[CONTRIBUTION FROM THE GIBBS LABORATORY, DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

A Further Examination of the Molecular Weight and Size of Desoxypentose Nucleic Acid

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Light scattering and low gradient viscometric studies of new sodium desoxypentose nucleate (DNA) samples from calf thymus have been carried out. These together with earlier measurements are interpreted on the basis of new values for the specific refractive index increment and the extinction coefficient. It is concluded that carefully prepared samples of DNA have a weight average molecular weight of about six million and a molecular shape resembling a stiff coil whose root-mean-square end-to-end separation is 5000 Å. and whose contour length is 20,000 Å. The following evidence corroborates these findings: (1) The molecular weight calculated fron the sedimentation constant–intrinisc viscosity formula of Mandelkern and Flory, (2) the end-to-end separation calculated from the Flory–Fox equation, (3) the agreement with the Riseman–Kirkwood relation between rotary diffusion constant and intrinsic viscosity for coils and (4) preliminary electron microscope studies by R. C. Williams. If intrinsic viscosity and rotary diffusion constants are interpreted in terms of an ellipsoid of revolution with no hydration axial ratios in the range of 400 to 500 are obtained but the literal interpretation of these values must be rejected.

Previous communications from this Laboratory[4,5] have dealt with the molecular weight, size and shape of four preparations of sodium desoxyribonucleate (DNA) from calf thymus. We wish to report here on the measurement of further samples of particular interest, to revise slightly our earlier molecular weight values on the basis of new determinations of the specific refractive index increment, dn/dc, and the extinction coefficient, ϵ, and to discuss other types of measurements which lead to the same conclusions.

I. Molecular Weight

Values of Specific Refractive Index Increment and Extinction Coefficient.—The determination of dn/dc requires a measurement of the refractive index difference between solvent and solution and of the concentration of the solution. This simple operation is made difficult for DNA because the reduced specific viscosity is so great that the maximum concentration employed cannot exceed about 1 mg./cc. thus limiting severely the refractive index difference to be measured, and because the complete removal of water from the sample proves to require special heat treatment. We have prepared approximately 0.1% aqueous solutions of the Varin and Simmons DNA samples described in the next section and using both a Zeiss interferometer and a Brice–Speiser differential refractometer obtained average values of Δn of 171×10^{-6} for Varin and 159×10^{-6} for Simmons DNA at 435 mμ. About 25 g. of each solution was then lyophilized and heated under vacuum at 56° for six hours and then at 110° for 108 hours. Further heating at 120° and 160° did not lead to further loss of weight. The concentrations so determined were 0.834 and 0.924 mg./cc. and this gives dn/dc values of 0.185 and 0.191 for the Varin and Simmons sample, respectively. For the purposes of this paper the average of these two values, 0.188, is used. This differs significantly from the value of 0.160[6] previously used.

The determination of the extinction coefficient (ϵ) at the peak of the 260 mμ absorption band is likewise complicated by the necessity of complete removal of water. Failure in this respect leads to low values. Preliminary investigations in this Laboratory by Dr. R. Varin showed that ϵ decreased with increasing ionic strength and that the decrease was a linear function of the logarithm of the ionic strength. The ratio of ϵ in water to that in 0.2 *M* NaCl solutions was found to be 1.21. Dr. Bunce[7] has found for the B–G DNA sample[4] an ϵ value of 245 in aqueous solution and 218 in 0.2 *M* NaCl. In recent determinations by Blout[8] and ourselves a value of 213 in 0.2 *M* NaCl is found and the ratio of the value in aqueous solution to this is the same as that found by Varin. Because of the agreement of this ratio and the indications that the B–G sample is somewhat degraded, the value of 213 for ϵ is used in this paper. This value is somewhat higher than that calculated from Chargaff's[9] value of 6650 for the average atomic extinction coefficient with respect to phosphorus (accepting 9.24% as the phosphorus content of DNA this gives $\epsilon = 199$) or the value previously used by us ($\epsilon = 175$).

A new investigation of dn/dc has just been reported[10] in which a value of 0.201 has been obtained. The difference between this value and ours (7%) appears to be outside probable experimental error. If it is not due to errors in calibration of the refractometers, it may arise from the incomplete removal of protein in the DNA used by these investigators. Since their concentration determination was based on phosphorus analysis, residual protein would not have been included whereas it would have contributed to the refractive index increment. The dry weight method we employed avoided this difficulty but, of course, substituted the requirement of complete removal of water.

Description of Samples.—All of the DNA samples considered here were prepared from fresh calf thymus. The first four listed in Table I have been described in the earlier publications.[4,5] The preparation of the Chargaff and Dounce samples have also been described,[11-13] whereas the Simmons sample which involves a number of improvements in preparation and which leads to quite reproducible results has not yet been described. In addition we have obtained a measure of the protein content of the Varin sample. Using bovine serum albumin as a standard in the Biuret test, this was found to be 1.2%, this being considerably higher but more reliable than that given by the Sakaguchi test.

Molecular Weights from Light Scattering.—The results of previous investigations corrected for the new dn/dc and ϵ values, and the results on the samples just described are summarized in Table I. In the later work the concentrations measured were in the range of 7 to 40 mg./l. DNA and the results were obtained from plots of the data as shown in Fig. 1 for the Simmons sample. Ignoring the B–G and Gulland samples which were obviously degraded, it is seen that the remaining five samples all prepared by different methods have molecular weights within 20% of the average. The differences found within this range are, however, real since the reproducibility of our measurements is considerably better. For example, three measure-

(1) U. S. Public Health Postdoctorate Fellow, 1951–1953.
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(4) P. Doty and B. H. Bunce, THIS JOURNAL, **74**, 5029 (1952).
(5) M. E. Reichmann, R. Varin and P. Doty, *ibid.*, **74**, 3203 (1952).
(6) H. G. Tennent and C. F. Vibrandt, *ibid.*, **65**, 424 (1943).
(7) Dr. Barbara H. Bunce, Oberlin College, private communication, 1952.
(8) E. K. Blout and A. Asadourian, *Biochim. et Biophys. Acta*, **13**, 161 (1954).
(9) E. Chargaff and R. Lipshitz, THIS JOURNAL, **75**, 3658 (1953).
(10) R. G. Northrop, R. L. Nutter and R. L. Sinsheimer, *ibid.*, **75**, 5134 (1953).
(11) E. R. M. Kay, N. S. Simmons and A. L. Dounce, *ibid.*, **74**, 1724 (1952).
(12) C. Tamm, M. E. Hodes and E. Chargaff, *J. Biol. Chem.*, **195**, 49 (1952).
(13) E. Chargaff and S. Zameuhof, *ibid.*, **173**, 327 (1948)

TABLE I

SUMMARY OF MOLECULAR CONSTANTS FOR NUCLEIC ACID SAMPLES

Based on $\epsilon = 213$, $dn/dc = 0.188$

Sample	$M_w \times 10^{-6}$	$\sqrt{\overline{R^2}}$, Å.	$\sqrt{\overline{R_g^2}}$, Å.	L_w	θ	$2a$, Å.	$[\eta]_{G=0}$	$R_{[\eta]}$, Å.	L_w/R_w
Signer VII	5.90	5400	2200	19,000	21.2	10,900	(51.0)	(5240)	4.3
Varin	6.85	5030	2050	22,000	4.64	19,000	48.0	5400	5.4
Simmons (B.)	5.85	4950	2020	18,700	18.2	11,200	53.4	5300	4.5
B-G	3.50	4000	1630	11,200	282	4,400	(30.7)	(3730)	3.4
Gulland	3.50	2850	1160	11,200	....	....	....	....	4.9
Dounce	4.70	4480	1830	14,900	....	....	....	....	4.2
Chargaff	4.65	4990	2036	15,000	....	....	....	....	3.7

M_w = molecular weight
$\sqrt{\overline{R^2}}$ = mean end-to-end distance
$\sqrt{\overline{R_g^2}}$ = radius of gyration
L = contour length assuming 2 nucleotides per 3.5 Å.
θ = rotary diffusion constant
$2a$ = length of major axis from θ
$[\eta]_{G=0}$ = intrinsic viscosity 100 cc./g.
$R_{[\eta]}$ = mean end-to-end distance from viscosity

ments on the Varin sample made a year after that reported in Table I give 6.48, 6.18 and 6.47 millions (5410, 5230 and 5480 Å.$\sqrt{\overline{R^2}}$). Allowing for a little degradation in the Dounce and Chargaff samples, it appears very probable that the weight average molecular weight of calf thymus DNA is 6 million ± 10%.

Recent considerations by Benoit[14] raise the question of whether it is the weight average molecular weights that are obtained from the ordinary extrapolation of Zimm-type plots in cases such as this. He shows that when the angular measurements extend down to 30° and when the value of $\sqrt{\overline{R^2}}$ exceeds about 2000 Å., the ordinary extrapolation leads to the weight average molecular weight only if the molecular weight distribution roughly approximates that for which the ratio of weight to number average is equal to 2. Otherwise, in a case such as this, our measurements determine an asymptote whose intercept yields the reciprocal of twice the number average molecular weight, so that the value assigned to M_w tends to be somewhat high. Although this point cannot be completely settled at the moment, it appears that this complication does not arise in this case because the intercept obtained for the much smaller molecular configuration which exists at *p*H 2.6 is the same as that obtained at neutral *p*H (see Figs. 1 and 3 of M. E. Reichmann, B. A. Bunce and P. Doty, *J. Polymer Sci.*, **10**, 109 (1953)). Such would not be the case if the molecular weight distribution in DNA differed greatly from that for which $M_w/M_n = 2$.

In at least three other cases molecular weights near this value have been obtained: 5.8 million by Katz,[15] 4.8 million by Steiner[16] and 5.0 million by Rowen.[17] The first value has been altered from the published figure by using the value of dn/dc adopted here. The other values cannot be corrected since the values of ϵ and dn/dc employed were not published.

Molecular Weights from Intrinsic Viscosity and Sedimentation Constant.—Since the molecular weight values reported above are from five to ten times those values reported in the earlier literature, an explanation of the difference is desirable and new methods of determining the molecular weight are very welcome. While there is undoubtedly less degradation in the newer preparative procedures, it appears likely that most of the difference arises from the physical methods employed. Most earlier reports of molecular weight determinations were based on the use of the Svedberg equation and required measurements of the sedimentation constant s and the diffusion constant D that could be reliably extrapolated to zero concentration. Since both of these are kinetic constants, it is necessary that the concentration range on which the extrapolation is based is sufficiently dilute that the molecules can execute independent motions. In other words, the molecular domains should not overlap. Now if we take the radius of gyration discussed below as the radius of the minimum molecular domain, it is found that these domains cease to overlap only at concentrations below 28 mg./l. (0.0028%). The sedimentation and diffusion constants reported have been measured at 20 to 100 times this value. Without proof, it is difficult to believe that the values of kinetic constants at such high effective concentrations permit extrapolations to infinite dilution.

Despite this difficulty it is important to recognize that two practical considerations make the extrapolation of sedimentation constants, in contrast to diffusion constants, a real possibility. First, the self-sharpening of the peak during sedimentation makes possible the determination of s down to about 0.2 mg./cc., whereas the spreading of a boundary which must be observed in a diffusion constant measurement with the same optical techniques requires at least ten times this concentration. Second, the concentration dependence of s for thread-like molecules has been sufficiently explored both in practice and in theory to ensure that a plot of $1/s$ against concentration is linear at low concentrations[18] whereas no method of plotting diffusion constants of such molecules against concentrations is known to ensure a limiting linear relation.[19] The result

(14) H. Benoit, *J. Polymer Sci.*, **11**, 507 (1953).
(15) S. Katz, THIS JOURNAL, **74**, 2238 (1952).
(16) R. F. Steiner, *Trans. Faraday Soc.*, **48**, 1185 (1952).
(17) J. W. Rowen, *Biochim. et Biophys. Acta*, **10**, 391 (1953).
(18) L. Mandelkern, W. R. Krigbaum, H. A. Scheraga and P. J. Flory, *J. Chem. Phys.*, **20**, 1392 (1952).
(19) L. Mandelkern and P. J. Flory, *ibid.*, **19**, 984 (1951).

is that the extrapolation of sedimentation constants in the range of 0.2 to 1.0 mg./cc. probably yields a good estimate of s_0, whereas no estimate of D_0 is now possible. Consequently Svedberg's equation cannot yet be implemented in the case of DNA.

Quite recently, however, a relation somewhat analogous to the Svedberg equation, relating s, M and the intrinsic viscosity $[\eta]$, has been derived for chain molecules and shown to have applicability.[18] This relation is

$$M = \left(\frac{s_0[\eta]^{1/2}\eta_0 N}{2.5 \times 10^6 (1 - \bar{v}\rho)}\right)^{3/2}$$

where η_0 is the viscosity of the solvent, $N = 6.02 \times 10^{23}$, $\bar{v}$ is the specific volume and ρ is the density of the solution.

Although earlier reports on other samples indicate values of s ranging up to 13×10^{-13} measurements on Simmons' sample[20] give values of 15.5 and higher. Using this and the value of $[\eta]$ listed in Table I and discussed below, one obtains with the above relations a value of 5.8 million for the molecular weight.

Preliminary Electron Microscopy Determinations of Molecular Weight.—Although there are several reports of the electron microscope photographs of DNA molecules, no attempt was made to determine dimensions with a view to obtaining molecular weights. Such an investigation is now being undertaken by Prof. Williams[21] using Varin's DNA sample. Preliminary investigation shows that the molecules appear to be threads moderately coiled with constant diameters of 20 Å. which have a length distribution characterized by a weight average length of 15,000 Å. and a number average of 11,000. If we assume these are cylindrical and have a density of 1.63, the molecular weight is found to be 4,600,000. Professor Williams points out, however, that this value is certain to be less than the actual weight average since he is more likely to miss the longest fibers because these will be seen less frequently in their entirety on a micrograph than will the shorter ones. With this in mind, together with the possibility that the density of the threads may be somewhat higher than the bulk density, it is clear that these preliminary results are in good agreement with the light scattering value of 6 million. On the other hand, if the M_w/M_n ratio proves to be closer to 1.4 than to 2.0, the light scattering values will have to be slightly lowered. If this is indeed the case, it would be apparent from the light scattering data alone if the measurements were carried to lower angles. Work along these lines is now underway. It seems likely that further work will bring light scattering and electron microscope values into still better agreement.

Discussion.—From the foregoing considerations it appears that the molecular weight of DNA cannot be determined by the classical absolute methods of sedimentation–diffusion and osmotic pressure. However, the light scattering method is applicable although the value determined may be more accurately described as equal to twice the number average than as the weight average. Professor Williams' electron micrographs of DNA indicate that the difference between these two interpretations is marginal and support the molecular weight by light scattering. Light scattering investigations carried out at lower scattering angles and further efforts to improve the preparative methods of DNA are nevertheless desirable.

For the best current preparations, we find an average value of 6 million for the weight average molecular weight and 3 million for the number average molecular weight. This latter figure may be used to compute tentatively the number of DNA molecules in the nucleus of the calf thymus cell. There is general agreement that the weight of DNA per cell in calf thymus is 7×10^{-12} g.[22] From this the number of DNA molecules is found to be 1.4 million distributed presumably among 48 chromosomes.

Although the results discussed here refer to DNA from calf thymus, it is interesting to note that the molecular weight of DNA in bacteria and bacteriophage may be approximately the same as that found for calf thymus. The evidence in the case of bacteria lies in the recent report of Fluke, Drew and Pollard[23] who found from ionizing radiation studies that the molecular weight of the Pneumococcus transforming principle (presumed to be nearly pure DNA) was 6,000,000. The evidence in the case of bacteriophage is in work currently in progress in this Laboratory, in coöperation with Dr. A. Garen, in which preparations of DNA from bacteriophage T2 are found to have molecular weights near 6 million. The implications of these results, if they prove to be correct and typical of other species, will be of considerable interest.

II. Molecular Shape

Light Scattering.—The square root of the quotient of the initial slope and intercept of the reciprocal scattering envelope obtained by the Zimm-type extrapolation provide at once the radius of gyration, that is, the sum of the products of the mass of each segment in the molecule by the square of its distance from the center of gravity. These quantities are listed as R_g in Table I and are seen to be about 2000 Å. indicating at once that the molecules are extremely large for their weight, that is, they are highly extended.

The shape of the complete reciprocal scattering envelope (see Fig. 1) depends essentially upon the shape of the scattering particle but it is affected, to a much smaller extent, by the molecular weight distribution. Of the simple geometrical models, the observed envelope corresponds most closely to that of a random coil. The possible effects of various molecular weight distributions are not sufficient to alter this general conclusion. Consequently, we list the root-mean-square end-to-end length, $\sqrt{\overline{R^2}}$ on the assumption that the molecules are randomly coiled. This is a z-average dimension, in that it is the average dimension of molecules corresponding to the z-average molecular weight, which will be higher than the weight average.

(20) Dr. N. S. Simmons, University of California, Los Angeles, Cal.

(21) Prof. Robley C. Williams, University of California, Berkeley, private communication.

(22) J. N. Davidson, "Biochemistry of Nucleic Acids," Methuen, London, 1950.

(23) D. Fluke, R. Drew and E. Pollard, *Proc. Natl. Acad. Sci.*, **38**, 180 (1952).

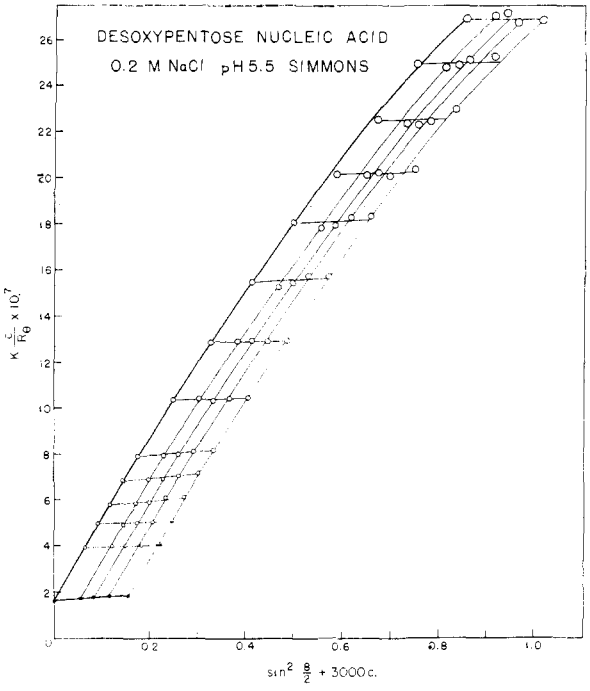

Fig. 1.—Plot of light scattering data for Simmons' sample in 0.20 M NaCl. All points except the lowest ones correspond to an error of $\pm 1\%$.

Since the extent to which the observed envelopes differ from those of random coils is within the range which can be produced by polymolecularity, it is not possible to decide from the light scattering evidence alone whether to interpret the deviation in terms of shape or polydispersity or a combination of both. If we assume the molecular weight distribution is very narrow, the envelope indicates that the distribution of segments with respect to the center of gravity averaged over all orientations is one in which the density is somewhat higher near the center and lower near the periphery than that of a Gaussian distribution of segments. Such a distribution could correspond to a highly swollen, spherically symmetrical particle with a high density near the center or to an asymmetrically shaped particle such as an ellipsoid or partially extended chain. On the other hand, if we seek to explain the deviation as due to a wide molecular weight distribution of randomly coiled chains, we find, using the analysis of Benoit,[14] the unusually high values of $M_z/M_w = 2$ and $M_w/M_n = 6$. Actually the preliminary electron microscope evidence points to a much narrower distribution. If this is the case, the shape of the reciprocal scattering envelope must therefore be related primarily to stiffness in the DNA molecules if the randomly coiled model is assumed. Now randomly coiled macromolecules do not reach the Gaussian limit until the contour length exceeds about three to four times their mean end-to-end length[24] and so we may inquire if this limit has been reached in the case of DNA. The last column in the table shows the ratio of the weight average contour length to the weight average end-to-end length, R_w (assuming M_z/M_w = 1.5). It is seen to average about 4. With the distribution we have assumed, this would mean that nearly half of the molecules in the sample lie below this limit and therefore their reciprocal scattering envelopes would exhibit the downward trend at high angles. Hence we conclude that the angular dependence of the scattering from DNA solutions is due to chain-like molecules which are sufficiently stiff to be somewhat non-Gaussian in character. This is to be expected from the L/R ratio which is determined by invoking the diameter (20 Å.) from extra-light scattering information.

Peterlin[25,26] has provided a detailed, but approximate, treatment of the scattering from random chains that are too stiff to be Gaussian. The parameter characterizing his model is denoted by x and is equal to the ratio of the contour length (L) to the persistence length (q), the latter being the average value of the projection of an infinitely long chain on the tangent to one end. The evaluation of x and q from the scattering data leads to the value of the contour length L. Now a comparison of the values of L_w from the table with Peterlin's calculations shows very good agreement in two cases (Varin and Gulland) and marked disagreement in two cases (Signer and B–G), the latter differing by a factor of 2.3. It is difficult to conclude from this whether the theoretical treatment of Peterlin or the angular dependence of our intensity measurements are at fault. It appears to us that polydispersity is probably the cause of the difficulty and that a precise application could only be made if M_w/M_n were very close to 2.0. There are two reasons for this. The reciprocal envelope for a Gaussian coil ($x = \infty$) is taken by Peterlin to be a straight line. This is only true if $M_w/M_n = 2.0$. If the ratio is less but not as little as even 1.4, the reciprocal envelope for Gaussian coils would curve upward from the abscissa to a very noticeable extent for molecules as large as DNA. Hence the base line is shifted and the value of x obtained in the prescribed way becomes subject to a large error. On the other hand, experimentally the high angle points on a reciprocal scattering envelope are very sensitive to small amounts of low molecular weight components and therefore to M_w/M_n. Therefore, it appears that the Peterlin interpretation is too sensitive to polydispersity to be applied in a quantitative manner unless the molecular weight distribution is known in detail.

Intrinsic Viscosity.—The interpretation of the intrinsic viscosity in terms of size and shape requires that the value at zero gradient be known. Because of the pronounced gradient dependence of the reduced specific viscosity of DNA, special procedures are required and these have only recently been applied. Instead of the Couette viscometer used by others[27,28] for low gradient measurements, we have employed capillary viscometers developed in the Laboratory in collaboration with Dr. N. S. Schneider. The capillary of these

(24) H. Benoit and P. Doty, *J. Phys. Chem.*, **57**, 958 (1953).

(25) A. Peterlin, *J. Polymer Sci.*, **10**, 425 (1952).

(26) A. Peterlin, *Makromolekulare Chem.*, **9**, 244 (1953).

(27) J. Pouyet, *J. chim. phys.*, **48**, 90 (1951); *Compt. rend.*, **234**, 152 (1952).

(28) B. E. Conway and J. A. V. Butler, *J. Chem. Soc.*, 3075 (1952); *J. Polymer Sci.*, **12**, 199 (1954).

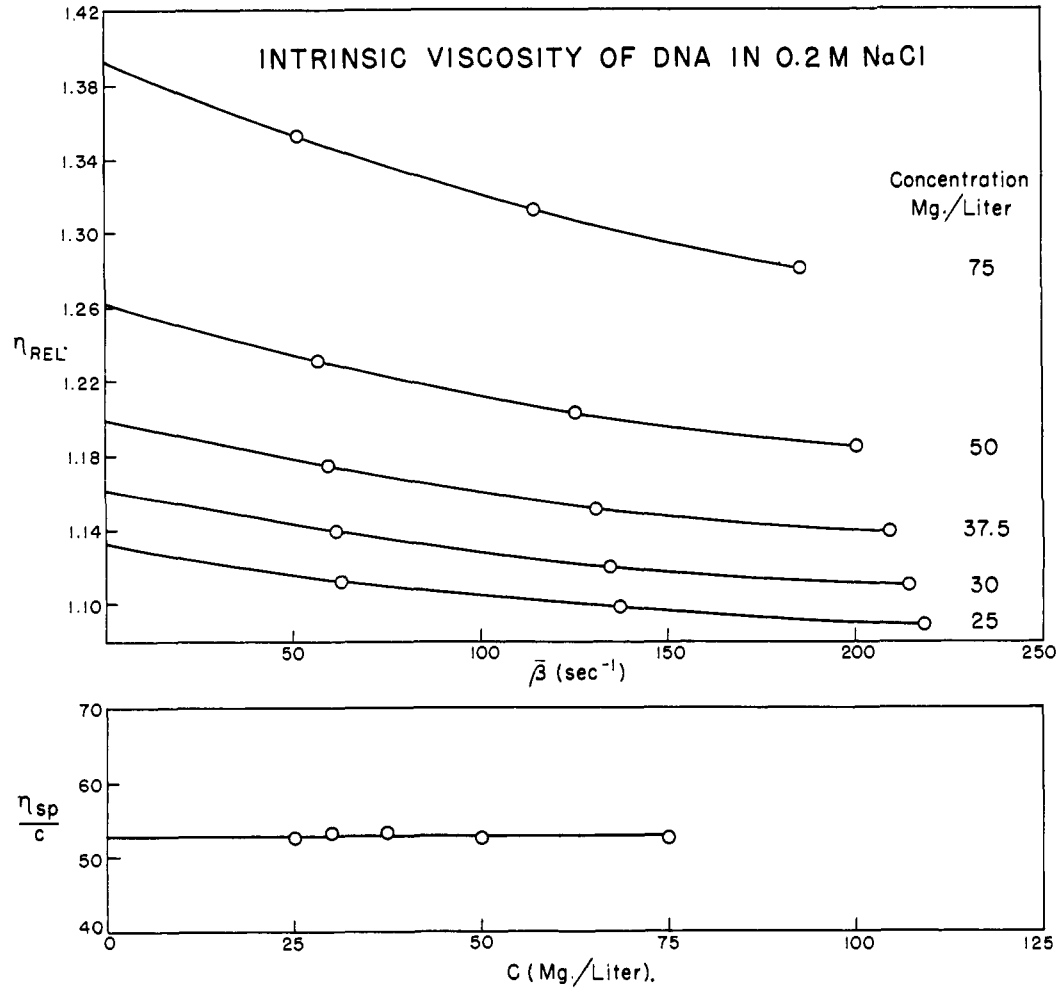

Fig. 2.—A summary of the determination of the intrinsic viscosity in zero gradient for Simmons' sample in 0.2 *M* NaCl.

viscometers is helically shaped (1 mm. in diameter) and about 150 cm. in length. Three bulbs are located above the capillary. In the 0.01 mg./cc. concentration range in which measurements on DNA are made, the times of efflux from each bulb correspond to mean gradients of approximately 60, 130 and 200 sec.$^{-1}$. The extrapolation of the data to zero concentration yields the intrinsic viscosity values listed in the eighth column of Table I. The two values in parentheses have been obtained by correcting earlier data at 1000 sec.$^{-1}$ by a factor of 2.51, this being the ratio of intrinsic viscosities at zero gradient and 1000 sec.$^{-1}$ for the Varin DNA. Although the gradients used here are not as low as those of a Couette viscometer, it appears that they are low enough to allow extrapolation at this salt concentration, *i.e.*, 0.2 *M* NaCl. Moreover, the results are in general agreement with the value of about 40 obtained on a similar preparation (Signer V) using the Couette apparatus.[27,28] Typical results are shown in Fig. 2 where the gradient dependence is shown at the top and a plot of the reduced specific viscosity at zero gradient below.

The interpretation of the values of the intrinsic viscosity so determined requires the assumption of a model of the solute particle. If we accept the conclusions reached from the light scattering studies, a random coil model should be applicable. The Flory–Fox relation[29] between the intrinsic viscosity and the mean end-to-end length has received general confirmation. This relation is

$$[\eta] = \Phi \frac{(R^2)^{3/2}}{M}$$

where Φ is a constant having a value of 2.1×10^{21}. Assuming that the molecular weight distribution is not wide enough to have a significant effect, substitution in this equation yields the values of R listed in the next to the last column of Table I. The agreement with the light scattering values of R is in all cases within 7%. This offers strong independent support of the conclusions reached by light scattering.

An alternative interpretation of the intrinsic viscosity is possible if one assumes the model of an ellipsoid of revolution and some relation between the actual molecular volume and the hydrodynamically effective volume. If we assume that these two volumes are identical and that the relation of Simha[30] is correct, the axial ratio is found to be 425 for an intrinsic viscosity of 50. If now the molecular volume is calculated from the molecular weight

(29) P. J. Flory and T. G. Fox, THIS JOURNAL, **73**, 1904 (1951).
(30) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(6×10^6) and the density (1.63), this value of the axial ratio leads to a length (major axis) of 12,750 Å. and a diameter (minor axis) of 30 Å. That is, rod-like particles of these dimensions and weight would produce the observed intrinsic viscosity. This interpretation is, however, untenable since the scattering envelope of the DNA is strikingly different from that of a rod and very close to that of a coiled chain. On this basis we therefore rule out the non-hydrated ellipsoidal model of high axial ratio.

However, it is possible that the ellipsoid model can be retained if it is so altered in concept to become the ellipsoid which is hydrodynamically equivalent to the mean configuration of the coiled model. It seems likely that the DNA molecule, although mildly coiled and convoluted, does not have the flexibility between segments and hence the rapid intramolecular Brownian motion that is associated with typical synthetic polymers. As a result, particular configurations are pictured as relatively stable with respect to the time scale of the gradients employed in the measurement of viscosity (and flow birefringence). That is, it is assumed that the individual configurations persist long enough to become oriented in the gradient. Hence the ellipsoidal model should, in this view, represent the average over the equivalent ellipsoids for each configuration weighted in proportion to the frequency of its occurrence. The volume of this hydrodynamically equivalent ellipsoid will be many times greater than that discussed above: a factor of 10^3 for this volume increase appears reasonable and we shall for purposes of illustration assume this value. Since the volume of solution occupied by these ellipsoids increases by the same factor, the axial ratio required to match the observed intrinsic viscosity is found to be diminished approximately a hundred-fold, that is, in this case it would have a value in the range of 5 to 10. This concept of the hydrodynamic behavior of DNA eludes sharp definition because there is no unique way of selecting one particular pair of values from the many self-consistent pairs of the two variables, volume and axial ratio, on which the intrinsic viscosity depends. Thus, although this concept, in contrast to the first ellipsoidal model described above, is acceptable, we prefer the randomly coiled model of Flory and Fox because it appears to represent the DNA molecule more faithfully and, moreover, relates size to intrinsic viscosity in an unambiguous manner. It should be emphasized, however, that the first ellipsoidal model, whose volume is equal to that of the DNA molecule, is the one which has been most widely used in interpreting viscosity and extinction angle measurements in the past. As pointed out, this model is quite incompatible with the angular dependence of scattered light and on this basis should be rejected.

Flow Birefringence.—Against the background just developed, it is of interest to examine the behavior of DNA in flow birefringence experiments where the extinction angle is measured as a function of gradient. It will be recalled that the orientation which gives rise to the extinction angle can be due either to the orientation of asymmetric molecules or to the deformation and orientation of molecules that at zero gradient are spherical in a hydrodynamic sense. Because of the structure of DNA and its large cross section (20 Å.), it is unlikely that it is deformed in the gradients usually employed and the gradient dependence of birefringence supports this view. Hence it is assumed that it is the orientation of hydrodynamically asymmetric, undeformed, molecules that gives rise to the observed extinction angle.

The only basis for interpreting the observed variation of extinction angle with gradient lies in assuming that the molecules are rigid ellipsoids of revolution. When this is done, the data can be interpreted in terms of a rotary diffusion constant from which the length of the axes can be derived provided the molecular volume is known.

Extinction angle measurements have been made on four of the samples reported on here. The details and interpretation of these measurements are presented elsewhere,[31] but some mention is necessary here for completeness. Proceeding on the assumption that the DNA molecules behave as rigid ellipsoids, the rotary diffusion constants, θ, have been evaluated and are listed in the table. The calculation of the lengths of the axes from these values of θ presents us with the same alternatives found in the interpretation of intrinsic viscosity. If the molecular volume is assigned the value corresponding to the dry molecule ($M/N\rho$) as is usually done, one finds the values listed in Table I for the major axis, $2a$. It is seen that the length of the semi-major axis, a, is approximately equal to the end-to-end length, $\sqrt{\overline{R^2}}$. This result would be expected, since a and $\sqrt{\overline{R^2}}$ are related to the radius of gyration in a nearly equal manner,[32] if this model were correct. However, its incompatibility with the light scattering envelope forces us to reject this model and hence view the near equality of a and $\sqrt{\overline{R^2}}$ as coincidental.

Turning to the second ellipsoidal model, the one which contains the DNA molecule and about 10^3 times its volume in solvent, it is found that the axial ratio which will produce the observed rotary diffusion constants is in the range of 10. Thus it is seen that this solvent immobilizing ellipsoidal model with an axial ratio of 10 fits both the extinction angle data and the intrinsic viscosity data provided we allow the volume of solvent immobilized to be about 10^3 times the molecular volume.

At this point it would be desirable to have a theory for the extinction angle-gradient relation of rigid coiled chain molecules so that one could dispense with the ellipsoidal model as was possible in the case of intrinsic viscosity. Since this desire is not fulfilled, the nearest approach lies in exploring a relation between θ and $[\eta]$ derived by Riseman and Kirkwood[33]

$$\theta = RT/KM\eta_0[\eta]$$

where R is the gas constant, η_0 the viscosity of sol-

(31) M. Goldstein and M. E. Reichmann, THIS JOURNAL, **76**, July (1954).

(32) When $(a/b) >> 2$ the radius of gyration of an ellipsoid of revolution is approximately equal to $a/\sqrt{5}$; for a random coil it is equal to $\sqrt{\overline{R^2}}/\sqrt{6}$.

(33) J. Riseman and J. G. Kirkwood, *J. Chem. Phys.*, **17**, 442 (1949).

vent, and K is a constant having values of 240, 400 and 3000 where the molecular or particle shape is that of a sphere, randomly coiled chain and thin rod, respectively. Upon substituting $M = 6 \times 10^6$, $[\eta] = 50$ and $\theta = 20$ into this equation, the value of K is found to be 411. Since the θ calculated from the measurements on DNA assumed an ellipsoidal model, the significance of the value of K found here is that it lies between that of a rod and a sphere in the range expected for not too asymmetric ellipsoids, and not that it lies close to the value of a random coil since we have not been able to evaluate θ for a random coil.

Discussion.—Let us now summarize the evidence on the shape of DNA molecules. From the light scattering measurements, we find, in addition to the molecular weight, that the radius of gyration is about 2000 Å. and that the distribution of mass in the DNA molecule is equivalent to a rather stiff chain whose (weight average) end-to-end length is about one-fourth of its contour length. We wish to see if the intrinsic viscosity and extinction angle-gradient data support this view or the ellipsoidal model used in the past. Using the Flory–Fox relation, it is found that the intrinsic viscosity is compatible with the stiff chain model having the dimensions deduced from light scattering. The absence of a theory prevents a comparison with the extinction angle–gradient data.

If the DNA molecule is assumed to be an ellipsoid which immobilizes no solvent, we can uniquely assign it an axial ratio and the lengths of the major and minor axes from both the intrinsic viscosity and rotary diffusion constant. The values obtained average 425 for the axial ratio and 13,000 Å. for the length of the major axis. This model would, however, scatter light in a manner very similar to that of a rod. The observed scattering is so different in its angular dependence that this model should be rejected on this basis.

As an alternative ellipsoidal model, a hydrodynamically equivalent ellipsoid has been examined. It is found that if the amount of solvent immobilized is of the order of 10^3 times the molecular volume and if the axial ratio is about 10, the average values observed for the intrinsic viscosity and rotary diffusion constant are accounted for. This model cannot be compared with the light scattering observations because its physical extent in space need not correspond to the dimensions of the equivalent ellipsoid. The ambiguity in assigning values to the amount of solvent immobilized and the axial ratio greatly limits the usefulness of this model.

Consequently, we return to our previously stated view: that the DNA molecule is a stiff chain whose weight and mean extension in space are derivable from light scattering observations. The intrinsic viscosity and extinction angle data are consistent with this view but can also be interpreted in terms of an ellipsoid of high axial ratio. This interpretation conflicts with the angular variation of scattered light and on this basis is excluded.

The confidence with which this last step is taken may be questioned. In answer, two arguments are presented. First, there is the auxiliary data mentioned in this paper: the moderate coiling observed in the electron microscope photographs, the consistent value of the molecular weight obtained when the sedimentation constant and intrinsic viscosity are employed in a relation derived for chain molecules and, finally, the intermediate value of K obtained in the Riseman–Kirkwood equation. The second argument is based upon the results obtained when similar investigative methods have been applied to cellulose derivatives in this Laboratory.[34] In particular the extinction angle–gradient data were quite analogous to those obtained for DNA and show, therefore, that DNA in extinction angle–gradient experiments behaves like molecules known to be stiff chains. We see, therefore, no reason for not accepting the chain model of DNA derived from light scattering measurements together with the weight and dimensions to which these lead.

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(34) N. S. Schneider, Ph.D. Thesis, Harvard University, 1953.