the primary chain lengths directly from the extent of reaction of -OH groups. Second, there is no measure here of the extent of ester interchange proceeding in the carbonate copolymers during condensation. Such interchange would be helpful in assuring the assumed random distribution of bonds throughout the polysperse copolymer. Lateral evidence (not presented) indicates essentially negligible ester interchange under the mild conditions employed in the present polycondensations. Finally, the finding of a branching efficiency less than unity suggests nonequivalence in the reactivities of the three phenolic -OH groups in phloroglucinol. Such nonequivalence should somewhat distort the branching complexity and thereby perturb the molecular distribution function. Such a perturbation is not even qualitatively estimated here, but it is believed not to have any appreciable influence on the above computation of branching efficiency.

The calculation of phloroglucinol's branching efficiency in phloroglucinol–bisphenol-A–phosgene condensations, by application of a theoretical treatment of intrinsic viscosity and weight-average molecular weight data, has led to self-consistent results. Although these results are not conclusive in themselves they are indicative of the utility and probable validity of the evaluation procedure.

Acknowledgment. The authors wish to thank Mr. Donald Papuga for assisting in the light scattering and solution viscosity measurements.

Molecular Weights of Homogeneous Samples of Deoxyribonucleic Acid Determined from Hydrodynamic Theories for the Wormlike Coil

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ABSTRACT: The molecular weights of a number of viral DNA's have been calculated using the hydrodynamic theories for the wormlike coil and the Flory–Mandelkern equation. The theoretical fit to the experimental sedimentation and viscosity data is good. The Kuhn statistical length of DNA has been calculated to be 805 Å in a solution with an ionic strength of 0.2. The ionic strength dependence of $1/\lambda$ is large and has been calculated. The range of molecular weights calculated for T-2 bacteriophage DNA is 94.4×10^6 to 105×10^6. The range calculated for T-7 bacteriophage DNA is 21.6×10^6 to 24.0×10^6.

Theoretical analysis of the hydrodynamic properties of dilute polymer solutions has been quite successful in the case of synthetic polymers. Flory[1] pointed out many years ago that the Kirkwood[2] theory for the translational friction factor for a random coil predicts a proportionality constant between $s_{20,w}$ and $M^{1/2}$ which is in precise agreement with experiment for polymers in θ solvents. Similarly, the Zimm[3] theory for the intrinsic viscosity of a random coil predicts a proportionality constant, ϕ, between $[\eta]$ and $M^{1/2}$ which is only 14% higher than that estimated by Flory[1] for a monodisperse polymer sample. This agreement is somewhat surprising in view of the assumptions which are required for the development of these theories. (1) The solvent is considered to be a hydrodynamic continuum. (2) The polymer chain is replaced by a string of beads which interact with each other as point disturbances. (3) The mathematical complexities of this problem have required the use of a premature average of the interaction tensor. (4) The intrinsic viscosity is calculated using a dynamic model (the Rouse chain) of balls connected by springs with average equilibrium lengths of zero. This model has $3N$ degrees of freedom where N is the number of beads in the chain, while any real chain would be expected to have only $2N + 1$ degrees of freedom.

Recognizing that the physical foundation for these theories is less than perfect, it remains true that their agreement with experiment has been impressive for ideal random coils. The extension of these theories to a molecular model which is reasonable for DNA (the wormlike coil) has been carried out in a series of papers,[4-8] using precisely the same assumptions which have been applied to the random coil. These newer papers provide results which apply to models with any properties intermediate between and including the properties of the rigid rod and the random coil. In addition, the effects of excluded volume have been treated.

Despite the good agreement between the theoretical proportionality constants and experiments, the Kirkwood–Riseman theory has frequently been considered incorrect because it predicts an intercept for the plot of $s_{20,w}$ *vs.* $M^{1/2}$ which is different from zero, while

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(1) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, Chapter 14.

(2) J. G. Kirkwood, *J. Polym. Sci.*, **12**, 1 (1954).

(3) B. H. Zimm, *J. Chem. Phys.*, **24**, 269 (1956).

(4) J. E. Hearst and W. H. Stockmayer, *ibid.*, **37**, 1425 (1962)

(5) R. A. Harris and J. E. Hearst, *ibid.*, **44**, 2595 (1966).

(6) J. E. Hearst, R. A. Harris, and E. Beals, *ibid.*, **45**, 3106 (1966).

(7) H. B. Gray, Jr., V. A. Bloomfield, and J. E. Hearst, *ibid.*, **46**, 1493 (1967).

(8) J. E. Hearst, E. Beals, and R. A. Harris, *ibid.*, **48**, 5371 (1968).

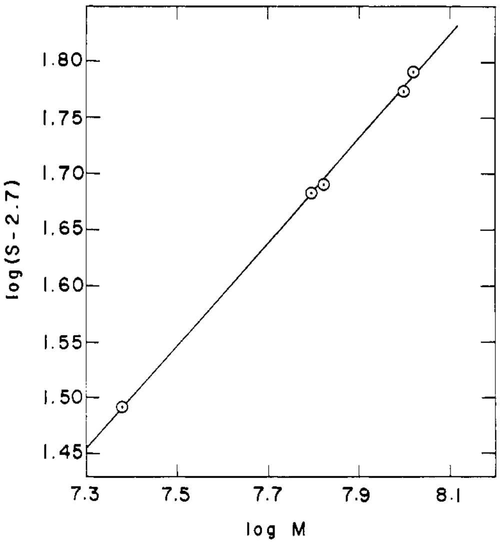

Figure 1. Plot of log ($s_{20,w}$ − 2.7) *vs.* log M for the determination of ϵ. The value calculated from the slope is ϵ = 0.072 ± 0.036; ionic strength μ = 0.2.

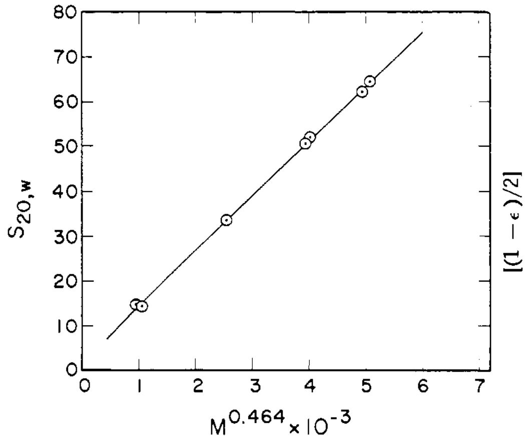

Figure 2. Experimental points (⊙) and the theoretical curve (—) for $s_{20,w}$ *vs.* M. Assuming a mass per unit length of the DNA to be 196 daltons/Å, the slope yields a value of the Kuhn statistical length of DNA in a solution of ionic strength μ = 0.2 of 1/λ = 805 Å. The highest molecular weight point on the plot is T-2 DNA, the lowest is Polyoma DNA.

Table I

Calculated and Experimental Intrinsic Viscosities[a] of Homogeneous DNA's

b, Å	T-2	T-4	T-5	T-5 sto	T-7
25.1	313	302	229	218	112
30.0	317	307	232	222	114
35.0	321	310	235	225	116
Exptl	316	313	240	226	111

[a] Units are deciliters per gram (1/λ = 805 Å).

experiments on synthetic polymers do not verify this prediction. A more careful analysis of this problem by Hearst and Stockmayer,[4] and Gray, Bloomfield, and Hearst[7] has shown that the fundamental assumptions of Kirkwood and Riseman[2] remain valid. In order to correct the prediction of the intercept, the average reciprocal distance between chain elements at small contour distances must not be obtained from a Gaussian distribution function. An interpolation scheme which provides more realistic values of $\langle 1/r \rangle$ for short contour distances was used by Hearst and Stockmayer[4] and has been incorporated into all subsequent manuscripts on the wormlike coil[7,8] with favorable results. Most synthetic polymers have flexibilities which theory predicts would lead to no intercept. Experimentally, stiff chain polymers such as DNA have finite intercepts in both the sedimentation and viscosity plots in agreement with the modified theory.

In this manuscript we analyze the best sedimentation and viscosity data presently available for monodisperse samples of native DNA in terms of these theories. Our purpose is twofold. First, the analysis provides an independent determination of molecular weights for the large DNA's. Second, the predicted properties of DNA solutions will ultimately provide a stringent test for the validity of these theories.

Methods and Results

We have calculated molecular weights from the Flory–Mandelkern[1] equation. This, of course, is not an original notion. In an analysis by Eigner and Doty,[9] the Flory–Mandelkern constant β has been estimated for DNA solutions. At the time the Eigner–Doty[9] manuscript was written, DNA had hydrodynamic properties which were not understood, so a reasonable value for β was not predictable. The β's were therefore calculated using molecular weights obtained by other methods.[10–12] Since the theoretical value of β has now been calculated from the wormlike coil,[8] there is no particular reason to suspect hydrodynamic predictions compared to the predictions of these other methods.

Our choice of β = 2.81 is precisely consistent with Table II of ref 8, but the range of molecular weights calculated by taking β = 2.65 and 2.85 and $\bar{v}$ = 0.555 ml/g is broad enough to encompass the value obtained by choosing ϕ to be 14% smaller. This results in molecular weights only 7% larger and thus does not account for the rather substantial discrepancy for T-2, T-4, and T-5 DNA between these results and most others obtained from the electron microscope and autoradiography.[13–15] It is our belief that these other methods provide results well outside the region of reasonable error in the hydrodynamic theories.

The excluded volume parameter, ϵ, has been determined from a log–log plot of $s_{20,w}$ −2.7 against M, Figure 1. The resulting value of ϵ is 0.072 ± 0.036 and is independent of the choice of β. There is substantial uncertainty in ϵ which will remain until data for a much broader molecular weight range are available. Once ϵ is determined, the molecular weight dependence of $s_{20,w}$, Figure 2, uniquely determines the

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(9) J. Eigner and P. Doty, *J. Mol. Biol.*, **12**, 549 (1965).

(10) D. J. Cummings and L. M. Kozloff, *Biochem. Biophys. Acta*, **44**, 445 (1960).

(11) I. Rubenstein, C. A. Thomas, Jr., and A. D. Hershey, *Proc. Nat. Acad. Sci. U. S.*, **47**, 1113 (1961).

(12) P. F. Davidson and D. Freifelder, *J. Mol. Biol.*, **5**, 635 (1962).

(13) C. A. Thomas, Jr., *J. Gen. Physiol.*, **49**, 143 (1966).

(14) J. Abelson and C. A. Thomas, Jr., *J. Mol. Biol.*, **18**, 262 (1966).

(15) R. B. Inman, *ibid.*, **25**, 209 (1967).

TABLE II
CALCULATED MOLECULAR WEIGHTS OF HOMOGENEOUS DNA'S[g]

DNA	$s_{20,w}$, S	Ref	$[\eta]$, dl/g	Ref	$M \times 10^{-6}$ $\beta = 2.65$	$M \times 10^{-6}$ $\beta = 2.85$	$M \times 10^{-6}$ $\beta = 2.81$
T-2	64.5	*a*	316	*a*	105	94.4	96.4
T-4	62.1	*b*	313	*c*	99.7	89.6	91.5
T-5	51.8	*b*	240	*c*	66.6	59.9	61.1
T-5 sto	50.7	*b, d*	226	*c*	62.3	56.0	57.2
T-7	33.7	*a*	111	*a*	24.0	21.6	22.1
ϕX174 RF	14.3	*e*				3.4[e]	
Polyoma	14.5	*f*				2.5[f]	

[a] D. Crothers and B. Zimm, *J. Mol. Biol.*, **12,** 525 (1965) (solvent 0.006 M Na_2HPO_4, 0.002 M $NaH_2PO_4^{2-}$, 0.179 M NaCl, and 10^{-3} M Na_2EDTA). [b] H. B. Gray, Jr., and J. E. Hearst, *ibid.*, **35,** 111 (1968) (solvent 0.044 M Na_2HPO_4 0.056 M NaH_2PO_4, 0.1 M NaCl and 10^{-3} M Na_2EDTA). [c] P. Ross and R. Scruggs, *Biopolymers*, **6,** 1005 (1968) (data interpolated to 0.2 M NaCl). [d] S. B. Leighton and I. Rubenstein, *Biophys. J.*, **8,** A-80 (1968) (s_{sucrose} = 49.2 T-5, 48.1 T-5 sto) $s_{20,w}$ for T-5 DNA in 0.2 ionic strength buffer measured in footnote *b* as 51.8 S. T-5 sto therefore corrected to 51.8(48.1/49.2) = 50.7 S. [e] A. Burton and R. L. Sinsheimer, *J. Mol. Biol.*, **14,** 327 (1962). The molecular weight was obtained by doubling the single-stranded DNA molecular weight obtained from light scattering. The $s_{20,w}$ for double-stranded rings with one strand open (16.2) has been corrected to a linear DNA value by multiplying by 0.88. [f] J. Vinograd, J. Lebowitz, R. Radloff, R. Watson, and P. Laipis, *Proc. Nat. Acad. Sci. U. S.*, **53,** 1104 (1965). [g] $\bar{v}$ for NaDNA = 0.555 (see footnote *b*).

TABLE III
STATISTICAL LENGTH (1/λ) AS A FUNCTION OF NaCl CONCENTRATION

	1 M	0.2 M	0.1 M	0.01 M	0.005 M
Slope of Figure 5 $\times 10^{-4}$	36.6	47.2	48.5	80.5	96.5
1/λ, Å	664	794[a]	805	1165	1315

[a] The discrepancy between this value of 1/λ at μ = 0.2 and the value of 805 obtained from Figure 2 is well within the experimental error of the two sets of data.

quantity $M_L^{1+\epsilon}\lambda^{1-\epsilon}$ where M_L is the molecular weight per unit length and 1/λ is the Kuhn statistical length of the coil. Thus all that has been calculated to this point is completely independent of the value of M_L and λ alone. The values of 1/λ calculated here assume M_L = 196 daltons/Å which is the mass per unit length of the Watson–Crick helix. This number may or may not apply to DNA in 0.2 M NaCl. Figure 3 shows the calculated intrinsic viscosity of monodisperse DNA's together with the experimental data. Figure 4 contains the calculated intrinsic viscosity over a broader range of molecular weights than presently available for DNA.

Table I contains the calculated intrinsic viscosity of the various DNA's using 1/λ′ = 805 Å which was determined from the slope of Figure 2. The uncertainty in 1/λ from Figure 2 is less than ±3% which introduces a maximum uncertainty in the calculated $[\eta]$ of ±4%. Table I demonstrates the weak dependence of $[\eta]$ on the parameter *b* as well as the close agreement with the experimental values of Table II.

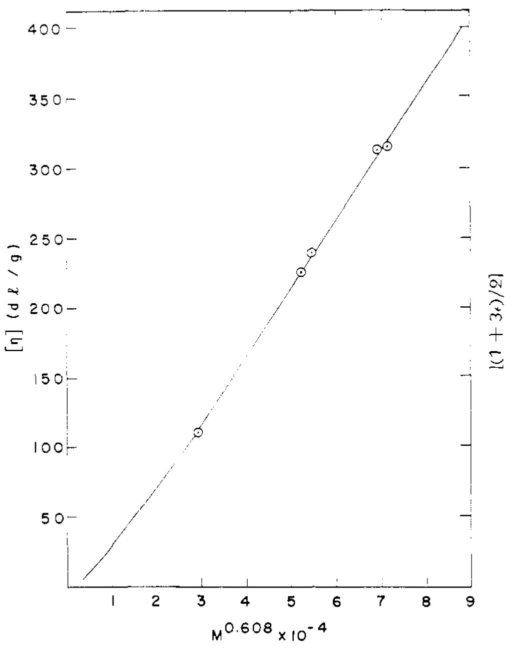

Figure 3. Experimental points (⊙) and the theoretical curve (—) for $[\eta]$ *vs.* M. The highest molecular weight point on the plot is T-2 DNA, the lowest is T-7 DNA.

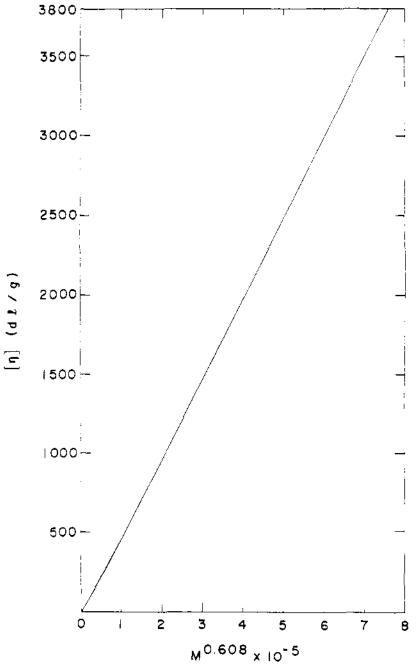

Figure 4. The theoretical plot of $[\eta]$ *vs.* M for DNA in a solution of ionic strength μ = 0.2 for molecular weights larger than those for which well-characterized DNA is presently available.

TABLE IV
FIRST RELAXATION TIMES (SECONDS) FOR HOMOGENEOUS DNA'S

	T-2	T-4	$^1/_2$T-4	λb2b5c	$^1/_2$λ
τ_1(calcd) $1/\lambda = 805$ $b = 30$ Å	0.525	0.482	0.151	0.0399	0.0126
τ_1(exptl)	0.45	0.43–0.49	0.107–0.160	0.029–0.034	0.0105–0.0125
Ref	*a, b*	*c*	*c*	*c*	*c*

[a] D. S. Thompson and S. J. Gill, *J. Chem. Phys.*, **47**, 5008 (1967). [b] R. E. Harrington, *Biopolymers*, **6**, 105 (1968). [c] P. Callis and N. Davidson, private communication.

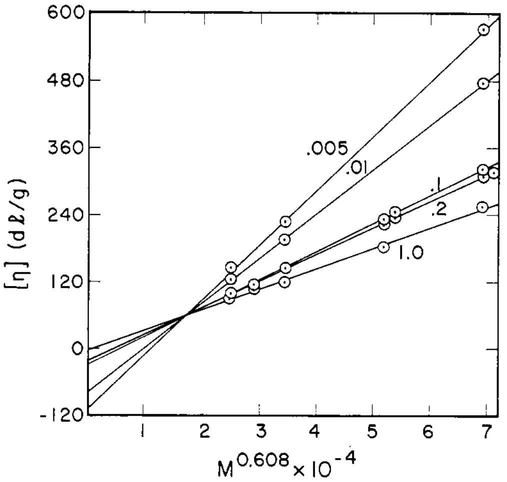

Figure 5. The ionic strength dependence of the plot of [η] *vs.* *M* from the data of Ross and Scruggs.[16] The negative intercepts of these experimental straight line plots are predicted by theory[8] as displayed in Figures 3 and 4.

Scruggs and Ross[16] have recently published studies on the ionic strength dependence of the intrinsic viscosity of DNA. They choose to interpret their data by allowing ϵ to change but as a result get a nonmonotonic trend in the ionic strength dependence of $1/\lambda$. Their data are not sufficiently abundant or precise to determine ϵ, so we have chosen to hold $\epsilon = 0.072$ and interpret the change as a function of $1/\lambda$. It is clear within the range of these data, either interpretation or a combination of both can be made to fit the data. Figure 5 shows the plot of [η] *vs.* $M^{0.608}$ for the different ionic strengths. Table III contains the slope of these lines and the calculated Kuhn statistical lengths, $1/\lambda$.

Table IV contains calculated first relaxation times and the experimentally observed longest relaxation times from flow birefringence and dichroism relaxation measurements. Agreement is generally good although the calculated relaxation times are slightly higher than those observed.

Discussion

Using sedimentation and viscosity data on homogeneous samples of DNA, we have calculated molecular weights and persistence lengths consistent with the hydrodynamic theory for the wormlike coil. The calculated molecular weights for T-2, T-4, and T-5 bacteriophage DNA's are about 20% lower than the molecular weights estimated from electron microscope contour lengths and autoradiography.

The molecular weight of the subunit of *E. coli* DNA isolated by Massie and Zimm[17] ($s_{20,w} = 81$, [η] $= 500 \pm 50$) has been calculated to be 1.7×10^8. The corresponding theoretical $s_{20,w} = 82.5$ and [η] $= 475$. These are in reasonable agreement with the experimental results. These data points have not been included in the above analysis, because this DNA is not monodisperse.

Acknowledgments. We wish to acknowledge the assistance of Eric Beals with the computer calculations. This research was supported by USPH Grant No. GM 11180, the Laboratory of Chemical Biodynamics of the AEC, and NASA Grants NsG 243 and NsG 479.

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(16) See Table II, footnote *c*.

(17) H. R. Massie and B. H. Zimm, *Proc. Nat. Acad. Sci. U.S.*, **54**, 1636 (1965).