duplicate this. Ingold and Chandrasena[24] reported a melting point of 128° for this material. Chloromaleic acid was prepared by hydrolysis of the anhydride.[25] It melted at 109.5–110° (reported 114–115°).[25]

All of the compounds gave excellent correspondence to the second-order rate equation and the theoretical halogen production, with the exception of chloromaleic acid which gave 97% of the theoretical chloride ion and rate constants which drifted down slightly at beyond 60% completion. These were corrected for by using the 97% figure as theoretical and determining rate constants before 60% completion.

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

The Molecular Weight and Shape of Desoxypentose Nucleic Acid

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The angular distribution of the reduced intensity of light scattered from solutions of three desoxypentose nucleic acid samples (Gulland and Jordan, Schwander and Signer, and our own) over the concentration range of 5 to 20 $\times 10^{-5}$ g./cc. in 0.2 *M* NaCl has been determined. From this it is deduced that the molecular weights are 4.0, 6.7 and 4.0 million, respectively, and the maximum dimension of the Schwander and Signer sample is 6400 Å. Molecular shapes that are consistent with the scattering envelope are discussed.

In contrast with the recent progress in the chemical investigation of the structure of desoxypentose nucleic acid, DNA, physical studies have not led to a consistent view of the molecular weight and shape of this critically important macromolecule. The obstacles to successful physical studies have been twofold. First, various methods of preparation undoubtedly produce samples having different physical properties. These differences appear to be due to varying degrees of degradation. However, the recent introduction of milder methods of extraction and protein removal together with an increasingly effective inactivation of degrading enzymes offers hope that samples approaching closely the native molecules of DNA can be prepared. Second, it appears that none of the older methods of molecular weight and shape determination are adequate for the present problem, at least in the form in which they have been employed. This conclusion deserves some elaboration.

Chemical studies show that DNA is principally a polynucleotide carrying both strong and weak electrolytic groups. The magnitude of the double refraction of flow and the sedimentation constant show that most preparations yield large, asymmetric molecules whose molecular weight must be of the order of magnitude of one million. Thus the problem lies in characterizing a polyelectrolyte of unknown macrostructure. Indeed this is a most demanding problem as the small number of successful studies of the molecular weight and shape of polymeric electrolytes of known chain structure testify. The methods that have been applied to the problem are the following: sedimentation velocity,[2-6] diffusion,[2b-4] streaming birefringence,[7-11] dielectric dispersion[12] and the Kerr effect.[13] There have been numerous investigations of the viscosity of DNA solutions but thus far no one has succeeded in extrapolating to zero gradient. Until this is done precise molecular information cannot be expected from this source. Of the methods applied, only the combination of sedimentation and diffusion using the Svedberg equation leads to a molecular weight determination. An examination of the diffusion data shows that it has not been possible to carry out the measurements at sufficiently low concentrations to permit an unambiguous interpretation. Indeed the Schlieren curves reveal such marked asymmetry and the concentration dependence of the diffusion constant differs so radically from one investigation to another that it appears that no reliable estimate of the molecular weight is possible by this method. The osmotic pressure method is clearly too insensitive for use here and apparently has not been tried. One concludes therefore that no satisfactory determination of the molecular weight of DNA has been carried out except possibly on very degraded samples which are not considered here. In contrast there is complete unanimity among the methods listed above concerning the marked asymmetry of the DNA molecule. The quantitative interpretation of the data is however severely limited because only ellipsoidal models have been employed in deriving the relation between size and experimental effect. Making the arbitrary assumption that the DNA molecule is rod-like, streaming birefringence measurements on what appears to be the least degraded sample[10,11] lead to lengths of about 8000 Å.

The recently established techniques of light

(1) National Institutes of Health Predoctoral Fellow (1950–1951).

(2) (a) K. O. Pedersen, T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, New York, N. Y., 1940, p. 443; (b) H. C. Tennent and C. F. Vilbrandt, THIS JOURNAL, **65**, 425 (1943).

(3) R. Cecil and A. G. Ogston, *J. Chem. Soc.*, 1382 (1948).

(4) H. Kahler, *J. Phys. Colloid Chem.*, **52**, 676 (1948).

(5) L. E. Krejci, L. Sweeny and J. Hambelton, *J. Franklin Inst.*, **248**, 177 (1949).

(6) G. Goldstein and K. G. Stern, *J. Polymer Sci.*, **5**, 687 (1950).

(7) R. Signer, T. Caspersson and E. Hammarsten, *Nature*, **141**, 122 (1938).

(8) A. Wissler, Dissertation, Berne, 1940.

(9) H. Schwander and R. Cerf, *Helv. Chim. Acta*, **34**, 436 (1951).

(10) H. Schwander and R. Signer, *ibid.*, **34**, 1344 (1951).

(11) M. Goldstein, private communication.

(12) I. Junger, *Acta Physiol. Scand.*, **20**, Suppl. 69 (1950).

(13) H. Benoit, *J. chim. phys.*, **47**, 720 (1950).

scattering[14,15] provide methods of determining the molecular weight, interaction and size and shape of macromolecules and therefore offer promise in the characterization of the DNA molecule. The complete exploitation of this method requires measurements over a wide range of scattering angle and a demonstration that the solutions are optically clean. This paper summarizes our efforts in this direction on three different DNA preparations. Some of our conclusions have been mentioned earlier[16] and simultaneously two other reports on light scattering investigations have appeared.[17,18]

Experimental Methods

DNA Samples.—Through the courtesy of Dr. Jordan a sample prepared according to the method of Gulland, Jordan and Threlfall[19] was made available. This sample is designated as G. A second sample was received as a gift from Prof. Signer. Its preparation has been fully described[20] and it is this sample, denoted as VII, which has been used in other investigations (*e.g.*, ref. 9, 10).

A third sample was prepared in this Laboratory with the collaboration of Dr. E. P. Geiduschek, following the procedure of Schwander and Signer.[20] This sample is given the designation B. As will be seen from the experiments recorded below, B is somewhat degraded in comparison with VII. On the other hand Dr. Roger Varin has more recently prepared by this same procedure a sample that compares very closely with VII. It is therefore of interest to mention the differences in the detailed application in this procedure in the preparation of B which appear to have led to some degradation. First, the glands were minced in the Waring blender for about three minutes in B and only about a half-minute in Varin's preparation. Second, the precipitation of NaCl along with DNA upon pouring the saturated NaCl solution into alcohol led to difficulties which were overcome by Dr. Varin by adding equal volumes of water to the saturated NaCl solution. This disposed of the need to carry out a third precipitation of the DNA in order to get a fibrous precipitate which easily wound onto a wooden dowel rod. Thirdly, the stirring at all stages was more vigorous than in Dr. Varin's preparation. Fourth, all operations were carried out at about twice the dilution recommended. These then appear to be the only points upon which our original interpretation of the Schwander and Signer procedure appears in retrospect to have been in error. It is likely that the longer time in the Waring Blendor was chiefly responsible for the apparent degradation.

Light Scattering Measurements.—The light scattering measurements were made in a Brice–Speiser photometer.[21] The calibration of the photometer to give reduced intensities in absolute units has been previously described.[21,22] Light having a wave length of 4370 Å. from the mercury arc was used. The value of dn/dc is taken as 0.160.[6] Consequently the value of K, equal to $2\pi^2 n_0^2 (dn/dc)^2/N\lambda^4$ becomes 4.07×10^{-7}.

The dissymmetry of DNA solutions proved to be so large that measurements of the angular distribution of the reduced intensity were obviously required in order to arrive at a precise value of the molecular weight as well as to deduce the maximum information about the shape of the DNA molecule. For these angular measurements a hand blown, thin-walled cell, shaped like an erlenmeyer flask proved quite satisfactory. The incident beam was cut to 3 mm. in width and the back of the cell was painted black. With these precautions residual reflections were negligible. The cell used in this work had a capacity of 66 cc. and required a minimum volume of 39 cc. for the light beam to clear the meniscus. For the zero degree reading the cell was removed. With the same slit system a calibration factor of 1.08 was required to transform the value of the reduced intensity determined in this way to that obtained in a square cell. The relationship between galvanometer deflection and R_θ is determined by the calibration mentioned above.[21,22] The absence of distortion of the angular envelope was ensured by measuring the scattering of fluorescein solutions of different concentrations. It was found that over the range of 35–135° the variation of the observed intensity, multiplied by sin θ to correct for the volume of scattering solution viewed, did not differ from constancy by more than ±2% when scattering from fluorescein was twice that of the scattering from water. The deviations were reduced to ±1% when the scattering was nine times that of water.

Preparation of Solutions.—For each scattering experiment a stock solution of the sample was prepared by dissolving in doubly distilled water. The solutions were magnetically stirred for at least 8 hours in the cold room to ensure complete solution. A concentrated NaCl solution was then added to bring the salt content to 0.2 M. In the case of the B and VII samples, the aqueous solutions were dialyzed against 0.2 M NaCl. Each solution was then centrifuged at approximately 20,000 g for at least 12 hours. A small pellet of gel was usually found at the bottom of the tube. This corresponded to only a small fraction of the DNA and did not increase in size with further centrifuging. Indeed the thermal gradients in the centrifuge tube prevented significant sedimentation of the DNA molecules. However, the removal of the gel component and dust appeared to be complete as evidenced by the reproducibility of the scattering envelope upon centrifuging aliquots of the same solution and by the constancy of the angular envelope upon successive periods of centrifuging.

The alteration of the DNA concentration in the scattering experiments was carried out in different ways. In measurements on the G sample the highest concentration, 20.4×10^{-5} g./cc., was measured first, then diluted with solvent and centrifuged for 12 hours before transference to the cell for measurement. This process was repeated for further dilutions. With sample VII all the dilutions were made in the cell. First the scattering envelope of the solvent was measured. The solvent was then removed in a single clean pipet and the minimum amount of the highest concentration DNA solution was introduced and measured. Dilutions were made by adding the previously measured solvent to the solution in the cell. The concentration with reference to the original solution was determined by weighing the cell upon each dilution. Magnetic stirring was employed to insure homogeneity of the diluted solution. With sample B measurements were made first on a minimum amount of the solvent. Then successive amounts of relatively concentrated solution, about 50×10^{-5} g./cc., were added to the cell with weighing and stirring. Of these three methods of dilution the last is generally the most desirable since the solvent scattering is most accurately known and the gradual accumulation of dust particles with successive manipulations occurs when the scattering is greatest, thereby minimizing that effect. On the other hand, due to the increase in viscosity with concentration, the cleaning of the more concentrated solutions becomes more difficult, forcing a compromise on the concentration range that can be covered in this way.

Viscosity Measurements.—Three viscometers were used to cover a sufficient range of mean gradient to allow the value at 1000 sec.$^{-1}$ to be obtained by interpolation. The characteristics of these viscometers as determined with two calibrating liquids, water and aniline, are given in Table I. Using these constants the viscosity of toluene and cyclohexane were found to differ by no more than 1.5% from the "International Critical Tables" values. The temperature of the viscosity measurements was 25.00 ± 0.02°. The kinetic energy correction, B/At_{obsd} where t_{obsd} is observed time of efflux, is subtracted from the observed time of efflux. The mean gradient is calculated from

$$\bar{\beta} = 8V/3\pi r^3 t_{cor}$$

Determination of Concentrations.—Concentration determinations were made by measurement of the optical

(14) P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).

(15) P. Doty and J. T. Edsall, "Advances in Protein Chemistry," Vol. IV, Academic Press, Inc., New York, N. Y., 1951, pp. 37-121.

(16) E. R. Blout and P. Doty, *Science*, **112**, 2918 (1950).

(17) D. B. Smith and H. Sheffer, *Canadian J. Research*, **B28**, 96 (1950).

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(20) H. Schwander and R. Signer, *Helv. Chim. Acta*, **33**, 1521 (1950).

(21) B. A. Brice, M. Halwer and R. Speiser, *J. Optical Soc. Am.*, **40**, 768 (1950).

(22) P. Doty and R. F. Steiner, *J. Chem. Phys.*, **18**, 1211 (1950).

TABLE I

VISCOMETER CONSTANTS

	Ostwald-Fenske No. 50	Ostwald-Fenske No. 100	Ubbelohde No. 1
Volume, cc.	3.20	3.20	5.72
Capillary length, cm.	7.2	7.6	9.0
Capillary radius, mm.	0.230	0.321	0.322
Mean pres. head, cm. H_2O	9.2	9.7	12.6
A	0.00432	0.01627	0.01018
B	2.29	2.39	1.27
B/A	530	147	125

density at the peak of the adsorption band at 2590 Å. We have used an extinction coefficient of 175 g. DNA/100 cc./cm. for the 0.2 M NaCl solutions. This value has been obtained by Dr. Stern[23] and by Dr. Varin in this Laboratory. The concentration of the DNA solution was determined after centrifuging and the concentrations of its dilutions were then based on weighing.

Results

Reduced intensities, R_θ, at various concentrations were measured for the three samples described and are reported in the form of plots of Kc/R_θ against $\sin^2 \theta/2 + kc$.[24] The data at angles up to 90° are shown as open circles in Figs. 1, 2 and 3 corresponding to samples G, VII and B, respectively. This method of plotting is in accordance with the basic equation

$$Kc/R_\theta = 1/MP(\theta) + 2BP(\theta)c$$

where $P(\theta)$ is the particle scattering factor and B is the second virial coefficient multiplied by M^2. The former is the source of information about the size and shape of the scattering unit and the latter measures the intermolecular interaction and is equivalent to the activity coefficient. As a con-

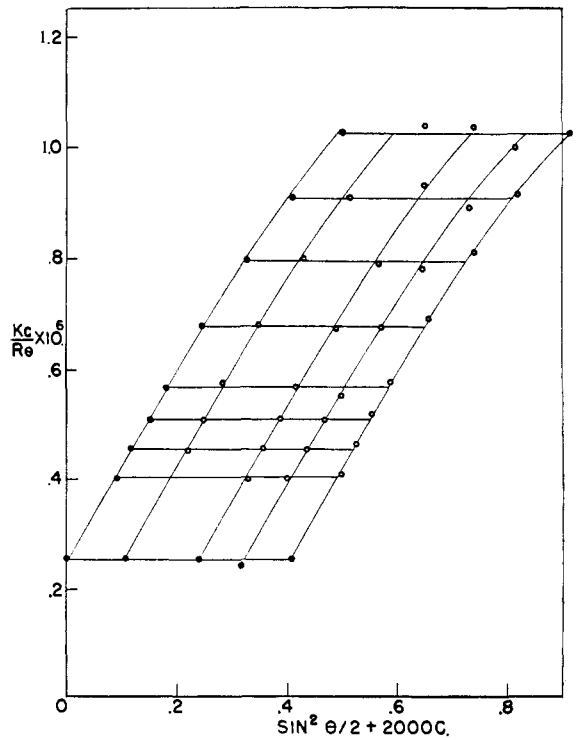

Fig. 1.—Reciprocal reduced intensity plot for sample G. Angular range is 35 to 90° and concentrations are 5.08, 11.9, 15.7 and 20.4 × 10^{-5} g./cc.

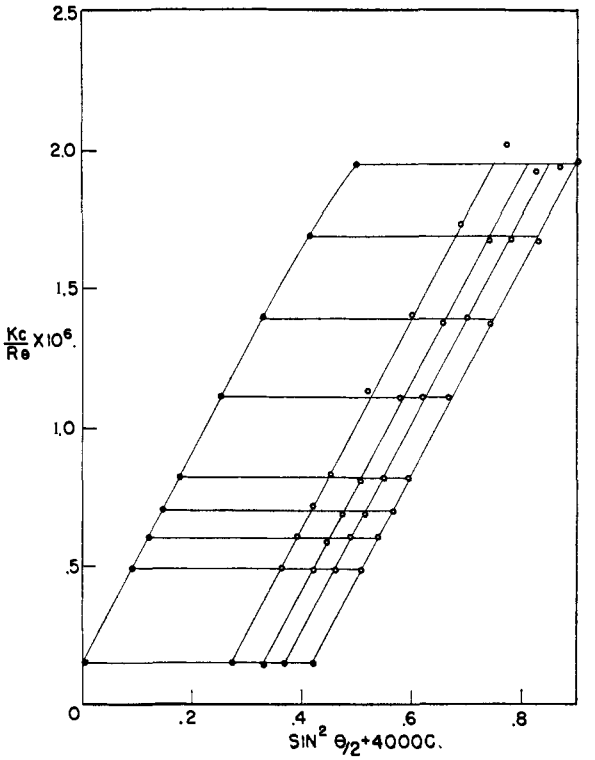

Fig. 2.—Reciprocal reduced intensity plot for sample VII. Angular range is 35 to 90° and concentrations are 6.83, 8.20, 9.24 and 10.4 × 10^{-5} g./cc.

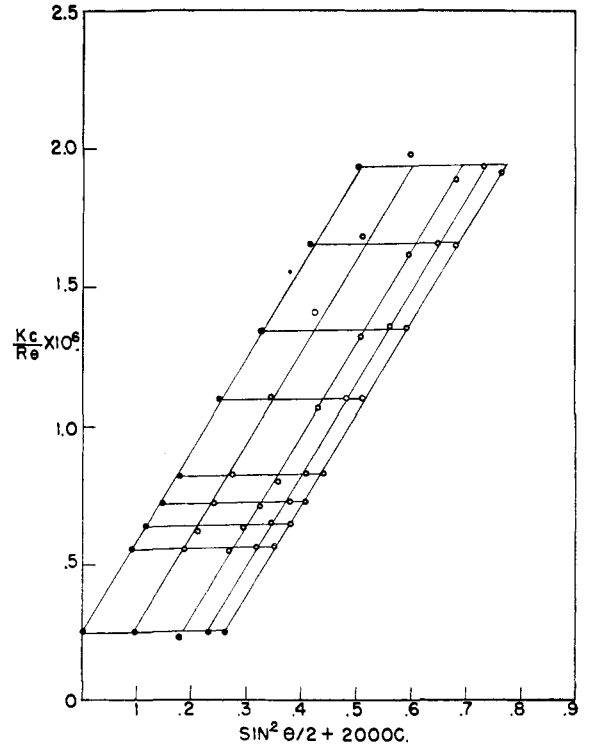

Fig. 3.—Reciprocal reduced intensity plot for sample B. Angular range is 35 to 90° and concentrations are 4.73, 8.90, 11.5 and 13.1 × 10^{-5} g./cc.

(23) Dr. K. G. Stern, Polytechnic Institute Institute of Brooklyn, private communication.

(24) B. H. Zimm, *J. Chem. Phys.*, **15**, 1093 (1948).

sequence of the linear dependence of $P(\theta)^{-1}$ on $\sin^2 \theta/2$ at low angles the data, plotted in this manner, is readily extrapolated in a grid-like fashion to zero angle giving a curve whose limiting slope is $2B$, and to zero concentration giving a line whose limiting slope is simply related to the product of the molecular weight and a characteristic dimension of the molecule. The two curves, drawn through the extrapolated points shown as filled circles, have a common intercept on the ordinate which is equal to the reciprocal of the weight average molecular weight. The molecular weights determined in this manner are recorded in Table II which summarizes our results.

TABLE II

SUMMARY OF RESULTS ON THREE DNA SAMPLES IN 0.2 M NaCl

	G	VII	B
Molecular weight	4,000,000	6,700,000	4,000,000
Limiting slope $\times 10^6$	1.7	3.7	3.3
$[\eta]$ at 1000 sec.$^{-1}$	.....	19.9	12.1
Dissymmetry	2.75	4.10	3.80
Radius of gyration Å.	1170	2200	1630
R for coil, Å.	2850	5400	4000
pH	4.4	6.5	6.6

The angular dependence of scattering from 35 to 135° and extrapolated to zero concentration for sample VII is shown in Fig. 4 as a plot of KcM/R_θ, which is equivalent to $P(\theta)^{-1}$, against $\sin^2 \theta/2$. The plot must be linear at low angles. The shape of the plot at higher angles reflects the distribution in space of the atomic groups making up the molecule. For any simple geometrical shape the size parameter(s) can be chosen to match the initial slope and the comparison of the remainder of the curve with the experimental one yields information about the possible shapes that the real molecule can possess. It is only by assigning a shape to the molecule that a characteristic dimension becomes defined and can be evaluated. Alternatively the radius of gyration is a generalized dimension which avoids specifying the particle shape and density distribution. It is simply related to the initial slope[25] and is well suited for use in cases such as this, where the details of shape and density are not yet assigned. However, it is helpful to examine the dimensions of any geometrical model which approximately fits the observed scattering envelope. The only simple model (*i.e.*, one having a single dimension parameter—sphere, rod or random coil), which is at all close, is that of a random coil. The $P(\theta)^{-1}$ plot for the coil curves gently upward while the observed data curves downward. Polymolecularity causes a change of the $P(\theta)^{-1}$ plot in the direction of the observed data. When the polymolecularity corresponds to a ratio of the weight to number average molecular weights of two, the $P(\theta)^{-1}$ plot becomes linear.[24] Inasmuch as there is other evidence indicating polymolecularity in our samples, the values of the root-mean-square end-to-end separation (z-average) of chain ends for this particular distribution of random coils, R, are given in Table II along with the radius of gyration, R_G, and values of the limiting slope and the dissymmetry at angles of 45 and 135°.

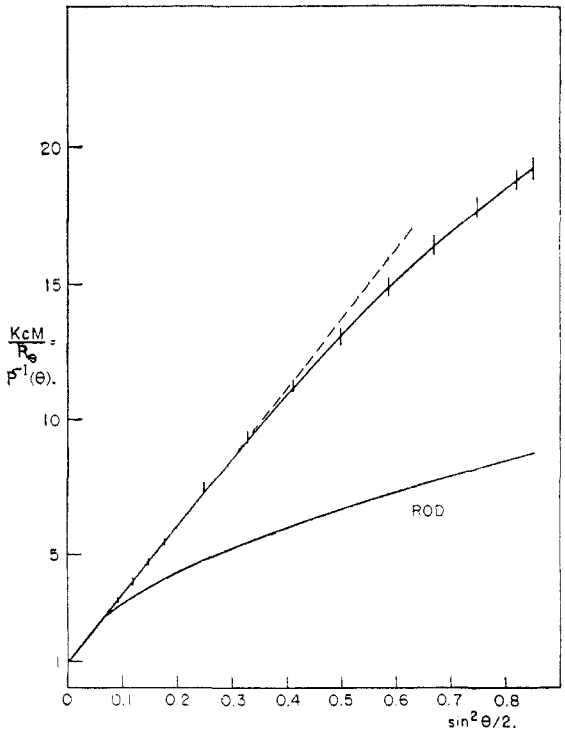

Fig. 4.—Reciprocal scattering envelope at infinite dilution for sample G.

The specific viscosity of two samples was measured at three gradients and over the concentration range of 5 to 20 $\times$ 10^{-5} g./cc. The reduced specific viscosity interpolated to a value of the mean gradient of 1000 sec.$^{-1}$ are independent of concentration. These values, which are the intrinsic viscosities, are listed in Table II.

All the solutions used were 0.2 M in NaCl. The pH values are entered in Table II.

Discussion

Interpretation.—The magnitude of the molecular weight found for these three samples is substantially greater than the estimates obtained by the methods noted in the introduction. In particular Cecil and Ogston[3] give a value of 820,000 for sample G and Schwander and Cerf[9] a value of 800,000 for a sample denoted as V which may be considered very similar to our sample B inasmuch as the method of preparation was very similar and the reduced specific viscosity had nearly the same value. If our determination is correct, earlier estimates appear to be low by about a factor of five. The significantly higher value of VII clearly shows the superiority of the Schwander and Signer method of preparation. As mentioned previously, Dr. Varin's repetition of this preparative procedure has led to a sample of essentially the same properties.[26] The results of a more comprehensive investigation of this new sample will be published later.

The scattering envelopes of these three samples tell us that the distribution of the nucleotide segments in DNA is surely three dimensional. Assuming the molecular size distribution is not too broad,

(25) P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).

(26) M. E. Reichmann, R. Varin and P. Doty, THIS JOURNAL, **74**, 3203 (1952).

the distribution of segments is similar to that for random coil with a moderate asymmetry. However, we could fit the envelope with any number of asymmetric shapes. For example, a cylinder of finite diameter and constant density could be fitted since calculations of the scattering factor of such forms have recently become available.[27] In this case the cylinder representing VII would be about 6000 Å. long and 1500 Å. in diameter. Since the molecular volume of VII is only about 1/10,000th that of such a cylinder, this possibility must be rejected. A cylindrical shell, which would conform to an open helical structure, is only slightly more attractive since even in this case the diameter would have to be several hundred ångstroms. In this form the successive spirals would be too far apart to stabilize the structure. Considering the predominantly polynucleotide structure of DNA, a linear chain that is only gently coiled, that is, one much too stiff to be considered random, might be suggested. However, this too appears untenable when one considers that such a linear chain would be, in the case of VII, about 60,000 Å. in length and yet the maximum dimension must be about one-tenth of this value (Table II). A linear chain coiled to this extent would be very similar to a random coil. So far we have thought of only two shapes that are consistent with the polynucleotide chain that seem to withstand objections similar to those used in these examples. One proposal is that of a branched polynucleotide chain. If the branches were long they would have to be few in number, say ten; if they were short a larger number, say a few hundred, could be accepted. Although the number of long branches required is so few that they would be undetectable by chemical methods, evidence has recently appeared that suggests that there are numerous branch points in DNA arising through either glycosidic or phosphoryl groups.[28] The other proposal is a parallel arrangement of polynucleotide chains which would produce a shorter and stiffer structure. For example, if three or four polynucleotide chains were so combined the contour length would be reduced to 20,000 or 15,000 Å. and this with an end-to-end separation of 6000 Å. would exhibit a radiation envelope like that observed. Some evidence for such a molecular fiber is found in the X-ray diagrams for more concentrated solutions.[29] The possibility of distinguishing between these two suggestions lies in the fact that due to its polyelectrolyte character, the shape of the former should expand when the ionic strength is decreased whereas the latter might be expected to be unaffected. As a consequence, the ionic strength dependence of the shape has been investigated and will be reported shortly.

Due to this inability to assign a precise geometrical shape to the DNA molecule, the meaning of its "dimensions" remains vague. Nevertheless one can show that the great majority of segments of the molecule must lie within a region whose maximum dimension lies between that of R and $3R_G$ in Table II. It can, therefore, be said that the maximum dimension of VII is quite close to 6,000 Å.

With this estimate of the size of the DNA molecule, we can return to the interpretation of the observation that the value of B and hence the second virial coefficient is essentially zero. Theoretical considerations of the meaning of this constant show that in polymer solutions generally it will be approximately equal to the effective volume of the macromolecule if there is no net attraction between the macromolecules. Therefore the very large size of the DNA molecule would lead to corresponding large positive values for B. The fact that these are not found (indeed, the solutions follow van't Hoff's law) shows that there is fairly strong net attraction between the DNA molecules in 0.2 M NaCl solutions.

The linearity of the zero angle curve is evidence against that aggregation since the presence of aggregates would be indicated by a tendency to dissociate with dilution, giving rise to upward curvature of the zero angle line. Such an aggregation would have to arise from unusually strong secondary bonds if dissociation were not to occur in this low concentration range.

Upon examining the results in Table II as a whole and allotting to each measurement a maximum of confidence, the following comparisons can be made. Although G and B have the same weight, G is considerably smaller. The only evident difference lies in the pH and this leads to the tentative conclusion that the DNA molecule shrinks with decreasing pH. If B is considered to be a degraded form of VII, the degradation could be considered to be of a random nature since the change in dimensions is intermediate between that of proportionality to M and $M^{1/2}$, as should be the case for a branched or cross-linked structure such as that suggested. However, these speculations are no more than indicative of the kind of information that detailed light scattering investigations can produce.

Sources of Error.—Thus far we have ignored the possibility or consequence of errors of any kind. In this section we consider all those errors which seem likely, and offer reasons that indicate that they are not sufficient to seriously alter the views just presented. First, of course, are the obvious systematic and random errors. These will tend to alter the absolute value of the scattering and affect, therefore, the molecular weight values. However, they do not concern the angular measurements nor the results derived from them. The absolute calibration of the photometer has a precision measure of about ± 5%. Random errors contribute about ± 3% in addition. The concentration determination depends upon the extinction coefficient. At present we can only estimate the precision of this to be ± 4%. These lead, therefore, to the possibility that the absolute value of the molecular weights may be in error by as much as ± 7%. In addition the error can be magnified by the extrapolation. Furthermore, DNA solutions have been found to be subject to erratic degradation during the time of carrying out a complete experiment. This effect cannot be simply

(27) N. Saito and Y. Ikeda, *J. Phys. Soc. Japan*, **6**, 305 (1951).

(28) See, *e.g.*, D. O. Jordan, *Ann. Rev. Biochem.*, **21**, 232 (1952).

(29) D. P. Riley and G. Oster, *Biochim. et Biophys. Acta*, **7**, 526 (1951).

characterized but might raise the uncertainty to ± 12%.

The extent to which we have eliminated errors due to residual dust and gel particles involves considerable subjective judgment based upon experience. Little more can be stated than that at every stage of the experiments the solutions were carefully examined for contaminants by looking for particulate scattering at angles very close to the incident beam. In addition, the reproducibility in cleaning DNA solutions served as an objective criterion, which, however, was probably not sufficiently exploited. The fact that a small fraction of the DNA did come out upon centrifuging indicated that any aggregates much larger than individual molecules would have sedimented out. On the other hand, the existence of aggregates of several molecules in significant quantities seems unlikely since, if present, they could also be detected in sedimentation diagrams.

It is well known that sufficient polymolecularity can significantly alter the scattering envelope. These effects have been investigated in detail by Zimm.[24] Increasing polymolecularity tends to increase the convexity of the $P(\theta)^{-1}$ plot: thus, the data in Fig. 4 could be considered to be derived from an extremely wide molecular weight distribution of linear, randomly coiled chain molecules. The width required of such a distribution is so extreme, being equivalent to a weight average to number average molecular weight ratio of more than ten, that this possibility seems very remote.

Finally, mention should be made of recent calculations[30] of the alteration of the scattering envelope brought about by the anisotropic character of rod-shaped particles. These results do indeed show that the magnitude of the effect can be quite large. However, for the range of anisotropies observed in real macromolecules including DNA, a computation of the effect based on their calculations shows it to be marginal. At most it would alter the size estimates given in Table II by 10%. Since the calculation of the correction requires the assumption of a rod-shaped molecule, it cannot be applied in the present case. The minor nature of this effect is consistent with the relatively small depolarization observed with DNA solutions. The depolarization at 90° with vertically polarized incident light was found to be about 0.017. The Cabannes correction, which this leads to, was also considered too small to apply.

Comparison with Other Light Scattering Studies.—Oster[18] in a very brief note states that he obtained molecular weights of 3.2, 1.3 and 0.5 million for three samples. The first value refers to an earlier Signer sample (probably V) the reduced specific viscosity of which is similar to our B sample with a molecular weight of 4 million. Beyond this there is little agreement, however, for he reports that the scattering envelope is that of a sphere but insists that this is an artifact and can indeed be explained as the scattering by a very anisotropic rod. This observation and interpretation appear unacceptable. The observation may be explained by assuming that some compact particles (*e.g.*, dust) have not been removed. Their contribution to the dissymmetry may not be very great, thereby permitting a good estimate of the molecular weight, but they would alter the low angle region of the scattering envelope in the direction corresponding to spherical particles.

The report of Smith and Sheffer[17] is more complete but direct comparisons are prevented because their samples are not otherwise characterized and their measurements are carried out at much lower salt concentrations (zero and 0.02 *M*). Moreover they measured only dissymmetry rather than scattering envelopes and were therefore unable to give other than minimum values for the molecular weight and size. Their dissymmetry values are in good agreement with ours for sample B and their minimum molecular weight, 4.4 million is also close. Actually, if we had calculated the molecular weight from our dissymmetry values as they did, we would have obtained 2.3 million. This illustrates the necessity of determining the scattering envelope rather than the dissymmetry for molecules of this size. Correspondingly, this restriction to dissymmetry measurements leads to somewhat of an over-estimate of the minimum dimension. Our choice of much higher salt concentrations than those used by Smith and Sheffer was based on the desire to avoid the complications which arise with polyelectrolytes at low ionic strength.[31] The existence of these complications are shown in Smith and Sheffer's data and do indirectly support the models suggested above.

Acknowledgment.—We should like to express our indebtedness to Drs. E. P. Geiduschek, M. E. Reichmann and R. Varin for their substantial help in the work reported here. In addition we acknowledge with pleasure the assistance received from the Institutional Grant to Harvard University by the American Cancer Society.

CAMBRIDGE, MASS.

(30) P. Horn, H. Benoit and G. Oster, *J. chim. phys.*, **48**, 530 (1951).

(31) A. Oth and P. Doty, *J. Phys. Chem.*, **56**, 43 (1952).