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# Low angle light scattering studies on whole, half, and quarter molecules of T2 bacteriophage DNA

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ABSTRACT Static light scattering measurements have been made at angles as low as 8° on whole, half, and quarter molecules of native, T2 bacteriophage DNA in 0.195 M $Na^+$. The fragments were obtained by high-speed stirring of the native DNA, and fractionated on methylated-albumin-kieselguhr columns. Accompanying measurements of sedimentation coefficients and intrinsic viscosities were made. Because linear extrapolations of light scattering data above 8° for these samples were suspect, the measurements were analyzed by fitting curves calculated from the theory of wormlike coils to experimental curves at $c = 0$. Results showed that the excluded volume parameter, $\epsilon$, must be used in analyzing the scattering curves; a reasonable value of $\epsilon$ was 0.08, in agreement with that found for T7 DNA (Harpst, J. A. 1980. *Biophys. Chem.* 11:295–302). The persistence length of all three DNAs in this paper was 50 ± 5 nm, showed no dependence on molecular weight, but was somewhat below that reported previously for T7 DNA (60 nm). Theoretical curves calculated with the preceding parameters had a clear upward curvature in scattering envelopes below 8° for quarter and half molecules, but such curvature was minimal for whole T2 DNA, so that linear extrapolations of experimental data above 8° gave a molecular weight and root-mean-square radius which were nearly the same as those from theory. The molecular weight and radius for whole T2, derived from the comparison of theory and experiment, were $115 \times 10^6$ and 1,224 nm, respectively. The measurements on T2 DNA were clearly at the upper limit of current techniques.

## INTRODUCTION

The unique double-helical structure of native DNA imparts to the molecule its essential biological functions. To elucidate and understand the details of these functions, it is necessary to determine the structural characteristics of DNA, some of which can be obtained from the size and other physico-chemical properties of the molecule in solution. Molecular weights have intrinsic importance as indicators of the size, and as the basis for manipulation, of DNA molecules in the chromosomes of various organisms (Roberts et al., 1975; Smith et al., 1986). Another indicator of molecular size is the extension of the molecule in solution, often measured by sedimentation, viscosity, diffusion, or other properties that are functions of the root-mean-square radius (Tanford, 1961; Bloomfield et al., 1974; Cantor and Schimmel, 1980). Measurements of this type clearly show that native DNA is a relatively rigid molecule. This realization led to recognition of the packing problem, which deals with the mechanism and energy required to condense a very large and relatively rigid DNA into the small volume in viruses or cellular nuclei. A complete understanding of packaging must include knowledge of the flexibility of DNA, one measure of which is the persistence length of the wormlike coil (Eisenberg, 1974; Bloomfield et al., 1974; Schurr and Schmitz, 1986). It is also apparent that a detailed understanding of packaging, as well as the biological functions of DNA in replication and transcription, requires knowledge of the inter- and intramolecular interactions of DNA, including its behavior as a polyelectrolyte (Manning, 1978; Anderson and Record, 1982; Schurr and Schmitz, 1986).

A variety of techniques has been developed for and applied to studies of native DNA (Bloomfield et al., 1974; Cantor and Schimmel, 1980; Rousseau, 1984). One of these, static (or Rayleigh) light scattering, was widely used in establishing basic solution properties of DNA (Geiduschek and Holtzer, 1958; Eigner and Doty, 1965; Eisenberg, 1974; Bloomfield et al., 1974), but it fell into disfavor because of problems with the technique and analysis of data (Geiduschek and Holtzer, 1958; Harpst et al., 1968; Schmid et al., 1971; Harpst, 1980). However, when these difficulties are appropriately addressed, static light scattering has several advantages over other methods, including the generation of a substantial amount of information from a single set of measurements. It is an independent method, which can provide molecular weights, $M$, without prior assumptions of molecular shape and without prior calibration of a measured property against samples with established molecular weights (Tan-

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ford, 1961; Roberts et al., 1975). Theoretically, it is applicable in a molecular-weight range between a few thousand to several millions. Light scattering is one of the few techniques that provide a direct measure of molecular shape or extension through the root-mean-square radius, $R$. In addition to the size of molecules, the method provides a measure of intermolecular interactions through the second virial coefficient, $A_2$. Further theoretical and experimental developments (summarized in Harpst, 1980) have shown how experimental scattering curves can be analyzed to provide estimates of the persistence length, $a$, and an excluded volume parameter, $\epsilon$, which is a function of intramolecular interactions (Sharp and Bloomfield, 1968; Bloomfield et al., 1974). This analysis has provided reliable values of $M$ and $R$ from static light scattering measurements on native DNA (Harpst, 1980).

This paper reports static light scattering measurements on native T2 bacteriophage DNA and on fractionated samples of its half and quarter molecules. Analysis of the data by the method described previously (Harpst, 1980) gives a molecular weight for T2 DNA of $115 \times 10^6$, in good agreement with values obtained by a variety of methods (Chapman et al., 1969; Freifelder, 1970; Lang, 1970; Roberts et al., 1975; Weissman et al., 1976; Bowen and Zimm, 1978). These results indicate that the analysis (Harpst, 1980) is reliable and establish an upper molecular-weight limit of $\sim 10^8$ for light scattering measurements at angles of 8° and above. The measurements on native T2 DNA, along with those on the fractionated half and quarter molecules, provide data on a series of native DNAs with molecular weights of $24 \times 10^6$, $64 \times 10^6$, and $115 \times 10^6$. Data from all three DNAs in the series indicate that the excluded volume parameter (Sharp and Bloomfield, 1968; Eisenberg, 1974; Bloomfield et al., 1974) for native DNA should be ~0.08, and that the persistence length (Kratky and Porod, 1949; Eisenberg, 1974; Bloomfield et al., 1974) is ~50 nm. Finally, the molecular-weight results are correlated with additional measurements of sedimentation coefficients and intrinsic viscosities.

## EXPERIMENTAL

## Materials

Commercially-available chemicals of the highest available purity were used. Phenol was freshly distilled under $N_2$ and saturated with 0.1 M buffered saline (0.033 M $NaH_2PO_4$, 0.067 M $Na_2HPO_4$, 0.1 M NaCl, pH 7.1). Methylated albumin was obtained from Worthington Biochemical Corp., Freehold, NJ. All DNA solutions (Harpst et al., 1968; Krasna et al., 1970) were in BPES buffer (0.06 M $Na_2HPO_4$, 0.002 M $NaH_2PO_4$, 0.001 M $Na_2$ · EDTA, 0.179 M NaCl, pH 6.8), unless otherwise noted.

## Methods

### Growth and purification of bacteriophage

T2 bacteriophage and its *E. coli* B host were obtained from Dr. L. Astrachan. *E. coli* B were grown at 37°C in a Microferm fermentor (New Brunswick Scientific Co., Inc., Edison, NJ) in five liter batches of 3XD (glycerol) medium (Fraser and Jerrel, 1953) with vigorous aeration. When the medium with cells reached 100–150 Klett units (measured with a red, No. 66 filter), it was infected with $10^8$ T2 bacteriophage per milliliter. After bacteriophage growth was complete, 50 ml $CHCl_3$ were added to complete lysis. Bacteriophage were harvested either by high-speed centrifugation or by a two-step polymer phase separation method (Albertsson, 1960). Purified bacteriophage were dialyzed into 0.4 M NaCl, 0.001 M $MgCl_2$ and stored at 4°C.

### Preparation of T2 DNA

DNA was prepared from the bacteriophage by extracting three times (Mandell and Hershey, 1960) with freshly-distilled, buffer-saturated phenol. Residual phenol was removed by exhaustive dialysis of the DNA extract against BPES buffer at 4°C. Stock solutions of DNA (300–600 μg/ml) were stored at 4°C over several drops of $CHCl_3$.

Concentrations were determined as described previously (Krasna and Harpst, 1964; Harpst et al., 1968; Dawson and Harpst, 1971*a*) from absorbances at 260 nm, $A_{260}$, and the specific absorption coefficient, $\epsilon_{260} = 18.1$ liter · $g^{-1}$ · $cm^{-1}$, for T2 DNA (Rubenstein et al., 1961). Dilutions of concentrated stock solutions were allowed to stand at least 1 d at 4°C with periodic, gentle swirling to allow dispersal of the DNA (Aten and Cohen, 1965; Bowen and Zimm, 1978).

Measurements of the ratios of absorbances at indicated wavelengths were used to estimate the purity of DNA preparations. The samples used in this study had ratios of $A_{260}/A_{280} = 1.8 \pm 0.1$ and $A_{260}/A_{230} = 2.5 \pm 0.3$. These are within or exceed the accepted values for protein-free DNA (Thomas and Abelson, 1966).

### Preparation and fractionation of T2 DNA fragments

Fragments of T2 DNA were prepared generally as outlined by others (Hershey and Burgi, 1960; Burgi and Hershey, 1961). Native T2 DNA at concentrations of 0.6 mg/ml in BPES buffer was sheared in a 5 ml flask of an homogenizer (model 16-200; Virtis Co., Gardiner, NY) with crossed blades on a microshaft (model 16-205). Stirring speed was calibrated with a stroboscope and controlled by a variable transformer and voltmeter.

Half molecules of T2 DNA were prepared by stirring for 1 h at 6,000 rpm and 4°C. Quarter molecules were obtained from whole T2 DNA by stirring for 1 h at 10,000–11,000 rpm in a 4°C coldroom. Fragmentation of the DNA was monitored at regular intervals by relative viscosity measurements (see below).

Both the half and quarter molecules were fractionated on methylated-albumin-kieselguhr (MAK) columns (Hershey and Burgi, 1960; Mandell and Hershey, 1960). Columns were prepared (30 mL) as described by Sueoka ad Cheng (1967). Appropriate amounts of the sheared DNA in BPES were loaded onto the columns, which were then eluted with a linear gradient (Sueoka and Cheng, 1967; Osawa and Sibatani, 1967) of buffered saline (0.5–0.9 M NaCl for half molecules and 0.4–0.9 M NaCl for quarter molecules). Elution profiles similar to ones described by others were obtained (Hershey and Burgi, 1960; Burgi and Hershey, 1961). Fig. 1 shows a column profile for halves of T2. After fractionation, appropriate peak fractions were selected (see Fig. 1) to eliminate the largest and smallest molecules. The fractions were combined and dialyzed against BPES.

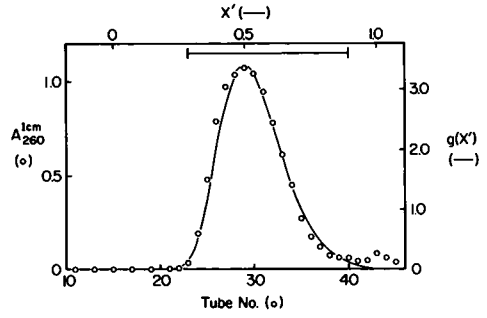


FIGURE 1 Fractionation and length distribution of T2 half-molecules from a MAK column. *Open circles* (O) show the column elution profile, $A_{260}^{1cm}$ versus Tube number. The *solid curve* (—) is the Schulz-Zimm distribution, calculated from Eq. 1 and the parameters given in the text. The bar (⊢⊣) indicates fractions pooled for subsequent measurements.

### Viscometry

Relative and instrinsic viscosities, $[\eta]$, were measured at 25.00 ± 0.02°C in a Couette viscometer as previously described (Dawson and Harpst, 1971*a*). For T2 DNA and its half molecules a rotor with low shear stress was used ($1.7 \times 10^{-3}$ dynes/cm$^2$, rotation time = 144.6 s in water at 25°C). Rotors with higher shear stresses (Dawson and Harpst, 1971*a*) were used for the quarter molecules.

### Sedimentation velocity analyses

Boundary sedimentation velocities of whole, native T2 DNA were measured at 20–25°C as described by Crothers and Zimm (1965). The samples were centrifuged at 15,000 rpm in the Spinco (model E; Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) ultracentrifuge in order to avoid the concentration-dependent speed effect (Aten and Cohen, 1965; Crothers and Zimm, 1965; Rosenbloom and Schumaker, 1967). Boundary sedimentation velocities were corrected to standard conditions, as described by Studier (1965), with the density (1.0085 g/cm$^3$, relative to water at 4°C) and viscosity (1.020, relative to water) of BPES, measured at 25.0°C in this laboratory. Values of $s_{20,w}$ were obtained over a range of concentrations (5–40 μg/ml) and extrapolated to zero concentration to give $s^{\circ}_{20,w}$.

Band sedimentation velocities were measured as described previously (Dawson and Harpst, 1971*a*), except that the rotor speeds used were 15,000 rpm for whole T2 DNA and 26,000 rpm for the smaller fragments.

### Light scattering

The instrument and techniques were the same as described elsewhere (Dawson and Harpst, 1971*a*).

Clarification of most DNA solutions was achieved by direct filtration into the light scattering cell through a 0.22 μm pore-size, type GS, MF-Millipore (Millipore Corp., Bedford, MA) filter (Harpst et al., 1968; Dawson and Harpst, 1971*a*). One preparation of whole T2 DNA could not be clarified by this method. Instead, a stock solution at ~50 μg/ml was clarified by passage first through a 0.45 μm, type HA filter, followed by four passes through a 0.22 μm membrane. Precleaned glassware and pipettes (Harpst et al., 1968; Krasna et al., 1970) were then used to dilute the clarified stock solution with filtered BPES and to make transfers to the light scattering cell.

Light scattering measurements were made at 546 nm on DNA solutions in BPES as described earlier (Krasna et al., 1970; Dawson and Harpst, 1971*a*). Data were processed by computer with the constants used in a preceding study (Harpst, 1980). Results have been analyzed in two ways. The first was by the reciprocal-intensity plots used previously (Harpst et al., 1968; Krasna et al., 1970; Dawson and Harpst, 1971*a*) in which linear extrapolations of $\mathcal{H}c/\mathcal{R}_\theta$ were made over the lowest accessible angles, $\theta$. $\mathcal{H}$ is an optical constant, $c$ is the concentration, and $\mathcal{R}_\theta$ is the Rayleigh ratio at the given angle. As shown in an earlier paper (Harpst, 1980), this method gives an apparent molecular weight, $M_A$, an apparent root-mean-square radius, $R_A$, and the second virial coefficient for high-molecular-weight T7 DNA. For later discussion, it should be noted that the value of $M$ determined by this procedure from light scattering is a weight-average value, $\overline{M}_w$, and $R$ is a $z$-average, $\overline{R}_z$, when the sample is heterogeneous. The second type of analysis was to compare the experimental light scattering curves, corrected to zero concentration (Harpst, 1980), with curves calculated from theory (Sharp and Bloomfield, 1968; Schmid et al., 1971). Details of this method, including the necessary parameters, have been described (Harpst, 1980). This procedure gives the correct (or theoretical $_T$), limiting molecular weight, $M_T$, limiting radius $R_T$, an estimate of the excluded volume parameter, $\epsilon$ (Sharp and Bloomfield, 1968), and the statistical segment length, $1/\lambda'$ (Kuhn, 1934; Sharp and Bloomfield, 1968). For the very long molecules here, the Kuhn length is twice the Kratky-Porod (1949) persistence length, $a$ (Eisenberg, 1974; Bloomfield et al., 1974).

## RESULTS

### Sample characteristics

Native T2 bacteriophage DNA was carefully isolated by the standard procedures described above, in order to provide solutions of unbroken molecules. Pertinent data included in following sections show that whole molecules were obtained, with the reservations indicated by the results.

The half- and quarter-molecule fragments were polydisperse, because they were obtained by mechanical shear. The distribution of half molecules is shown in Fig. 1. Comparison with earlier results of Hershey and Burgi (1960) indicated that the small peak near the trailing edge of Fig. 1 is unbroken T2 DNA. To obtain a quantitative estimate of the polydispersity of the fragments, an effort was made to identify the distribution in Fig. 1. Burgi and Hershey (1961) reported the use of a normal (Gaussian) distribution function $[f(x)]$ and weight distribution $[x \cdot f(x)]$ to relate the distribution of lengths of similarly-fractionated DNA (Hershey and Burgi, 1960) to sedimentation coefficients. However, the normal distribution has a narrower peak, and broader base, and does not fit that shown in Fig. 1. Comparison of the data in Fig. 1 with different types of distributions given by Tung (1967) shows that the Schulz-Zimm distribution closely approximates the observed DNA lengths. This distribution by weight is given in the general form (Brandrup and Immergut, 1975; Kamide, 1977)

$$g(X) = \frac{y^{h+1}}{\Gamma(h+1)} X^h \exp(-yX), \qquad (1)$$

where

$$y = \frac{h}{\overline{X}_n}, \tag{2}$$

$$h = \left(\frac{\overline{X}_w}{\overline{X}_n} - 1\right)^{-1}, \tag{3}$$

$X$ is the degree of polymerization, $\overline{X}_w$ = weight-average $X$, and $\overline{X}_n$ = number-average $X$.

To fit Eq. 1 to the fractionation data on T2 halves (Fig. 1), it is assumed with reasonable certainty that the fractions off the MAK column are proportional to fragment length (Hershey and Burgi, 1960; Burgi and Hershey, 1961). The origin of $g(X)$ may be transposed by letting $X = X' - X_{min}$, where $X'$ is a new length scale from zero to $X' = 1$ for whole T2, which is well separated from half molecules. $X_{min}$ is a minimum (adjustable) length for which $g(X) = 0$. If $\overline{X}'_n = 0.5$ at the peak for T2 halves (Fig. 1), the parameters $h$ and $y$ can be adjusted by iteration to fit the data. The best fit, as judged by the sum of the square of deviations, is shown in Fig. 1 for $X_{min} = 0.15$, $h = 9$, and $y = 25.7$. From the Schulz-Zimm distribution (Eqs. 1 and 3), the heterogeneity of half-fragments may be estimated as the ratio, $\overline{X}'_w/\overline{X}'_n = 1.08$, with the parameters above. This polydispersity index should be slightly above that for the pooled fractions indicated in Fig. 1 and used in later experiments. An index <1.1 indicates the sample has a narrow molecular-weight distribution (Godfrey and Eisenberg, 1976; Rabek, 1980). The pooled fractions (Fig. 1) noticeably skew the sample toward heavier fragments.

The fractionation profile for quarter molecules was similar to that for halves (Fig. 1), with the peak fraction appearing in tube 23. The profile was broader and even more skewed toward higher $M$ than Fig. 1. As a result, neither the Schulz-Zimm nor other standard distribution functions (Tung, 1967; Brandrup and Immergut, 1975) would exactly fit the data. The closest fit with Eq. 1 was obtained as above with $\overline{X}'_n = 0.25$, $X_{min} = -0.25$, $h = 6$, and $y = 12$. For this case $\overline{X}'_w/\overline{X}'_n \approx 1.33$, which indicates the polydispersity of quarter molecules is significant and greater than halves.

## Hydrodynamic measurements

Table 1 summarizes the results of hydrodynamic measurements on the DNA samples after Millipore filtration. Uncertainties indicated in the table are average deviations of at least two determinations. The maximum intrinsic viscosity obtained for whole, native T2 DNA, before or after filtration, was 282 dl/g, found for one preparation after clarification. The lowest value of $[\eta]$ = 272 dl/g was observed for the preparation that was subjected to multiple filtrations before transfer by pipette to the light scattering cell (see Methods). Sedimentation coefficients, obtained both by boundary and by band methods, are also included in Table 1.

## Light scattering

The analysis of static light scattering data from relatively rigid, high-molecular-weight DNA must address the difficulties that were briefly summarized in the introduction. The preferred method of analysis is to use linear extrapolations of reciprocal-intensity data from the limiting regions of angles and concentrations (Zimm, 1948; Geiduschek and Holtzer, 1958; Tanford, 1961; Harpst et al., 1968). It is well-known that the limiting angular range is attained when the scattering function, $P^{-1}(\theta)$, is <1.33 (Zimm, 1948; Schmid et al., 1971). However, in this

**TABLE 1 Experimental light scattering and hydrodynamic results for native T2 DNA after clarification**

| T2 DNA | $M^*_A \times 10^{-6}$ | $R^*_A$ | $A^*_2 \times 10^4$ | $[\eta]^‡$ | $s_{20,w}$ |
|---|---|---|---|---|---|
| | | *nm* | *mol cm³g⁻²* | *dl/g* | *S* |
| Wholes | 117 ± 12 | 1,350 ± 120 | 2.0 ± 0.4 | 277 ± 5 | 64.5§ |
| | | | | | 63.0 ± 0.8‖ |
| Halves | 70.2 ± 0.5 | 1,020 ± 20 | 2.5¶ | 182** | 44.0 ± 1.3‖ |
| Quarters | 26.9 ± 0.9 | 620 ± 10 | 3.0 ± 1.1 | 113¶ | 31.3‖‡‡ |

Full experimental details and symbols are given in the text.

*Results obtained at room temperature from linear extrapolations of light scattering data in reciprocal-intensity plots at the lowest accessible angles (see text).

‡Values at 25.00 ± 0.02°C.

§Sedimentation coefficient determined from boundary sedimentation data on two preparations. Value given is extrapolated to infinite dilution ($s^0_{20,w}$).

‖Sedimentation coefficients from band experiments. Indicated uncertainty is the average deviation.

¶Identical results from duplicate determinations.

**Value from one set of measurements on a single preparation.

‡‡Single determination.

investigation the minimum experimental value of $P^{-1}(\theta)$ for whole T2 is ~4.5, and that for the smallest, quarter molecules is 1.8, still above the theoretical limit. Clearly, linear extrapolations of the lowest attainable angular measurements can only be expected to give apparent values of $M$ and $R$. For this reason the scattering data here are analyzed as follows. The low-angle data for all samples are observed to define a linear range (see Figs. 2 and 4) from which standard extrapolations (Zimm, 1948; Tanford, 1961; Harpst et al., 1968) can be made to yield the apparent values, $M_A$ and $R_A$. The observed linear angular range may then be fitted easily to a theoretical curve for wormlike coils (Sharp and Bloomfield, 1968; Harpst, 1980) with persistence and excluded volume. The scattering curves for quarter and half molecules can also be fitted over the full angular range for which the theory is valid, to give reasonable estimates of $a$ and $\epsilon$. The appropriate theoretical curves then provide the correct or theoretical, limiting values, $M_T$ and $R_T$.

Values of the second virial coefficient, $A_2$, are derived by standard procedures from linear extrapolations of the low-angle data to zero angle (see Figs. 2 and 4). These results are taken as accurate and correct ones, because the concentration extrapolations are linear and the lines at each angle are parallel with that at $\theta = 0$ (Figs. 2 and 4). Because of scatter in the data at higher angles (see below), it is not possible to discern a systematic change in the virial coefficient with angle.

A representative reciprocal-intensity plot of light scattering data on whole, native T2 DNA at low angles is in Fig. 2. Values of $M_A$, $R_A$, and $A_2$ were obtained by extrapolations (Zimm, 1948) to $c = 0$ and to $\theta = 0$ from the observed linear angular range, 8–14°. The results in Table 1 are the average of determinations on two

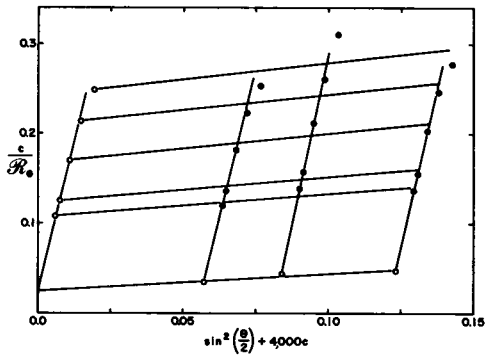


FIGURE 2 Reciprocal-intensity plot of light scattering data on native T2 DNA, filtered directly into the scattering cell. Measurements shown are at angles of 9–16° and concentrations of 14.3, 21.0, and 30.8 μg/ml. *Filled circles* (●) are experimental points and *open circles* (○) are extrapolated points at $c = 0$ or $\theta = 0$.

different preparations. Scattering data at angles above 20° gave generally well-defined curves at each concentration, but it was not possible to obtain reliable reciprocal-intensity plots due to uncertainties, caused primarily by a precipitous drop in intensities for these samples at higher angles. Therefore, the scattering curve at $c = 0$ for whole T2 DNA was approximated as done earlier (Harpst, 1980). Two scattering curves (at concentrations near 15 μg/ml) were selected to illustrate and emphasize the range (maximum to minimum) of all experimental curves. Each of the two curves was then translated to $c = 0$ by use of the virial coefficient, derived as described above from the same experiment. The average of the two curves was used as the scattering envelope at $c = 0$ for whole T2 DNA, as shown in Fig. 3. The figure illustrates the relatively large variation obtained in the curves for T2 DNA.

Light scattering data on half molecules of T2 DNA are plotted in Fig. 4 for the linear angular range, 8–18°. Average values of $M_A$, $R_A$, and $A_2$ are included in Table 1. Higher angle measurements from the experiment illustrated in Fig. 4 gave nearly parallel lines for concentration extrapolations at all angles between 8° and 70°, indicating little change in $A_2$ with angle. However, high angle data in other experiments were too scattered to extrapolate to $c = 0$, much as observed for T7 DNA

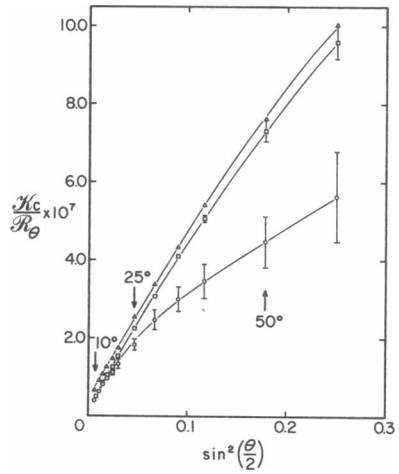


FIGURE 3 Average experimental scattering envelopes [$\mathcal{K}c/\mathcal{R}_\theta$ versus $\sin^2(\theta/2)$] at $c = 0$ for native T2 DNA and its fragments. Points are averages, derived as described in the text, for whole (○), half (□), and quarter (△) molecules. All data are on samples clarified by filtration directly into the scattering cell, except whole T2 results include measurements on filtered samples transferred to the cell by pipette. Symbols encompass maximum observed variations in data, except where larger uncertainties (maximum/minimum) are indicated by the bars (I). Angular range shown is 8–60°. Some points are omitted at lowest angles for clarity. Solid lines are the best visual fit to the points.

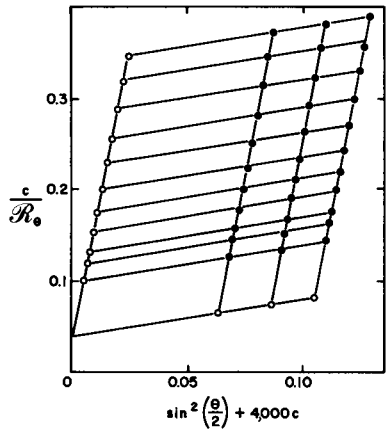


FIGURE 4 Reciprocal-intensity plot of light scattering data on half molecules of native T2 DNA, filtered directly into the scattering cell. Measurements shown are at angles of 8–18° and concentrations of 15.8, 21.4, and 26.2 μg/ml. Symbols are the same as described in the legend to Fig. 2.

(Harpst, 1980) and as noted above for whole T2 DNA. Consequently, the scattering curve at high angles was determined, as described for whole molecules, by selecting two curves at concentrations near 20 μg/ml to represent the maximum and minimum in the experimental data from two determinations. After correcting to $c = 0$ with the virial coefficient, the average of the curves was plotted as shown in Fig. 3.

Two determinations on one preparation of quarter molecules of T2 DNA gave reciprocal-intensity plots (not shown) similar to those obtained earlier for T7 DNA (Harpst et al., 1968). Linear extrapolations over the low angle range of 9° to 20° gave the parameters summarized in Table 1. The values of $M$ and $R$ are somewhat lower than those obtained for T7 DNA (Krasna et al., 1970). The scattering data at higher angles for quarter molecules closely resemble those for T7 DNA (Harpst, 1980). The curve at $c = 0$, derived as described above from one set of measurements at 18.5 μg/ml, is included in Fig. 3.

Experimental uncertainties in the results obtained from linear extrapolations of light scattering data have been discussed earlier (Dawson and Harpst, 1971*a*). These considerations hold for the data on half and quarter molecules of T2 DNA. However, the errors encountered in the measurements on whole T2 DNA are larger. As indicated in Fig. 3 there is increasing uncertainty in intensity measurements, due to the sharp decrease in intensity as angle increases, up to ~20% error at the highest angles. The more extreme curvature, also, limits the linear range for extrapolations, so that the error in $M_A$ for whole T2 DNA is at least ±15%, as estimated by the precision in making the linear extrapolations by eye.

## Comparisons with theory

### Without excluded volume

Theoretical calculations with $\epsilon = 0$ (Harpst, 1980) have been made for comparison with the light scattering results in Table 1 and with the curves at $c = 0$ for whole, half, and quarter molecules of T2 DNA (Fig. 3) as described earlier (Sharp and Bloomfield, 1968; Schmid et al., 1971; Harpst, 1980). Experimental scattering curves showed that low angle regions were linear over the ranges 8–14°, 8–18°, and 9–20° for whole, half, and quarter molecules, respectively. Calculated curves were extrapolated linearly from these angular ranges by a least squares fit to give apparent values of $M_A$ and $R_A$. Matching these apparent parameters with the experimentally determined ones in Table 1 allowed selection of appropriate values of $M_T$, $R_T$, and $1/\lambda'$.

To further the comparison of experimental and calculated curves, the sum of the squares of the deviations, $\Sigma d^2$, in $\mathcal{K}c/\mathcal{R}_\theta$ between theoretical and average experimental curves (Fig. 3) was calculated and included in Table 2. Equal weighting for points at 5 to 7 angles was assumed for the two angular regions shown (Table 2). A minimum in the value of $\Sigma d^2$ allows selection of the theoretical curve which best fits the experimental points.

Results of the calculations without excluded volume ($\epsilon = 0$) are summarized in Table 2 and illustrated in Figs. 5 and 6. Only the parameters of theoretical curves which provide the best fit to experimental points in the linear, experimental ranges are indicated in Table 2. Even though experimental scattering envelopes ($c = 0$) for half and quarter fragments (Fig. 3) have a less pronounced curvature than that observed for whole T2 DNA, the curves calculated for all samples with $\epsilon = 0$ deviate upward from the experimental points both above 20° and below 8° (Table 2, Figs. 5 and 6). These results clearly confirm the observation (Harpst, 1980) that theoretical curves, calculated without excluded volume, cannot fit experimental scattering envelopes for large, native DNA samples, except over a limited angular range.

### With excluded volume

Scattering curves were also calculated with $\epsilon > 0$ from the equations of Sharp and Bloomfield (1968), as described by Harpst (1980). Table 2 summarizes these calculations but includes only those results that give $M_A$ and $R_A$, which are close to the experimentally-determined values in Table 1.

Results from the calculations for whole T2 DNA (Table 2) show that appropriate values of $M_A$ and $R_A$ can be obtained for a variety of values of $\epsilon$. All calculated curves deviate markedly from the experimental points above 15° (Fig. 5), although increasing $\epsilon$ causes the

**TABLE 2 Results from calculated scattering curves for whole, half, and quarter molecules of T2 DNA with and without excluded volume**

| T2 DNA | $M_T \times 10^{-6}$ | $\epsilon$ | $1/\lambda'$ | $R_T$ | $M_A^* \times 10^{-6}$ | $R_A^*$ | $(\Sigma d^2)^\ddagger \times 10^{14}$ (Angular range) | |
|---|---|---|---|---|---|---|---|---|
| | | | *nm* | *nm* | | *nm* | | |
| | | | | | | | (8–14°) | (16-30°) |
| Wholes | 80.0§ | 0 | 130 | 932 | 115 | 1,349 | 0.0003 | 1.2941 |
| | | | | | | | (8–18°) | (20–50°) |
| Halves | 52.0§ | 0 | 125 | 736 | 69.9 | 1,023 | 0.0006 | 1.5580 |
| | | | | | | | (9–20°) | (25–50°) |
| Quarters | 23.0§ | 0 | 130 | 496 | 27.2 | 632 | 0.0036 | 1.6939 |
| | | | | | | | (8–14°) | (16-30°) |
| Wholes | 114.0 | 0.08 | 100 | 1,218 | 116.3 | 1,370 | 0.0015 | 0.8491 |
| | 115.0‖ | 0.08 | 100 | 1,224 | 117.0 | 1,375 | 0.0015 | 0.8479 |
| | 130.0 | 0.10 | 90 | 1,320 | 116.5 | 1,355 | 0.0002 | 0.5696 |
| | 140.0 | 0.11 | 80 | 1,344 | 119.5 | 1,330 | 0.0032 | 0.2281 |
| | | | | | | | (8–18°) | (20–50°) |
| Halves | 60.0 | 0.06 | 100 | 819 | 70.2 | 1,013 | 0.0005 | 0.0982 |
| | 64.0‖ | 0.08 | 95 | 870 | 70.3 | 1,023 | 0.0001 | 0.0389 |
| | 68.0 | 0.10 | 90 | 924 | 69.3 | 1,024 | 0.0016 | 0.0110 |
| | | | | | | | (9–20°) | (25-50°) |
| Quarters | 23.5 | 0.06 | 110 | 519 | 26.6 | 628 | 0.0087 | 0.7162 |
| | 24.0‖ | 0.08 | 100 | 523 | 26.7 | 620 | 0.0007 | 0.0995 |
| | 24.5 | 0.10 | 95 | 538 | 26.8 | 626 | 0.0027 | 0.0397 |

Full experimental details and symbols are given in the text.

*Apparent values of $M$ and $R$ calculated from linear extrapolations over the low-angle ranges noted in the text and in this table.

‡$\Sigma d^2$ is the sum of the squares of the deviations in $\mathcal{K}c/\mathcal{R}_\theta$ between the theoretical and experimental curves for the angular ranges indicated. Additional details are given in the text.

§Values calculated without excluded volume ($\epsilon = 0$) are shown only for those which most closely coincide with experimental results in Table 1.

‖Values calculated with excluded volume ($\epsilon > 0$) from the best fit curve, selected as described in the text.

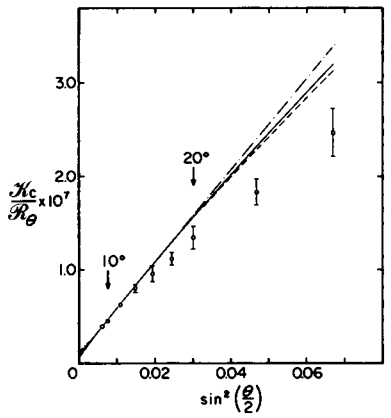


FIGURE 5 Plot of $\mathcal{K}c/\mathcal{R}_\theta$ versus $\sin^2(\theta/2)$ at $c = 0$ over the angular range of 0° to 30° for whole T2 DNA. Symbols and experimental points are the same as those in Fig. 3 for whole molecules. The curves were calculated, as described in the text, to give the best fit at $c = 0$ to the 8–14° data from the reciprocal-intensity plot (Figs. 2 and 3, Table 1). The following parameters were used: (—), $\epsilon = 0.08$, $M_T = 114 \times 10^6$, $1/\lambda' = 100$ nm; (– – – –), $\epsilon = 0.11$, $M_T = 140 \times 10^6$, $1/\lambda' = 90$ nm; and (— · —), $\epsilon = 0$, $M_T = 80 \times 10^6$, $1/\lambda' = 130$ nm.

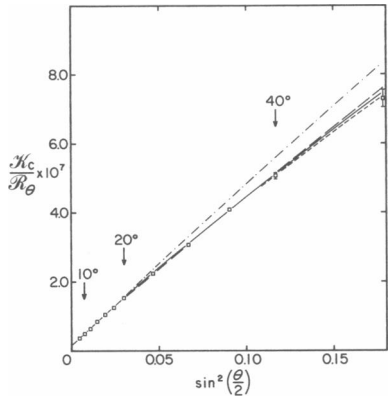


FIGURE 6 Plot of $\mathcal{K}c/\mathcal{R}_\theta$ versus $\sin^2(\theta/2)$ at $c = 0$ over the angular range, 0–50°, for half molecules of T2 DNA. Experimental points (□) and bars (I) are the same as in Fig. 3. The curves were calculated, as described in the text, to give the best fit at $c = 0$ to the 8–18° data in Fig. 4 with the following parameters: (—), $\epsilon = 0.08$, $M_T = 64 \times 10^6$, $1/\lambda' = 95$ nm; (– – – –), $\epsilon = 0.10$, $M_T = 68 \times 10^6$, $1/\lambda' = 90$ nm; (— —), $\epsilon = 0.06$, $M_T = 60 \times 10^6$, $1/\lambda' = 100$ nm; and (— · —), $\epsilon = 0$, $M_T = 52 \times 10^6$, $1/\lambda' = 125$ nm.

calculated envelopes to approach the experimental one (see $\Sigma d^2$, 16–30°, Table 2). Therefore, curve-fitting alone does not provide a unique value of $\epsilon$. Below 9° the behavior of the curves is noticeably different from that observed for T7 DNA (Harpst, 1980). The curve with $\epsilon = 0.08$ closely follows the linear extrapolation to $\theta = 0$ from the 8–14° range (Fig. 5, Table 2). Increasing $\epsilon$ to 0.11 increases downward curvature below 8° (Fig. 5) and increases $M_T$ and $R_T$ beyond reasonable limits (Table 2).

Results of calculations with $\epsilon = 0.06$ to 0.10 for half and quarter molecules are given in Table 2 and Fig. 6. Comparison of $M_T$ and $R_T$ with $M_A$ and $R_A$ in the table show that the calculated envelopes curve upward at angles below 8°, but this curvature decreases with increasing $\epsilon$. Above 20° it is clear that any one of the chosen values of $\epsilon$ gives a calculated curve which nearly coincides with the experimental points, similar to what was observed for T7 DNA, except that the sensitivity of curvature to different values of $\epsilon$ is less than for T7 (Harpst, 1980). The curves with $\epsilon = 0.06$ have slightly less curvature (20–50°) than the experimental points, but the results in Fig. 6 and Table 2 show that $\epsilon$ between 0.08 and 0.10 gives reasonable agreement between the calculated and experimental curves. Thus $\epsilon$ cannot be as precisely defined as for T7 DNA (Harpst, 1980). In addition, this curve-fitting process limits $1/\lambda'$ to a range between 90 and 100 nm.

Uncertainties in the parameters used for the calculations are indicated by the criteria used to fit the experimental data. Iterations were performed until calculated values of $M_A$ were within ~1% of the experimentally-determined values. Experimental and calculated values of $R_A$ were fitted within ±2%. Values of $1/\lambda'$ were usually selected in 10 nm increments, although half of this was used in some cases (Table 2). Calculations were normally made with $\epsilon$ in steps of 0.02, because the curve-fitting described above showed that smaller increments were not needed. Because calculations under these conditions gave parameters (Table 2) which coincided with experimental results well within experimental uncertainty (Table 1), no further refinements were necessary.

## DISCUSSION

Hydrodynamic measurements of $[\eta]$ and $s^\circ_{20,w}$ have been related by other workers to the molecular weight of DNA. In order to compare these earlier results with the DNA preparations and molecular weights obtained in this investigation, appropriate hydrodynamic data are needed.

The intrinsic viscosity obtained in this laboratory for whole, native T2 DNA in BPES was 277 ± 5 dl/g (Table 1). This is close to the values (250, 295 dl/g) obtained by some workers for T2 and T4 DNA (Burgi and Hershey, 1961; Aten and Cohen, 1965). However, our value is below the range, 300–320 dl/g, obtained from low-shear measurements on these DNAs by others (Schumaker and Bennett, 1962; Crothers and Zimm, 1965; Ross and Scruggs, 1968; Bowen and Zimm, 1978). Two factors that could cause the lower viscosity obtained in this study might be that the measurements were made at too high a shear stress (Crothers and Zimm, 1965), or that filtration sheared the DNA. However, both these effects were eliminated by use of a viscometer rotor which had a shear stress that should give the correct viscosity within 1% (Crothers and Zimm, 1965), and by measuring $[\eta]$ before and after filtration. Usually $[\eta]$ showed little or no change. The only sample which exhibited the expected decrease in $[\eta]$ if shear degradation had occurred (Krasna and Harpst, 1964) was that which was filtered several times before transfer by pipette to the cell. For this sample the lowest $[\eta] = 272$ dl/g indicated that there was only a small amount of degradation. These observations indicated that shear breakage during filtration did not significantly decrease the values of $[\eta]$. Other explanations for low viscosities are: (*a*) a systematic error in the measurements, due to temperature differences or wall effects in the viscometer, (*b*) a deletion mutant with a smaller DNA, (*c*) degradation during sample preparation, or (*d*) incomplete disaggregation of DNA after dilution (see Methods). Most of these difficulties can be eliminated on the basis of control experiments or the techniques used, except that the careful work on T2 DNA by Bowen and Zimm (1978) suggests that our sample preparation and handling might have been improved.

Confidence in the preparations of whole T2 DNA is supported by the sedimentation velocity measurements. The value of $s^\circ_{20,w} = 64.5$ (Table 1) from boundary experiments on filtered samples is the same as that obtained on unfiltered ones by others (Aten and Cohen, 1965; Crothers and Zimm, 1965). Band sedimentation measurements give only a slightly lower value (Table 1), which is likely to be due to a concentration effect, observed as a slight downfield skewing of the sedimentation band (Vinograd and Bruner, 1966). All bands had this expected shape and provided no evidence of other sedimenting components, thus indicating the DNA was normal and essentially homogeneous. Taken together, the viscosity and sedimentation results suggest that the whole T2 samples were nearly homogeneous, with a small amount of degradation detectable only in the viscosity measurements.

Hydrodynamic measurements for the half and quarter molecules (Table 1) appear to be reasonable when related to molecular weights derived from them (Table 3) and

TABLE 3 **Comparison of molecular weights of whole, half, and quarter molecules of native T2 DNA**

| T2 DNA | $M_A^* \times 10^{-6}$ | $M_T^\ddagger \times 10^{-6}$ | $M_S^\S \times 10^{-6}$ | $M_V^\S \times 10^{-6}$ | $M_S^\| \times 10^{-6}$ | $M_V^\| \times 10^{-6}$ |
|---|---|---|---|---|---|---|
| Wholes | 117 | 115 | 120 | 99 | 113 | 95 |
| Halves | 70 | 64 | 54 | 54 | 48 | 53 |
| Quarters | 27 | 24 | 24 | 26 | 20 | 27 |

Full details are given in the text.

*Experimental values derived from linear extrapolation of low angle light scattering data (Table 1).

‡Limiting values calculated with $\epsilon = 0.08$ from the curves fitted to low angle light scattering data (Table 2).

§Subscripts indicate molecular weights are calculated from S = sedimentation coefficients or from $V$ = intrinsic viscosities of samples with equations given by Crothers and Zimm (1965).

‖Subscripts are as defined above. Molecular weights are calculated from equations given by Reinert et al. (1971).

when compared with earlier work (Burgi and Hershey, 1961). Individual values of $[\eta]$ and $s$ vary markedly with the degree of shear of each sample and cannot, themselves, be used as criteria for heterogeneity. However, the sedimenting bands were all single peaks, indicating there were no detectable whole molecules or other discrete fragments (data not shown). The bands for fragments were broader than those for whole T2, as expected from the heterogeneity of the samples defined above (Fig. 1) and in earlier work (Burgi and Hershey, 1961).

In order to evaluate some of the results, as done in the following paragraphs, it is necessary to know the molecular weight of whole T2 DNA, determined by other workers. Bowen and Zimm (1978) give a molecular-weight range of (106–132) $\times 10^6$, derived from work in a number of laboratories. The molecular weight of T2 DNA can be estimated as $110 \times 10^6$ from the molecular weight of T4 DNA (Schmid et al., 1971) and the length ratios of T2 and T4, obtained by electron microscopy (Lang, 1970). Investigations by Weissman et al. (1976) and by Bowen and Zimm (1978) gave T2 DNA molecular weights of $(114 \pm 5) \times 10^6$ and $(126 \pm 5) \times 10^6$, respectively. The average of these three determinations is $(117 \pm 6) \times 10^6$, which we use as the accepted value for $M$ of T2 DNA in the following comparisons.

Linear extrapolation of the light scattering data for whole T2 DNA from the 8–14° range gave $M_A = (117 \pm 12) \times 10^6$ (Table 1), in remarkable, albeit fortuitous, agreement with the accepted value. Comparison of theoretical and experimental scattering curves (Fig. 5, Table 2) showed that, while the curves can be made approximately coincident in the 8–14° range, all calculated envelopes diverge from the experimental above 14°. It was expected that the calculated curves would fit the experimental ones up to 55° (V. A. Bloomfield, personal communication). It may be tempting to conclude that the wormlike coil model does not fit whole T2 DNA. However, the observed deviations most likely result from the large experimental uncertainties in the high angle data for T2 DNA (Fig. 3) or from the lack of a true curve at $c = 0$ (see Results). Resolution of this question must await development of an instrument capable of making more reliable high angle measurements.

It is clear from Fig. 5 that the process of matching curves cannot provide a unique value of $\epsilon$. However, if the 8–14° data are taken to be reliable, along with the accepted $M$ of T2 DNA ($117 \times 10^6$), a reasonable $\epsilon$ can be selected. The best theoretical curve fits the experimental (8–14°) data (cf. $M_A$ and $R_A$, Table 2) with $\epsilon = 0.08$, $1/\lambda' = 100$ nm, and $M_T = 115 \times 10^6$, which is closest to the accepted molecular weight for T2. This estimate of $\epsilon$ and $1/\lambda'$ is consistent with more definitive results on the smaller T7 DNA (Harpst, 1980) and fragments of T2. The best calculated curve (Table 2, Fig. 5) also shows that linear extrapolation of the 8–14° data gives an apparent molecular weight, $M_A$, which is nearly the same as the limiting value, $M_T$. This explains why linear extrapolations of 8–14° scattering data, as used here and in an earlier report (Dawson and Harpst, 1971*b*), provide an essentially correct molecular weight for T2 DNA. Clearly the measurements on whole T2 DNA do not give a completely independent determination of molecular weight. T2 DNA is at or beyond the upper limit of the light scattering techniques used here.

The results (Tables 1 and 2) for all three double-helical, linear DNAs investigated here indicate that $\epsilon$ should be between 0.08 and 0.10. In fact, close examination of these results suggests that $\epsilon$ increases (0.08 to 0.10) as $M$ decreases. This trend is most likely due to the increasing heterogeneity of the smaller fragments and the resultant increase in downward curvature of the scattering curves at high angles (Geiduschek and Holtzer, 1958; Kratochvil, 1972). At present the best value for all three DNAs seems to be $\epsilon = 0.08$, because it agrees with the result on T7 DNA (Harpst, 1980) and is indicated by the results on whole T2 DNA. This value of $\epsilon$ is below that ($\epsilon = 0.11$) used by Sharp and Bloomfield (1968) in earlier calculations of scattering curves, but is in the range of $\epsilon$

(0.07 ± 0.04) obtained by Hearst et al. (1968) from hydrodynamic measurements.

With the preceding selection of $\epsilon$, a value can be obtained for the Kuhn statistical segment length, $1/\lambda'$, or Kratky-Porod persistence length (Kuhn, 1934; Kratky and Porod, 1949; Bloomfield et al., 1974; Eisenberg, 1974). The results in Table 2 for $\epsilon = 0.08$ show that $1/\lambda'$ is 95–100 nm for whole, half, and quarter molecules of T2 DNA. Careful analysis of the scattering data suggests that $1/\lambda'$ decreases slightly (100 to 90 nm) as DNA size decreases. This effect is considered to be due to increasing heterogeneity of the smaller fragments, as noted above for $\epsilon$. On this basis, we conclude that the persistence length shows no significant change over the molecular-weight range of $24 \times 10^6$ to $115 \times 10^6$ or for sheared versus unsheared samples. This is consistent with the constancy of persistence length with molecular weight for smaller DNAs, reported previously by Godfrey and Eisenberg (1976) and by Elias and Eden (1981), with the reservations indicated recently (Lewis et al., 1986).

The best value for $1/\lambda'$ from these data on T2 DNA and its fragments are 100 ± 10 nm, or 50 ± 5 nm for the persistence length, where the range indicated is the uncertainty in the iterations. This is lower than $1/\lambda' =$ 120 nm, obtained for T7 DNA (Harpst, 1980), and is below the average value of $a = 60 \pm 6$ nm, determined for low-molecular-weight DNAs by light scattering and hydrodynamic methods (Godfrey and Eisenberg, 1976). Our persistence length of 50 nm agrees with results obtained without excluded volume corrections from earlier light scattering measurements on small DNAs (Godfrey and Eisenberg, 1976; Voordouw et al., 1978). The same laboratory has published more recent light scattering results with excluded volume corrections which give $a = 37$–41 nm (Borochov et al., 1981; Kam et al., 1981; Borochov and Eisenberg, 1984), significantly below our value of 50 nm. Interestingly, a different treatment of excluded volume effects (Manning, 1981) raises the value of $a$ obtained by Borochov et al. (1981) to 46 nm, which agrees with our results within experimental uncertainty. Further, we note that results (in 0.2 M $Na^+$, 20–25°C) for large DNAs and small fragments from a variety of other techniques (Hagerman, 1981; Rizzo and Schellman, 1981; Cairney and Harrington, 1982) and under different conditions (Elias and Eden, 1981; Maret and Weill, 1983) are all consistent with a persistence length of ~50 nm (Schurr and Schmitz, 1986). The close agreement of this value with our determinations lends strong support to the validity of our result. Further, it indicates that the theory and methodology used for analyzing the light scattering data and accounting for excluded volume effects are essentially correct.

A potential problem with the light scattering data could result from anisotropy in the scattering measurements (Geiduschek and Holtzer, 1958; Schmid et al., 1971). For the high-molecular-weight DNAs used here, these effects are negligible (Godfrey and Eisenberg, 1976; Harpst, 1980).

Heterogeneity in the DNA preparations used here has been discussed in earlier paragraphs, and must be considered in comparisons of these results among themselves or with other measurements. Because of the importance of sample heterogeneity, our initial expectation was to verify the polydispersity indexes, obtained from fractionation data, with values of $\overline{M}_w$ and the number-average molecular weight from low and high angle light scattering data, respectively (Bloomfield et al., 1974). However, this was not possible in the present study, because of the experimental uncertainty in high-angle data for whole and half molecules of T2. To achieve this objective, instrumentation must be improved to provide more precise high-angle measurements, and the theory should be extended to include effects of polydispersity, persistence, and excluded volume.

Some effects of heterogeneity, particularly in determining $a$ and $\epsilon$, have been noted in previous sections. An additional influence on the values of $A_2$ might be expected to be alteration of the relationship between $A_2$ and $M$, often given in the form, $A_2 = A_0 M^{-\nu}$, where $A_0$ and $\nu$ are constants (Yamakawa, 1971; Nagasawa and Takahashi, 1972). A log–log plot of $A_2$ from Table 1 versus the appropriate values of $M_T$ (Table 2) can be fitted reasonably well with a straight line having $\nu = 0.26$. The effect of polydispersity on $A_2$ may be estimated for our DNA samples as described by Yamakawa (1971), if we assume they behave as hard spheres. For quarter molecules, the most heterogeneous of our samples, a polydispersity index of 1.33 in the Schulz-Zimm distribution (see earlier section) would lead to an observed $A_2$ only 3.3% higher than that for a monodisperse sample with the same $\overline{M}_w$. This change in $A_2$ alters $\nu$ to 0.24 in the $A_2$ versus $M$ relationship. Clearly, the polydispersity of the DNA samples utilized in this study has a negligible effect on values of $A_2$.

The consistency of the hydrodynamic and light scattering data can be tested by comparing calculations of the parameter, $\beta$, from the Mandelkern-Flory-Scheraga relation (Mandelkern and Flory, 1952; Scheraga and Mandelkern, 1953). A number of workers have discussed $\beta$ in detail for native DNAs (Eigner and Doty, 1965; Reinert et al., 1971; Godfrey, 1976; Godfrey and Eisenberg, 1976) and have found it to be between $2.4 \times 10^6$ and $2.6 \times 10^6$ for high-molecular-weight samples. Values of $\beta$ for the three DNAs used in this investigation have been calculated, as detailed by Eigner and Doty (1965), from the hydrodynamic parameters in Table 1 and from the values of $M_T$ ($115 \times 10^6$, $64 \times 10^6$, and $24 \times 10^6$ for whole, half, and quarter molecules, respectively) for $\epsilon =$

0.08 in Table 2. Respective values of $\beta$ for whole, half, and quarter molecules are $2.38 \times 10^6$, $2.11 \times 10^6$, and $2.47 \times 10^6$. The $\beta$-values for wholes and quarters are within the expected range, but that for halves is low, most likely reflecting recognized inaccuracies in the measured parameters.

An early objective of this work was to provide data that could be used to calibrate measurements of $s$ and $[\eta]$ with high molecular weights of DNA (Crothers and Zimm, 1965). The hydrodynamic data (Table 1) and correct $M$'s from light scattering (Table 2) fall very close to the average line derived by Crothers and Zimm (1965). A more instructive comparison of results obtained here with others in the literature can be made by using molecular-weight dependences established previously (Crothers and Zimm, 1965; Reinert et al., 1971). Table 3 compares values of $M_T$, derived from light scattering data by calculations with $\epsilon = 0.08$ (Table 2), with molecular weights calculated from the hydrodynamic parameters in Table 1 and the relationships given by the workers just cited. Molecular weights calculated from $[\eta]$ for T2 DNA are lower than $M_A$ or $M_T$, which reflects the low intrinsic viscosity obtained in this study. All calculated values for half molecules are lower than $M_A$ and $M_T$, which is reflected in the low $\beta$-value for halves. Our results from quarter molecules are remarkably close to calculated $M$'s. This comparison of molecular weights from light scattering with those calculated from the cited relationships in the literature (Table 3) indicates that the constants in the empirical relationships would be modified somewhat if our data had been included. This suggests that the empirical molecular weight relationships should be modified and updated. An appropriate review could include our own and other results from light scattering (Godfrey and Eisenberg, 1976; Voordouw et al., 1978; Borochov et al., 1981; Kam et al., 1981) and other techniques (Weissman et al., 1976; Bowen and Zimm, 1978), including DNA sequencing, and consideration of polydispersity and glucosylation effects (Reinert et al., 1971).

The major conclusions from this investigation may be summarized as follows: (*a*) This study confirms the validity and applicability of the procedures developed previously (Harpst, 1980) as a method for obtaining limiting values of $M$ and $R$ from light scattering measurements above 8° on DNA in the high-molecular-weight range, $24 \times 10^6$ to $115 \times 10^6$, even in the absence of experimental data in the linear, limiting angular region. The approach also provides estimates of the persistence length and an excluded volume parameter, $\epsilon$. (*b*) The results substantiate the earlier finding (Harpst, 1980) that the excluded volume must be used to obtain a proper fit of calculated light scattering curves to experimental data. A value of $\epsilon = 0.08$, as determined for T7 DNA (Harpst, 1980), adequately fits all the data. (*c*) Native T2 DNA with a molecular weight of $115 \times 10^6$ is at or above the upper limit of light scattering measurements with present instrumentation. (*d*) Results on half and quarter molecules of T2 DNA confirm the earlier conclusion (Harpst, 1980) that linear extrapolation of scattering data on high-molecular-weight, native DNA from angles above 8° gives overestimates of $M$ and $R$ (Geiduschek and Holtzer, 1958; Schmid et al., 1971). (*e*) Comparison of experimental curves for whole T2 DNA with ones calculated with $\epsilon = 0.08$ shows, fortuitously, that linear extrapolations of 8–14° data nearly coincide with the theoretical curve and give essentially correct, limiting values of $M$ and $R$. (*f*) The Kuhn statistical segment length ($1/\lambda' = 100 \pm 10$ nm) or persistence length ($a = 50 \pm 5$ nm), calculated with $\epsilon = 0.08$, is the same for the DNA samples used in this investigation over the entire molecular-weight range, $24 \times 10^6$ to $115 \times 10^6$.

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