3, comparison cannot be made with values obtained by other workers. However, comparisons can be made between the morphologically similar and serologically related T7 phage (Kerby et al. 1949, Putnam et al. 1952).

From figure 1 it is seen that T3 and T7 are serologically related. Also, they are very similar in size and shape when viewed with the electron microscope. Possessing such similarities, they might be expected to be similar in some other respects. Their $s_{w20}$ values (480S for T7 and 469S for T3) differ by approximately 2 per cent. The specific volumes (0.68 for T7 and 0.70 for T3) agree within 3 per cent and the particle weights (31 x $10^6$ for T7 and 28 x $10^6$ for T3), derived from sedimentation,

| Type | Ultracentrifuge | | Diffusion | | Specific Volume | Infectivity | | Particle Weight | |
|---|---|---|---|---|---|---|---|---|---|
| | $S_{w20}$ $10^{-13}$ sec. | $d_{eq}$ mu | $D_{w20}$ $10^{-7}$ $cm^2$ $sec^{-1}$ | $d_{eq}$ mu | ml/g | gN per phage | $d_{eq}$ mu | $S_{w20}$ $10^6$ | Biol. $10^6$ |
| T1 | | | | | | | | | |
| T5 | | | | | | | | | |
| T2 | 1000<br>700 | 59<br>50 | | | 0.66 | 1.3 x $10^{-16}$ | 107 | | 584 |
| T4 | | | 0.80 | 53 | | | | | |
| T6 | 1050<br>825 | 61<br>54 | 0.45 | 94 | 0.66 | 1.0 x $10^{-16}$ | 98 | 167 | 460 |
| T3 | 469 | 45 | 1.19 | 36 | 0.70 | 0.3 x $10^{-16}$ | 76 | 28 | 199 |
| T7 | 480 | 43 | | | 0.68 | 0.5 x $10^{-16}$ | 80 | 31 | 246 |
| Staph | 650 | 77 | 0.18 | 235 | 0.83 | 1.0 x $10^{-16}$ | 93 | 530 | 300 |

Figure 1. Particle constants of some purified bacteriophages. (after Evans, 1952).

agree within less than 10 per cent. Putnam et al. (1952) report $s_{w20}$ values for T7 which varied from 450S to 490S with a mean of 465S.

Kerby et al. (1949) reported that the T7 bacteriophage at very dilute concentrations (0.025 mg. per ml.) exhibits a much lower sedimentation rate than at the commonly employed concentration range (5 mg. to 0.5 mg. per ml.). These authors stated that they performed the sedimentation experiments on T7 by an absorption method which permits measurements of bacteriophages at dilute concentrations. This was possible because of the bacteriophage's high absorbancy for ultraviolet light. The instrument used in the present investigation for the sedimentation of T3, employed the refractive index method and did not allow measurements of very dilute suspensions. Therefore, it was not possible to determine whether T3 displayed similar sedimentation behavior with the refractive method. Sharp et al. (1950) reported that this anomaly occurs with dilute suspensions of polystyrene latex particles also. These authors conclude that it is due to instability of the boundary between the supernatant fluid and the sedimenting solute.

Dual sedimentation has been reported for the bacteriophages T2 and T6. With T2 a single boundary of

about 1000 S is always found below pH 5.8 and a single boundary of about 700 S is obtained above pH 5.8. An immediate transition from the slow rate to the fast can be accomplished by pH adjustment and the transition is reversible. A similar effect of pH on sedimentation behavior is observed with the T6 phage, except that both forms frequently have been found together over a wide range of pH. The fast component is seen singly at pH 4.9 and has a value of about 1050 S while the slower component has a value of about 800 S.

Taylor et al. (1955) claimed that in their work on the T2 phage they found, by indirect methods, that the dual sedimentation behavior was not due to reversible aggregation or hydration but to a reversible change in shape of the particles.

The stability of the sedimentation rate for T7, when subjected to changes in pH, covered a fairly wide range (Kerby et al. 1949). T7 sedimentation was found to be constant from pH 5 through pH 10. In comparison, the stability for T3 ranged from pH 6 through pH 10.

A peculiar behavior by T3 was observed when the pH was adjusted to 5. At this pH, the sedimentation rate increased greatly. At pH 11, T3 showed signs of degradation. This was evidenced by the appearance of a multiple boundary. T7 also displayed another boundary at pH 11 (Kerby et al. 1949)

In the pH region where sedimentation stability was apparent, small variations occurred in the sedimentation constants. Upon plotting the data, a correlation appeared to exist between sedimentation constant and the type of buffer system utilized. This same phenomenon was reported for T7 (Kerby et al. 1949) and these workers pointed out that the small differences observed may be due to differences in hydration of the bacteriophage particles.

The close agreement between $s_{w20}$ values of different preparations of T3 is satisfying. This indicates that the method of preparation used in this study is quite reproducible. Indeed, when one considers the extreme variability that may be encountered in biological materials, such agreement is almost amazing.

Electron micrographs of the T3 and T7 particles reveal structures almost morphologically identical. The irregularities observed in shadowed preparations and the inability to depict defined, internal structures in the unshadowed samples were reported also for T7 by Kerby et al. (1949).

It had been mentioned before that electron micrographs of the T3 particles displayed short processes extending outward from the main structures of the particles.

These might be considered short "tails", analogous to the "tails" of T1, T2, T4, T5 and T6. These "tails" were visible in only a fraction of the population but, probably, this could have been correlated with the plane of orientation of the "tails". T7 displayed the same short, stubby "tail" (Kerby et al. 1949).

The difference in the two particle size measurements, made from shadowed and unshadowed preparations, could be due to the high contrast accomplished by shadow-casting, thereby rendering the outer edges of the particles visible. Since the extent to which matter scatters electrons depends on its density, it would appear that the T3 bacteriophage is not homogeneously dense but contains a less dense periphery circumscribing a more dense core.

Kerby et al. (1949) reported particle sizes for T7 measured from shadowed and unshadowed specimens. Their values were 73 mu for the shadowed and 51 mu for the unshadowed samples, resulting in a difference of 31 per cent between the two measurements. Electron micrographs of T3 have been published by Williams and Fraser (1953). These authors utilized frozen-dried preparations. Their particle size measurements from shadowed samples gave a value of 47 mu. They presented excellent

photographs which demonstrated the short tails and hexagonal shapes. In another publication, Fraser and Williams (1953) photographed T3 from air-dried preparations. The particle measurements from these air-dried, shadowed samples were 50 mu. Earlier measurements (Anderson 1946) gave a value of 45 mu.

Upon comparing the bacteriophages T3 and T7, it becomes evident that they are closely related and very similar. It appears that the only significant difference between the two lies in the degree of their response to homologous antisera.

## SUMMARY

The T3 bacteriophage has been investigated in relation to some of its physical properties and, particularly, its sedimentation behavior.

The $s_{w20}$ was found to be 469 S when the values from three determinations were averaged. A single, sharp, symmetrical boundary, migrating at a constant rate, was observed.

Sedimentation stability extended through pH 6 - pH 10. At pH 11, degradation of the virus occurred. Increased sedimentation rate was observed at pH 5.

The specific volume of the T3 particle, derived from three measurements and averaged, was 0.70 ml. per gram.

Micro-Kjeldahl analyses revealed 9.3 per cent nitrogen for the T3 bacteriophage and $0.3 \times 10^{-16}$ gram of nitrogen per infective particle. These values were lower than those found for T7, and could be indicative of less extraneous material in the T3 preparations.

Equivalent diameters, calculated from sedimentation and infectivity data, gave values of 45 mu and 76 mu, respectively, while particle weights, computed from

sedimentation and infectivity data, gave values of $28 \times 10^6$ and $199 \times 10^6$, respectively.

Particle sizes of T3 measured directly from electron micrographs averaged 50 mu.

The shape of the T3 particle in air-dried preparations was roughly spherical but, on closer observation, it was noticed that the particles displayed hexagonally-shaped structures and possessed a short, stubby "tail". The particles were not of uniform density.

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# SEDIMENTATION CHARACTERISTICS OF THE T3 BACTERIOPHAGE OF ESCHERICHIA COLI B

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AN ABSTRACT

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## ABSTRACT

Some of the physical properties of the T3 bacteriophage of Escherichia coli B were investigated and, in particular, its sedimentation behavior. The specific volume of the T3 particle was 0.70 ml. per gram. Nitrogen analyses of the T3 bacteriophage revealed 9.3 per cent nitrogen. The nitrogen content of the infective T3 particle was $0.3 \times 10^{-16}$ gram of nitrogen. The sedimentation constant was 469 Svedberg units. A single, sharp, symmetrical boundary, migrating at a constant rate, was observed. Sedimentation stability extended through pH 6 - pH 10. At pH 11 degradation of the bacteriophage occurred. Increased sedimentation rate was observed at pH 5. Equivalent diameters, calculated from sedimentation and infectivity data, were 45 mu and 76 mu, respectively. Particle weights, calculated from sedimentation and infectivity data, were $28 \times 10^6$ and $199 \times 10^6$, respectively. The particle size of the T3 bacteriophage, measured directly from electron micrographs of air-dried specimens, was 50 mu. The T3 particle's morphology was roughly spherical in air-dried preparations. Stubby "tails" were observed on some of the particles.