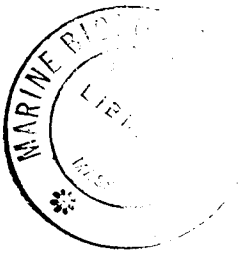

SEDIMENTATION RATE AS A MEASURE OF MOLECULAR WEIGHT OF DNA

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ABSTRACT Zone centrifugation of mixtures of two labeled DNA's at low concentrations in density gradients of sucrose permits accurate measurement of relative sedimentation rates. The individual rates are constant during the run. Measurements with DNA's from phages T2, T5, and lambda conform to the relation $D_2/D_1 = (M_2/M_1)^{0.46}$, where D and M refer to distances sedimented and molecular weights of the DNA pair. The results show that high molecular weight DNA's sediment artificially fast in the optical centrifuge, owing to a hitherto unknown effect of molecular interactions. The molecular weight of lambda DNA is 31 million, measured either from sedimentation rate or from tests of fragility under shear.

INTRODUCTION

Doty, McGill, and Rice (1958) proposed the relation

$$S = aM^k \tag{1}$$

between sedimentation coefficient (S) and molecular weight (M) of DNA's. Their measurements for samples of thymus DNA ranging in molecular weight from 0.3 to 7 million required the constants $a = 0.063$ and $k = 0.37$.

Rubenstein *et al.* (1961), on the other hand, measured $a = 0.0024$ and $k = 0.543$ for T2 DNA in the molecular weight range from 30 to 130 million. The discrepancy could mean that the character of equation 1 varies with molecular weight or that difficulties of measurement remain to be overcome.

The difficulties are formidable. There is no commonly accepted method for measuring molecular weights of DNA, particularly when they are high. Measurements of sedimentation coefficient are equally unsatisfactory, because effects of molecular interactions are not understood (Hearst and Vinograd, 1961; Burgi and Hershey, 1962), and because convective disturbances are difficult to avoid or even to recognize (Burgi and Hershey, 1961).

In the present paper we explore the generality of equation 1 on the basis of the following principles.

1. The DNA's of T2, T5, and lambda phages can be isolated as uniform, linear, double-helical structures (Burgi and Hershey, 1961; Hershey *et al.*, 1962; Hershey, Burgi, and Ingraham, 1963).

2. Such molecules can be broken into half-length fragments identifiable without recourse to molecular weight measurements (Burgi and Hershey, 1961; Levinthal and Davison, 1961).

3. Relative rates of sedimentation of isotopically labeled DNA's can be accurately measured in the absence of convection and molecular interaction in solutions stabilized by a density gradient of sucrose (Burgi, 1963).

4. Procedures (2) and (3) combine to give a hydrodynamically meaningful estimate of k in equation 1 for any molecular weight range in which suitable DNA preparations are available.

5. The molecular weight of T2 DNA is about 130 million (Rubenstein *et al.*, 1961), giving a fix on the scale of M.

6. DNA's of about 30 s behave well in the optical centrifuge, giving a fix on the scale of S.

7. Items (5) and (6) combine to yield an estimate of a in equation 1.

8. Equal molecular weights of two DNA's sedimenting at equal rates can be verified by matching with respect to fragility under hydrodynamic shear (Hershey *et al.*, 1962).

METHODS

DNA was extracted from phage particles labeled from H^3-uridine or P^{32}-orthophosphate by extraction with phenol in one of the ways described by Mandell and Hershey (1960), Burgi (1963), and Hershey, Goldberg, *et al.* (1963). The variations are important only as necessary to avoid breakage and radiochemical damage, and to ensure that lambda DNA is obtained in the form of linear monomers (Hershey, Burgi, and Ingraham, 1963).

Sedimentation coefficients were measured at 10 μg DNA/ml in 0.7 M NaCl in cells fitted with aluminum centerpieces (Burgi and Hershey, 1961). They are reported without correction except where $S_{20,w}$ is specifically designated.

DNA molecules were broken by stirring solutions containing 2 to 15 μg DNA/ml in 0.1 or 0.6 M NaCl at 5°C (Hershey *et al.*, 1962).

Zone sedimentation was observed in linear concentration gradients of 5 per cent to 20 per cent (w/v) sucrose in 0.1 M NaCl, 0.05 M phosphate, pH 6.7 (Britten and Roberts, 1960). A sample usually containing 0.2 μg or less of DNA in 0.1 or 0.2 ml of 0.1 M buffered saline was layered on 4.8 ml of sucrose and spun in an SW39L rotor of a Spinco model L centrifuge at 10°C. Samples were prepared and radioactivity assayed according to Hershey, Goldberg, *et al.* (1963). To control the size of drops collected from the tubes, a holder was constructed in which the tube bottom rested on a rubber washer holding a short, silicone-treated glass cannula. The tube was punctured by passing the point of a pin up through the cannula, which delivered 35 μl drops. Distances sedimented were measured between the centers of gravity of the initial and final positions of the DNA band.

When DNA is spun in an untreated cellulose tube, about 15 per cent of it rolls to the

bottom after sedimenting against the tube walls, and tends to contaminate the liquid collected from the tube in an erratic manner. To avoid that hazard, we treated the tubes before use with a silicone preparation (Siliclad, Clay-Adams, Inc., New York), which caused the DNA striking the walls to remain attached there. The practice proved effective when the total amount of DNA in the tube did not exceed 1 μg. Sedimentation against the tube walls probably does not cause errors in rate measurements. It does introduce errors in analysis of the composition of mixtures, because more of a faster sedimenting component is lost than of a slower.

In the course of the present work we again noticed subtle effects of radiochemical damage to DNA. Some P^{32}-labeled preparations of both T2 and lambda DNA that sedimented normally showed extreme fragility under shear and yielded first breakage products with abnormally high median and abnormally widely distributed sedimentation rates, as if radiochemical action had introduced randomly placed weak spots into the molecules. To avoid such effects, we prepared radioactive phage in small amounts when possible, minimized centrifugation time during purification, and promptly extracted and diluted the DNA, usually in solvents containing 0.2 per cent ethanol. Criteria of satisfactory preparations were: equal fragility on stirring in mixture of H^3- and P^{32}-labeled DNA's; and consistent measurements, independent of isotopic label and specific radioactivity, of the type reported in Table III.

EXPERIMENTAL PRINCIPLE

Our basic measurement is the ratio of distances through which two DNA's sediment when spun in the same tube. It is interpreted as follows (Burgi, 1963).

The coefficient a can be eliminated from equation 1 to give

$$\frac{S_2}{S_1} = \left(\frac{M_2}{M_1}\right)^k \tag{2}$$

where the subscripts refer to the two DNA's. In the limit as either ratio approaches unity,

$$\frac{D_2}{D_1} = \left(\frac{M_2}{M_1}\right)^k \tag{3}$$

in which D stands for distance sedimented in a concentration gradient of sucrose. As we shall show, equation 3 is valid in practice even for two DNA's sedimenting at very different rates. This is so because field and solvent effects oppose each other to give a constant rate of sedimentation from the top to the bottom of the tube (Britten and Roberts, 1960).

RESULTS

Estimate of k from Breakage of T2 DNA. We first present measurements for a series of T2 DNA fragments of various lengths prepared by breakage under shear and made as homogeneous as possible by chromatographic fractionation. The characterization of the fragments is given in Table I. The relative molecular lengths shown there are based on the principle of breakage into halves; that is,

TABLE I

CALIBRATED FRAGMENTS OF T2 DNA

Stirring speed	Sedimentation coefficient	Relative length
RPM	S	
Unstirred	62.0	1.0
1200	41.2	0.46
1500	38.3	0.40
1800	30.3	0.25

All four samples were passed through fractionating columns (Mandell and Hershey, 1960), and one of the middle fractions was selected for analysis. Fractional molecular lengths were calculated from $2L = (S/43)^{1.96}$ (Burgi and Hershey, 1961).

the sedimentation coefficients are used to interpolate between the unit, half, and quarter molecular lengths, not otherwise as measures of molecular weight.

The measurement of relative sedimentation rates in sucrose is illustrated in Fig. 1. Owing to the compact bands of sedimenting DNA and the 4 cm height of

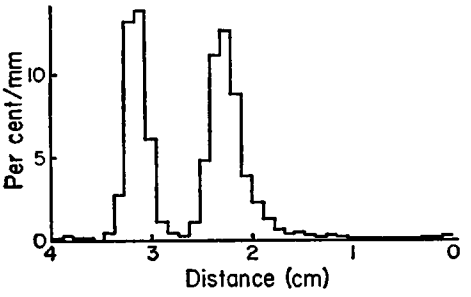

FIGURE 1 Sedimentation of a mixture of T2 DNA (left band) and 38.3 s fragments. Distance is measured from the meniscus.

the liquid column, the distances sedimented can be measured rather precisely. In a satisfactory run, the bulk of a native phage DNA is recovered from a zone 2 or 3 mm wide. Broken DNA forms a wider band reflecting a diversity of lengths which, in the example shown, has been largely eliminated by chromatographic fractionation.

All the measurements with calibrated T2 fragments are collected in Table II. They yield estimates of k ranging from 0.33 to 0.38 with an average of 0.352 $\pm$ 0.005. The fluctuations are random and do not depend noticeably on the molecular lengths of the fragments compared, suggesting that k is independent of molecular weight, that the molecular weights are correct, and that the rate of sedimentation does not depend on distance sedimented.

Estimate of k from Breakage of Lambda DNA. For the corresponding measurements with lambda DNA, we relied more directly on the assumption (in fact easily proved) that breakage of the DNA at a critical rate of shear produces

TABLE II

SEDIMENTATION OF T2 DNA FRAGMENTS IN SUCROSE SOLUTION

Centrifugation	L_2	L_1	D_2	D_1	k
$\times 10^9$ RPM^2 *hr.*			*cm*	*cm*	
3.6	1.0	0.46	3.32	2.53	0.34, 0.36
3.6	1.0	0.40	3.32	2.39	0.35, 0.37
3.6	1.0	0.25	3.49	2.17	0.33, 0.35
4.6	0.46	0.25	3.19	2.56	0.34, 0.35, 0.38
				Average	0.352 ± 0.005

Each row of figures refers to two or three centrifuge tubes containing P^{32}-labeled T2 DNA fragments of lengths L_2 and L_1, respectively (see Table I). D_2 and D_1 are the average distances sedimented. The exponent k in equation 3 is given for each tube.

fragments whose number-average length is half the length of the unbroken molecules. After stirring a solution of P^{32}-labeled DNA for 30 minutes at 1820 RPM and 10 μg/ml to break only part of the DNA, we determined the relative rates of sedimentation of the broken and unbroken components of the mixture. A series of such measurements with a single DNA preparation served to counter possible sources of error. First, the distance sedimented was varied to check the constancy of the rate of sedimentation. Second, H^3-uridine was added to the solutions to permit monitoring of the size of drops collected, because variation in these could introduce systematic errors.

Results of a single run are illustrated in Fig. 2, which shows that the drop size

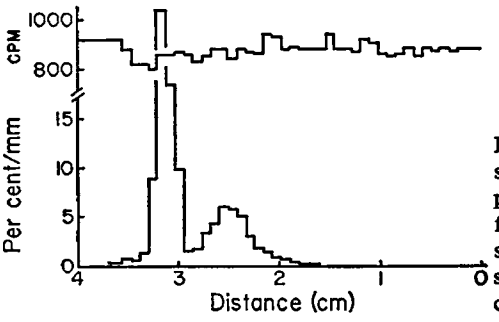

FIGURE 2 Sedimentation of a partly broken sample of P^{32}-labeled lambda DNA. The upper plot shows CPM per 3 drop sample originating from H^3-uridine added to the sucrose and DNA solutions. Other experiments show that the sedimentation of the unbroken DNA is not changed when part of the DNA is broken.

is virtually constant, and that the ratio of distances sedimented by unbroken and broken DNA is 1.27. For the entire series of measurements to be presented, the average ratio computed from drop numbers and from the tritium counts agreed within 1 per cent.

Results of all the measurements are summarized in Table III. The best estimate of D_2/D_1 is 1.267, and the ratio does not depend on distance sedimented. To

TABLE III

SEDIMENTATION OF PARTLY BROKEN λ DNA IN SUCROSE SOLUTION

Centrifugation	D_2	D_2/D_1	Centrifugation	D_2	D_2/D_1
$\times 10^9$ RPM^2 *hr.*	*cm*		$\times 10^9$ RPM^2 *hr.*	*cm*	
6.1	3.16	1.26	4.5	2.34	1.28
6.1	3.18	1.27	4.5	2.23	1.28
6.1	3.05	1.27	4.5	2.30	1.25
6.1	3.07	1.27	4.2	2.27	1.25
6.1	3.28	1.26	4.2	2.32	1.31
6.1	3.20	1.27	4.2	2.24	1.26
	Average	1.267 ± 0.002			1.272 ± 0.009

D_1 and D_2 are distances sedimented by the broken and unbroken components in the mixture whose sedimentation is illustrated in Fig. 2.

compute k, one must assign a median (weight-average) relative length to the broken DNA. The limits are 0.50, for uniform lengths, and 0.52, for lengths normally distributed with a coefficient of variation of 20 per cent as found for T2 fragments (Burgi and Hershey, 1961). (A larger coefficient of variation would be incompatible with the experimental fact that continued stirring at the critical speed does not diminish the size of the broken fragments. In fact, the variation in lengths of broken lambda DNA is less than that of T2 DNA, as judged by the widths of the sedimenting bands.) The exponent k in equation 3 therefore falls between the limits 0.34 and 0.36, from which we conclude that it is the same for the DNA's of T2 and lambda.

The breakage of T5 DNA is also compatible with the conclusion that k in equation 3 is 0.35. This case is complicated, however, by preferential breakage points in the molecules, concerning which we hope to report at another time.

Application to Molecular Weight Measurements. In Table IV we compare sedimentation rates of T5 and lambda DNA's with those of previously calibrated fragments of T2 DNA. Measurements were made with pairs of H^3- and P^{32}-labeled DNA's, or with P^{32}-labeled materials only, as convenient. We also include in the table data for small fragments of T2 DNA (27.2 s and 14.8 s, prepared by stirring at 2000 and 6000 RPM, respectively). Molecular weight ratios are computed according to equation 3, in which $k = 0.35$.

These and other data are consistent with the following assignment of molecular weights: 130 million for T2 DNA, 77 million for T5 DNA, 31 million for lambda DNA, 23.5 million for 27.2 s fragments of T2 DNA, and 4.3 million for 14.8 s fragments of T2 DNA. The figure given for T2 DNA is our reference molecular weight (Rubenstein *et al.*, 1961), and does not come into question in this paper. The figure given for T5 DNA may be compared with 80 to 84 million estimated by boundary centrifugation and tests of molecular fragility (Hershey *et al.*, 1962).

TABLE IV

RELATIVE MOLECULAR WEIGHTS OF SEVERAL DNA'S

Centrifugation	DNA_2	D_2	M_2	DNA_1	D_1	M_1
$\times 10^9$ RPM^2 *hr.*		*cm*	$\times 10^6$		*cm*	$\times 10^6$
3.7	T2	3.17	130	λ	1.95	32.5
3.0	T2	2.52	130	λ	1.58	34.0
5.1	30.3s T2	2.75	32.5	λ	2.71	31.2
5.4	30.3s T2	2.82	32.5	λ	2.77	31.0
3.6	T2	3.20	130	T5	2.67	78.0
3.6	T2	3.25	130	T5	2.72	78.5
5.3	λ	2.42	31.0	T5	3.31	75.5
4.3	λ	1.83	31.0	T5	2.52	77.0
5.4	λ	2.79	31.0	27.2s T2	2.53	23.5
3.8	T2	3.41	130	14.8s T2	1.03	4.3
6.4	λ	3.29	31.0	14.8s T2	1.61	4.0

Each row represents a centrifuge tube containing a pair of DNA's, and shows distances sedimented and molecular weights. M_2 is in each case the reference weight from which M_1 is calculated according to equation 3, $k = 0.35$.

The figure given for lambda DNA was checked as described in the following section of this paper. Figures given for the small fragments are unsupported, but will be useful in another connection.

Molecular Weight of Lambda DNA from Tests of Molecular Fragility. Molecules of lambda DNA sediment only slightly less rapidly in sucrose than quarter-length fragments of T2 DNA. If the sedimentation rates are measures of length, the two materials should break at about the same rate of shear. The prediction was tested by stirring mixtures of H^3-labeled lambda DNA and P^{32}-labeled fragments of T2 DNA.

The test proved somewhat inelegant because we found that short, uniform fragments of T2 DNA could not be isolated by chromatographic fractionation, as was evident from the relatively broad bands observed in zone centrifugation. Probably after breakage, heterogeneity in composition of the fragments intervened to prevent effective fractionation with respect to size. As a result, quarter-length (in contrast to longer) fragments failed to separate into two distinct sedimenting species after further breakage under shear. Instead, one saw only a gradual decrease in sedimentation rate as the speed of stirring was increased.

Nevertheless, it was possible to verify that lambda DNA is about equally as fragile as 30.3 s (quarter-length) fragments of T2 DNA, which sediment 1.02 times faster than lambda DNA in sucrose, or 28.9 s fragments of T2 DNA, which sediment 1.02 times slower than lambda DNA in sucrose, and that it is distinctly more fragile than 27.2 s fragments of T2 DNA, which sediment 1.1 times slower than lambda DNA in sucrose. A comparison with the 30.3 s fragments is illustrated in Fig. 3. It shows that the median sedimentation rate of the T2 fragments

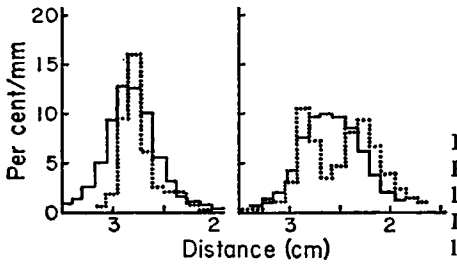

FIGURE 3 Sedimentation pattern of a mixture of P^{32}-labeled, 30.3 s fragments of T2 DNA (solid line) and H^3-labeled lambda DNA (dotted line). Left, unstirred. Right, stirred to break half of the lamba DNA.

was reduced on stirring by the factor 1.14, corresponding to a 1.45-fold reduction in weight-average molecular weight (equation 3). If the distribution of lengths was not altered by stirring, reduction in number-average weight was the same. Thus breakage of half the lambda DNA molecules was matched by breakage of roughly 45 per cent of the T2 fragments, mainly, of course, the longer ones.

The conclusion follows that lambda DNA resembles quarter-length fragments of T2 DNA both in sedimentation rate and in fragility, and consequently also in molecular weight and length. The molecular weight of 31 million cited previously is therefore about right.

Boundary Sedimentation and Solution of Equation 1. In Table V we collect sedimentation coefficients measured in the optical centrifuge for DNA's that have been studied by zone centrifugation. The DNA's are arranged in pairs to permit calculation of k by equation 2 for different molecular weight ranges.

Recall that zone-sedimentation rates for all these materials substitute into equation 3 to give a constant $k = 0.35$. The optically measured sedimentation coefficients also require an exponent of 0.35 (equation 2) for the molecular weight range below 32.5 million. Above this range, k rises sharply, indicating that the sedimentation coefficients are artificially high owing to a disturbance that augments as molecular weight increases.

The anomaly detected in this way apparently disappears abruptly below the molecular weight 32.5 million. This is shown particularly well by the 27.2 s and 14.8 s fragments of T2 DNA. Although the molecular weights given for these fragments in Table IV are doubtful extrapolations, their use in Table V shows that the fragments sediment at the same rate in relation to quarter-length fragments whether the comparison is made by boundary or zone centrifugation. A further indication that the smaller sedimentation coefficients may be reliable comes from the report of Meselson and Nazarian (1963) that sedimentation coefficients of lambda and T7 DNA's agreeing with the conventional ones can be measured from the approach to equilibrium in density-gradient centrifugation.

We therefore choose lambda DNA of molecular weight 31 million as a reference relating sedimentation rates measured by the two methods. The sedimentation coefficient 30.5 s (or 33 s in 0.1 M NaCl) becomes $S_{20,w} = 32$ when corrected to standard conditions, and should be increased to $S^0_{20,w} = 33.6$, in lieu of extrapola-

TABLE V

DEPENDENCE OF OPTICALLY MEASURED SEDIMENTATION COEFFICIENT ON MOLECULAR WEIGHT

DNA_1	S_1	M_1	DNA_2	S_2	M_2	k (eq. 2)	Reference
	s	$\times 10^6$		s	$\times 10^6$		
T2	63	130	T2 halves	43	65	0.55	*
T5	48.5	84	T5 halves	32.8	42	0.56	‡
T2 halves	43	65	T2 quarters	30.3	32.5	0.51	*
λ	30.5	31.0	λ halves	24.0	15.5	0.35	§
T2 quarters	30.3	32.5	T2 fragments	27.2	23.5	0.33	||
T2 quarters	30.3	32.5	T2 fragments	14.8	4.3	0.35	||

* Burgi and Hershey, 1961.
‡ Hershey *et al.*, 1962.
§ Hershey, Burgi, and Ingraham, 1963.
|| This paper, Table IV.

tion to zero concentration, according to the data of Eigner *et al.* (1962). Adopting the latter figure, we complete equation 1 by setting $a = 0.080$ and $k = 0.35$, from which it follows that T2 DNA at very low concentrations should exhibit a sedimentation coefficient of 55.5 instead of the $S_{20,w} = 66$ observed at 10 μg/ml. This conclusion is qualitatively verified by measurements to which we now turn.

Effect of DNA Concentration. The sedimentation rate of DNA's of about 30 s decreases as the concentration is raised (Eigner *et al.*, 1962; Davison and Freifelder, 1962). As Eigner *et al.* remark, one might expect a more severe effect in the same direction for DNA's of higher molecular weight. When DNA concentration is varied in zone centrifugation, however, the direction of the effect is seen to depend on molecular weight.

TABLE VI

EFFECT OF CONCENTRATION OF DNA ON RATE OF SEDIMENTATION IN SUCROSE

Centrifugation	DNA_1	Concentration of DNA_1	D_1	DNA_2	D_2
$\times 10^9$ RPM^2 *hr.*		μg/ml	cm		cm
5.1	λ	2	2.74	λ fragments	1.86
		12	2.61		1.84
		22	2.51		1.82
3.7	T2	0.2	3.17	λ	1.95
		10	3.34		1.94
		15	3.48		1.96

Each row represents a centrifuge tube containing DNA_1 at the specified initial concentration and DNA_2 at less than 1 μg/ml. DNA_2 serves to check the distance measurements.

Table VI shows that for lambda DNA (30.5 s) the sedimentation rate in sucrose falls distinctly and progressively as the initial concentration in the band is increased from 2 to 22 μg/ml. This is the expected result. For T2 DNA (63 s), an equally clear effect of the opposite kind appears. For either DNA, varying the concentration in the range between 1.0 and 0.1 μg/ml is without noticeable effect.

The influence of concentration on rate of sedimentation is also visible in the shape of the bands. At low concentrations, homogeneous DNA's yield symmetrical bands. At high concentrations, the band of sedimenting lambda DNA is skewed in the direction expected if the center of the band moves more slowly than its edges. The sedimenting band of T2 DNA is distorted in the opposite sense. These effects are illustrated in Fig. 4.

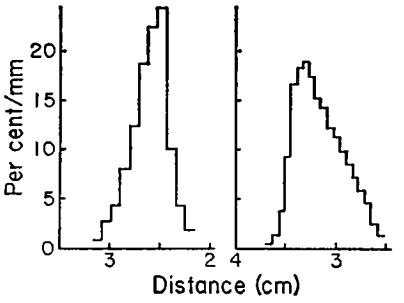

FIGURE 4 Distorted shape of sedimenting bands at high DNA concentrations. Left, lambda DNA at 12 μg/ml initial concentration. Right, T2 DNA at 15 μg/ml. See Figs. 1 and 2 for results at low concentrations.

We also tested the effect of concentration on rate of sedimentation of quarter-length fragments (30 s) of T2 DNA. In the range up to 15 μg/ml initial concentration, no significant influence on rate of movement of the band was detected, perhaps because, owing to the heterogeneity of the material, the actual concentration in the sedimenting band was too low. The distorted shape of the band clearly showed, however, that 30 s fragments of T2 DNA resemble lambda DNA in that the sedimentation rate falls as the concentration is increased. It is likely, therefore, that the nature of the concentration effect depends on molecular weight, not on the species of DNA as such. This conclusion is necessary, in fact, to explain the results already shown in Table V.

DISCUSSION

Interpretations given in this paper are based on the assumption that by zone centrifugation at low DNA concentrations one measures authentic sedimentation rates of individual molecules. Besides intrinsic plausibility, the assumption has the virtue of leading to a number of mutually consistent conclusions.

For one, we find that the molecular weight of one isotopically labeled DNA can be measured in terms of that of another from the ratio of distances sedimented according to the relation

$$\frac{D_2}{D_1} = \left(\frac{M_2}{M_1}\right)^{0.35} \tag{4}$$

Equation 4 works over the tested range from molecular weight 130 million for T2 DNA to 15.5 million for half-length fragments of lambda DNA, and for DNA's of three species. Its generality accords with other evidence that the materials mentioned consist of threadlike molecules whose hydrodynamically important variable is length (Hershey *et al.*, 1962). This is not to say, of course, that species-specific properties are necessarily irrelevant. For one thing, the partial specific volume, and therefore sedimentation rate, must depend to some extent on composition. For another, lambda DNA can be prepared in two different monomeric forms, only one of which sediments according to equation 4 (Hershey, Burgi, and Ingraham, 1963). But for typical, linear DNA molecules, considerations other than length are probably minor in relation to errors that must be tolerated for the present.

In the lower part of its tested range (molecular weights 15.5 to 32.5 million) equation 4 also holds for optically measured sedimentation coefficients, generating the relation

$$S^0_{20,w} = 0.080\ M^{0.35} \tag{5}$$

Equation 5 is based on the measurements $S^0_{20,w} = 33.6$ and $M = 31 \times 10^6$ for lambda DNA. It resembles the equation of Doty *et al.* (1958) and agrees within 15 per cent with their measurements for thymus DNA of molecular weight one million, suggesting that, when remaining errors are corrected, a universal relation will emerge.

One can also measure sedimentation coefficients by zone sedimentation, with a reproducibility of perhaps ±5 per cent with existing equipment, from

$$S^0_{20,w} = \frac{6.45 \times 10^{10} D}{\omega^2 t} \tag{6}$$

where D is measured in centimeters and $\omega^2 t$ in RPM^2 hours. The numerical coefficient is taken from the data of Table III and assigns to lambda DNA an $S^0_{20,w}$ of 33.6. The equation is valid, of course, only for the conditions, including temperature, sucrose solutions, and low DNA concentrations, that we used (*cf.* Nomura *et al.*, 1960). At the present time, equation 6 offers the only means of measuring sedimentation coefficients greater than 33.6 that make sense in terms of equation 5.

The limitation of equation 5 to molecular weights below 32.5 million can now be explained in terms of a molecular interaction, dependent on molecular weight, that causes abnormally fast sedimentation of T2 and T5 DNA's at optically measurable concentrations.

We think it likely that molecules of T2 and T5 DNA cohere or intertwine reversibly in solution by a non-specific, molecular weight–dependent process. No doubt the sedimentation of all DNA's is affected to some extent by aggregation of this kind, just as the sedimentation of all DNA's must be subject to a viscous effect working in the opposite direction. The net effect seen as DNA concentration is varied

thus depends on the molecular weight of the DNA and the viscosity of the solvent.

Aggregation of the same type may help to account for the fact that breakage of T2 DNA causes abnormal broadening of the bands seen in equilibrium-density-gradient centrifugation (Hershey and Burgi, 1961; Thomas and Pinkerton, 1962). Very likely it also contributes to self-protection against breakage under shear, another phenomenon that depends on concentration and molecular weight but not on the species of DNA (Burgi and Hershey, 1962).

Reversible aggregation can also explain, we believe, several other anomalies encountered in the boundary sedimentation of very long DNA molecules (Hearst and Vinograd, 1961; Burgi and Hershey, 1961; Eigner *et al.*, 1962). It is only necessary to assume (1) that the broad, asymmetrical boundary seen under conditions known to suppress convection is a direct result of the postulated aggregation, and (2) that such boundaries are abnormally sensitive to convective disturbance. Assumption (1) is supported by results presented in this paper. We verified assumption (2) by showing that the apparent sedimentation rate of T2 DNA in the optical centrifuge can be reduced (and the boundary sharpened) by heating the rotor slowly and continuously during the run, whereas the same or more rapid heating does not affect the sedimentation of fragmented T2 DNA, which normally exhibits a sharp boundary.

It should be added that extrapolation of measurements made by the optical boundary method to zero concentration does not correct for the excessive rate of sedimentation of T2, T4, and T5 DNA's seen at high concentrations. According to Hearst and Vinograd (1961) and Thomas and Pinkerton (1962), the sedimentation rate of these DNA's is nearly independent of concentration over the range accessible to optical measurements, and at rotor speeds usually considered adequate to suppress convection. Such measurements, corrected in any reasonable way for the viscosity of the solution, show that the sedimentation coefficient rises as the concentration is increased. This fact itself, together with the shape of the boundaries, first suggested to us the possibility of reversible aggregation.

In view of the limited applicability of equation 5, it is a useful feature of equation 4 that it makes sedimentation coefficients superfluous. Nature has provided in the phages a series of readily available DNA's of virtually precalibrated molecular weights. These, once measured, can serve as standards against which unknown molecular weights may be determined. Or equations 5 and 6 can be combined for absolute but less accurate measurements.

An inconvenience arises when the DNA under study is difficult to prepare in radioactive form. Then one may resort to equation 5 or, for molecular weights between 32.5 million and 130 million, to the equation of Rubenstein *et al.* (1961). This equation reflects the anomalous sedimentation of high molecular weight DNA's but is nevertheless correct, provided sedimentation coefficients are measured as Rubenstein *et al.* prescribe, and is in fact confirmed by a third method in

this paper, inasmuch as molecular weights computed by it (Table I) are consistent with equation 4. The only known error attending use of the Rubenstein equation resides in the fact that the exponent 0.543 is not quite constant over the usable range of the relation, as noted by Burgi and Hershey (1961) and confirmed in the present work (Table V). Since the exponent falls very sharply in the region around 32.5 million molecular weight, extrapolation below that weight cannot be made. One may, in addition, question the generality of an equation depending on molecular interactions that are not understood, but the equation works at least for the DNA's of T2, T5, and lambda phages.

The relations proposed here can be tested most critically by further experience with equation 4, which contains a minimum of ambiguities. The pressing need is for reference DNA's of known molecular weights. We have relied entirely on the DNA of phage T2. According to the scale so established, and equation 5, the *Escherichia coli* DNA of Eigner *et al.* (1962) should have a molecular weight of 30 million instead of the 16 million assigned to it by those authors, and the DNA of phage T7 should have a molecular weight of 27 million instead of the 19 million measured by Davison and Freifelder (1962). As noted by the latter authors, such discrepancies merely revive the question of how molecular weights should be measured. Sufficiently accurate measurements of DNA content per phage particle would be one answer.

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