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A Flow Birefringence Study of Size and Size Distribution in Desoxypentose Nucleic Acid

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Flow birefringence data have been obtained on DNA preparations that have also been studied by light scattering. It is found that on lower molecular weight preparations (2–4 million) the two methods are in agreement; but as the molecular weight increases, the dimension calculated from flow birefringence increases much faster than the light scattering dimension, becoming greater by a factor of as much as 3. Some explanations for this are considered; none are held to be completely satisfactory, but it is likely that a moderate flexibility of the DNA molecule is responsible. Flow birefringence is found to be highly sensitive to differences between DNA preparations that are barely detectable by light scattering.

Although thymus desoxypentose nucleic acid (hereafter referred to as DNA) solutions have been studied by various techniques of high polymer chemistry[2] including sedimentation and diffusion, viscosity, flow birefringence[3–5] and light scattering,[6–8] some questions remain as to the detailed shape of these molecules, and their size distribution.

Most results so far obtained are consistent with the idea that the molecules are built on a linear plan, with the stiffness undetermined. To the extent that these molecules can be treated as rods, the recent calculations by Scheraga, Edsall and Gadd[9] of flow birefringence parameters considerably increase the potential usefulness of flow birefringence measurements in this problem. In particular, detailed information as to length and length distribution may be obtained from flow birefringence measurements at a range of gradients.

Many different preparations of DNA made from calf thymus have been described in the literature; these preparations are characterized by somewhat different molecular weights[8]; most modern values are in the range from 1 to 8 million.

It is clearly desirable to have some means of ascertaining whether any of these preparations represent something close to a "native" or undegraded material. Molecular weight alone might represent one such criterion; however, the possibility of aggregation of the fundamental units exists. A second criterion is uniformity of size distribution. It is plausible, though by no means certain, that an undegraded material would be composed of molecules all of the same length, and that either degradation or aggregation would show itself by the presence of a distribution of sizes. For the purpose of exploring this possibility experimentally, a series of flow birefringence measurements has been carried out on four different DNA preparations whose molecular weights and characteristic dimensions also have been determined by light scattering, in an attempt to see whether a consistent model relating size, shape, and size distribution may be found for the DNA molecule.

Theory of Flow Birefringence.—The theory of flow birefringence has been summarized elsewhere.[10,11] A few brief facts are mentioned here in the interest of clarity. In a birefringence measurement two experimental quantities are determined: the extinction angle χ and the amount of birefringence Δn, both as functions of velocity gradient and concentration. For molecules that may be represented as rigid ellipsoids, and for which the axial ratio is about 15 or greater, χ is a function of G/θ alone, where G is the velocity gradient, a quantity determined by the known dimensions and rotational speed of the instrument, and θ is the rotary diffusion constant of the molecule, given by Perrin's formula for prolate ellipsoids (valid for axial ratio greater than 5)

$$\theta = \frac{\rho RT}{6\eta M}\frac{3}{2\left(\frac{a}{b}\right)^2}\left[-1 + 2\ln\left(2\frac{a}{b}\right)\right] \qquad (1)$$

where ρ is the density of the molecule, η the solvent viscosity, M the molecular weight, and a and b the semi-major and semi-minor axes of the ellipsoid.

The amount of birefringence Δn is given by

$$\Delta n = \frac{2\pi c'}{n_s}(g_1 - g_2)f(G/\theta) \qquad (2)$$

where c' is the volume fraction of the macromolecular component, n_s the refractive index of the solution at rest, and g_1 and g_2 are optical constants of the molecule, being measures of the intrinsic and form anisotropy. $\chi(G/\theta)$ and $f(G/\theta)$ are functions given in tabular form in the paper of Scheraga, Edsall and Gadd. The dimensionless ratio G/θ is conventionally represented by the symbol α.

The Influence of Polydispersity.—The equations relating χ and Δn in a heterogeneous system to the values one would observe for the individual components have been given by Sadron[12]

$$\tan 2\chi = \frac{\sum_i \Delta n_i \sin 2\chi_i}{\sum_i \Delta n_i \cos 2\chi_i} \qquad (3a)$$

$$(\Delta n)^2 = \left(\sum_i \Delta n_i \sin 2\chi_i\right)^2 \Big/ \left(\sum_i \Delta n_i \cos 2\chi_i\right)^2 \qquad (3b)$$

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(2) References are given in D. O. Jordan, *Ann. Rev. Biochem.*, **21**, 209 (1952).

(3) (a) R. Signer, T. Caspersson and E. Hammersten, *Nature*, **141**, 122 (1930); (b) H. Schwander and R. Cerf, *Helv. Chim. Acta*, **32**, 2356 (1949).

(4) H. Schwander and R. Cerf, *ibid.*, **33**, 436 (1950).

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

(6) P. Doty and B. H. Bunce, THIS JOURNAL, **74**, 5029 (1952).

(7) M. E. Reichmann, R. Varin and P. Doty, *ibid.*, **74**, 3203 (1952).

(8) M. E. Reichmann, S. A. Rice, C. A. Thomas, III, and P. Doty, *J. Phys. Chem.*, forthcoming.

(9) H. A. Scheraga, J. T. Edsall and J. O. Gadd, Jr., *J. Chem. Phys.*, **19**, 1101 (1951).

(10) J. T. Edsall, "Advances in Colloid Science," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1942.

(11) R. Cerf and H. A. Scheraga, *Chem. Revs.*, **51**, 185 (1952).

(12) C. Sadron, *J. phys. radium*, **9**, 381, 384 (1938).

The subscript i denotes the values of χ and Δn for the ith component. These equations are valid for any type of heterogeneity provided the orientation of the molecules of a given species is not influenced by the presence of the molecules of another species.

For systems composed of a given chemical species, possessing a distribution of molecular sizes, it is often assumed that $(g_1 - g_2)$ is independent of molecular weight. When this is so, simplifications of Sadron's equations are possible; for example, Donnet[13] gives as a limiting formula for vanishing gradient

$$\frac{1}{\theta} = \Sigma \frac{C_i}{\theta_i^2} \Big/ \Sigma \frac{C_i}{\theta_i} \qquad (4)$$

the C_i's being the volume concentrations and θ being the apparent diffusion constant at zero gradient.

From equation 1 it can be seen that the type of average length obtained from flow birefringence does not lend itself to any ready classification. If, however, a further approximation based on the well-known sensitivity of the formula for θ to the length a and its insensitivity to axial ratio is used, one may regard θ as varying as a^{-3} mainly, provided the molecules are sufficiently elongated. If this is done, the sort of average a obtained from flow birefringence measurements at zero shear, which we may symbolize by $a_{f.b.}(0)$, may be shown from equation 4 to be given by

$$a_{f.b.}(0) = (\langle a^6 \rangle / \langle a^3 \rangle)^{1/3} \qquad (5)$$

where the brackets denote weight averages over the distribution.

At very high gradients, when orientation is nearly complete, the optical factors f_i have reached their saturation values, and we may set $\tan 2\chi_i = \sin 2\chi_i$ and $\cos 2\chi_i = 1$. Sadron's equations then reduce to the form

$$\chi = \Sigma C_i \chi_i \qquad (6)$$

To proceed further, it is necessary to know how χ_i approaches zero as a function of G/θ_i. This problem has not been solved explicitly by the exact method of Scheraga, Edsall and Gadd; but Burgers[14] gives an analysis leading to the conclusion that the angle of the maximum in the orientation distribution function goes to zero as $\alpha^{-1/3}$ (his equations 15.5 and 15.18). χ_i is an average value over this distribution function and would equal the angle of the maximum in the limit of complete orientation. If it is assumed that χ_i goes to zero in the same way that the maximum does, we obtain

$$a_{f.b.}(\infty) = \langle a^{-1} \rangle^{-1} \qquad (7)$$

i.e., the number average length. This is contrary to the view of Scheraga[15] that at infinite gradient the weight average value of θ is observed, which would lead to $a_{f.b.}(\infty) = \langle a^{-3} \rangle^{-1/3}$ a rather heavy weighting by the smaller molecules. It is doubtful if distributions obeying all the assumptions mentioned, up to infinite shear, are likely to occur in nature; yet the reasoning leads us to expect a great sensitivity of the apparent dimension to the gradient used for measurement.

As a reasonable model for degradation of DNA, values of the flow birefringence parameters have been calculated for a polydisperse system of rods having a Montroll–Simha distribution.[16,17] This distribution of sizes occurs when molecules of a constant initial length are subjected to a random breaking of bonds, all of which are assumed equivalent and equally spaced during the molecules. The distribution is expressed in terms of two parameters: the number of bonds in the undegraded parent molecule and the fraction of bonds in the system broken. An approximation has been made in the formula that permits the distribution to be expressed in terms of a single parameter only, the average number of bonds broken per parent molecule. The dependence on some assumption as to the size of the parent molecule is suppressed; however, the approximation limits the applicability of the results to the case of molecules with a large number of bonds initially, and for which the degradation is not too far advanced (when γ, the average number of bonds per parent molecule broken, is less than 5).

The calculation of χ and Δn as functions of γ and gradient and the application of the results to DNA solutions is dependent also on certain additional assumptions. (1) The theoretical treatment of the optical and hydrodynamic properties of large molecules[9,10] is valid with sufficient precision at all gradients to permit an experimentally observed deviation to be interpreted in terms of polydispersity. The experiments of Donnet on tobacco mosaic virus[13] were not carried out over a wide enough range of gradients to test this, the lowest value of χ in his work being about 35°. (2) The axial ratio of the undegraded molecule is greater than 100, so that θ may be regarded as depending on the half-length of the molecule alone, and not on the axial ratio. (3) The optical factor $g_1 - g_2$ is independent of size. This has been shown to be true for highly asymmetric molecules whose length is small compared to the wave length of light.[18,19] When the molecules are of the dimensions of a wave length (as is the case for nucleic acid molecules of large molecular weight) it is not clear whether this relation will hold or not.[20] (4) DNA contains a large number of bonds equivalent from the point of view of degradation, and equally spaced along the molecule. Evidence for the existence of a relatively unattackable core in DNA found by Chargaff and

(13) J. B. Donnet, *Compt. rend.*, **229**, 189 (1949). Donnet has recently reported an experimental test of this formula (*J. Chim. Phys.*, **50**, 377 (1953)) on polydisperse solutions of tobacco mosaic virus, in which the size distribution was determined by electron microscopy. The observed and calculated θ's agree to 15%, corresponding to an agreement in calculated length of 5%.

(14) J. M. Burgers, Second Report on Viscosity and Plasticity of the Amsterdam Academy of Sciences (Nordemann, New York, 1938). Chapter III.

(15) H. A. Scheraga, *J. Chem. Phys.*, **19**, 983 (1951).

(16) E. W. Montroll and R. Simha, *ibid.*, **8**, 721 (1940).

(17) M. Goldstein, *ibid.*, **20**, 677 (1952).

(18) W. Kuhn, *Z. physik. Chem.*, **A161**, 1 (1932).

(19) A. J. De Rosset, *J. Chem. Phys.*, **9**, 766 (1941).

(20) It may be mentioned that this question can be answered experimentally: in a monodisperse system, the extinction angle will not depend on the optical anisotropy of the particle because of symmetry considerations; in a polydisperse system each particle makes a contribution that depends on its optical anisotropy; if there is a change in $(g_1 - g_2)$ with wave length, the extinction angle will be different at different wave lengths. In DNA solutions, a preliminary investigation indicated no dependence of χ on wave length. It would be desirable to perform more experiments of this type on a wider variety of substances of known polydispersity and size.

others[21,22] stands in contradiction to this assumption. (5) The molecules must be completely rigid. If there is even a slight degree of flexibility, such that some molecules are in a bent configuration while others are straight, the different effective lengths would cause the system as a whole to appear polydisperse. (6) No fractionation occurs in the extraction of DNA from tissue.

Accepting the assumptions stated above, the test of the theory by these results is straightforward. The logarithm of the rotary diffusion constant θ is plotted against the extinction angle χ. This plot is then superimposed over a plot of the theoretical values of log (θ/θ_0) *vs.* χ at various values of the degradation parameter γ. The value of γ giving the best fit is thus uniquely determined. Once this is done the value of θ_0, the rotary diffusion constant of the undegraded molecule, can be calculated from the data on a given preparation independently of the values obtained from any other preparation.

It has been shown[23] that the average length for polydisperse rods obtained from light scattering is $(<L^3>)/(<L>)^{1/2}$. It is of interest to compare this to the lengths calculated from flow birefringence for the Montroll–Simha distribution to see how different the results of the two methods may be expected to be in the region of experimental interest. The calculation of the ratios of the lengths obtained by the two methods to the length of the undegraded molecules is straight-forward. The results are given in Table I for the cases $\gamma = 1$ and $\gamma = 3$. The latter is the most degraded and most polydisperse system for which calculations were made.

TABLE I

γ	α_0	$\chi_{calcd.}$	a/a_0	$L_{l.s.}/L_0$
1	1	41.0	0.954	0.872
	8	24.9	.905	
	80	10.4	.781	
3	1	42.2	.835	0.661
	8	30.5	.771	
	80	16.2	.570	

In Table I, α_0 is a measure of the gradient[17]; $\chi_{calcd.}$ is the extinction angle observed; a/a_0 and $L_{l.s.}/L_0$ are proportional to the lengths obtained by flow birefringence and light scattering, respectively. We see that over a wide range of χ values, spanning the region in which experimental measurements are made, the differences between particle dimensions obtained by the two methods do not exceed 30% for systems obeying the assumptions listed previously.

Experimental

The instrument used in this study was of the small Edsall type; it has been described in detail elsewhere.[24] Plastic cylinders were used; no portion of the instrument in contact with the solutions was of metal. Unmounted polaroid sheets, type HN-22, 0.005 inch thick, were obtained from the Polaroid Corporation, Cambridge, Mass. These sheets were mounted without pressure between discs cut from Letiz 2 × 2 cover slides. The performance of the instrument under these conditions is described in reference 24. The annular gap employed was 1 mm.

Extinction angles χ were measured in all four quadrants. One sense of rotation only was used, as this was found to be sufficient when a split-field polarizer was used to align the plane of polarization of the light. Amount of birefringence (expressed in terms of δ, the phase difference in degrees, δ being twice the angular rotation of the Senarmont compensator) was measured on the same solution following the extinction angle measurements. χ's were not considered reliable when δ was less than 3 degrees.

Inasmuch as the use of a Senarmont compensator is based on the assumption of monochromatic light and since for speed and convenience white light was used, the measurements of $(n_2 - n_1)$ possess a systematic error, probably not exceeding 20%. Comparisons of readings with and without a green filter agreed more closely than this estimate. A wave length of 5000 Å. was used in calculations, as this is approximately the wave length appropriate to the compensator employed. Using this wave length, and the height of the cylinder of 8.5 cm., the conversion factor for converting δ to Δn is 1.6×10^{-8}.

The highest gradients used in this study were of the order of 1600 sec.$^{-1}$. The critical gradient for the onset of turbulence in this instrument was 5000 sec.$^{-1}$ for water.

Stock solutions were prepared as follows: an aliquot sample of DNA was suspended in water and stirred magnetically for 36 hours in a cold room. Concentrated NaCl solution was then added to bring the concentration to 0.1 *M*. Before the birefringence measurements were carried out, the solutions were kept for several hours in a room thermostated at 25° in order to attain temperature equilibrium.

Four DNA preparations were studied. They were prepared respectively by R. Varin, R. Signer (his preparation VII), N. S. Simmons and B. H. Bunce and E. P. Geiduschek. For convenience they will be symbolized by V, S-VII, NS and BG. The details of the preparation and the light scattering results are given in detail elsewhere.[5-8]

Dilutions in 0.1 *M* NaCl were made up by weighing aliquots of this stock solution into weighed portions of solvent and stirring each dilution so prepared for 15 minutes with a magnetic stirrer.

The reason for this procedure rather than one based on serial dilutions from a stock solution is that if serial dilutions are made with shaking at each stage, the DNA in the most dilute solution has been subjected to a greater number of shakings than that in the more concentrated solutions. This has been found to produce changes in extinction angle that were first attributed to concentration dependence in the solutions.[25] The origin of this degradation is unknown; it might not be observed in other physical measurements, the sensitivity of flow birefringence to the numbers of large molecules being so marked. It is interesting to note that most methods of preparing DNA involve treatment of the thymus with a Waring blendor. The influence of such treatment on the native material might be considerable.

The solutions were examined for degradation at the gradients used for measurement, with essentially negative results. At moderate concentrations there was some tendency for χ values to be temporarily higher at low gradients after an intervening measurement at high gradients, but this disappeared at lower concentrations. At high rotational speeds than those used in these studies, permanent rises in χ values could be produced; in addition, as reported by Schwander and Signer[5] permanent changes were readily produced at moderate speeds in the absence of electrolyte. This latter phenomenon may be connected with the protective effect of salt in STN solutions against heat changes observed by Miyaji and Price.[26]

Measurements were made in a temperature-controlled room at 25°.

Results

Experimental data for χ as a function of gradient are plotted in Fig. 1. Curve V represents the Varin preparation; the concentrations plotted vary from 0.0061 to 0.0014%. A second experiment on this

(21) S. Zamenhof, E. Chargaff and G. Braverman, *J. Biol. Chem.*, **187**, 1 (1950).

(22) E. Chargaff, *J. Cellular and Comparative Physiol.*, **38** Supp. 1, 41 (1951).

(23) B. H. Zimm, *J. Chem. Phys.*, **16**, 1099 (1948).

(24) J. T. Edsall, A. Rich and M. Goldstein, *Rev. Sci. Instr.*, **23**, 695 (1952).

(25) M. Goldstein, 119th Meeting, American Chemical Society, Boston, Mass., April, 1951.

(26) T. Miyaji and V. E. Price, *Proc. Soc. Exper. Biol. Med.*, **75**, 311 (1950).

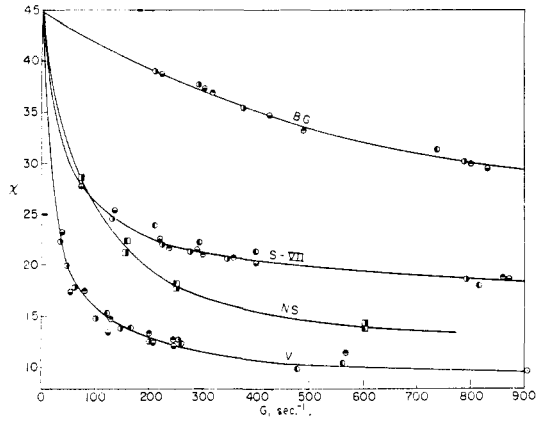

Fig. 1.—Extinction angle χ as a function of velocity gradient G. The concentrations in weight per cent. of DNA are as follows: for preparation V: ◒, 0.0061%; ◑, 0.0044%; ◓, 0.0026%; ◐, 0.0014%. For preparation S-VII: ◒, 0.0106%; ◑, 0.0080%; ◓, 0.0059%, ◐, 0.0041%. For preparation NS: ◨, 0.0056%; ◧, 0.0045%. For preparation BG: ◒, 0.0123%; ◑, 0.0098%; ◓, 0.0071%; ◐, 0.0053%.

preparation gave results in complete agreement with these. Curve S-VII is for Signer preparation; the concentrations plotted vary from 0.0106 to 0.0041%. Curve NS is Simmons sample; just two concentrations were studied, 0.0057 and 0.004%. Results for Bunce–Geiduschek preparation are given by curve BG; the concentrations varied from 0.0123 to 0.0053%. In Fig. 2, δ, the phase difference in degrees, divided by the concentration c, is plotted for the four preparations.[27] The essential independence of both χ and δ/c of concentration in the range of concentrations studied is clearly revealed by these figures.

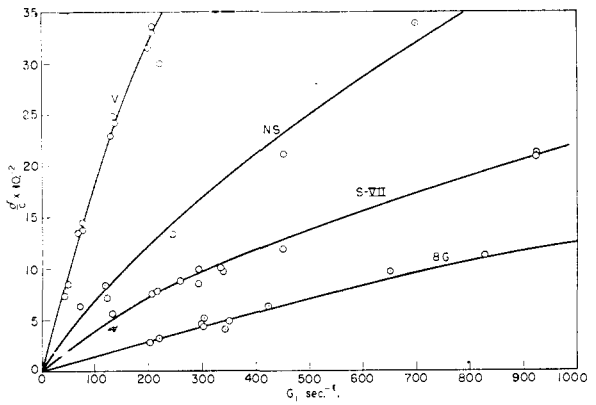

Fig. 2.—Amount of birefringence divided by concentration, plotted against velocity gradient. Birefringence is expressed as δ, twice the rotation in degrees of the Senarmont compensator, concentration C is in weight per cent.

For the purposes of examining the data for polydispersity and comparing them with the curves calculated for a Montroll–Simha distribution, the logarithm of the apparent θ, obtained from the tables of reference 9 and the assumption that the axial ratio was greater than 15, is plotted against χ for the three preparations in Fig. 3. In order to fit the curve for preparation BG in the same graph it was necessary to subtract 1 from the log of θ for this preparation. A cursory comparison of Fig. 3 with Fig. 3 of reference 17 reveals that the dependence of θ on gradient for the three preparations cannot be accounted for by the assumption of a Montroll–Simha distribution with γ between 0 and 3. All the preparations appear to be extremely polydisperse if the assumption of complete rigidity of all sizes of molecules is accepted. There does not even seem to be a consistent trend in the amount of polydispersity, as judged qualitatively from the change of θ with gradient, from high molecular weight samples to low ones.

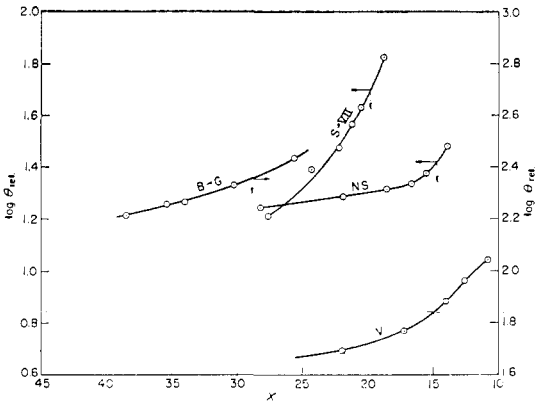

Fig. 3.—Logarithm of the apparent rotary diffusion constant θ, plotted against the extinction angle χ at which θ was determined. Curve BG has been shifted down by one unit in log θ.

For the calculation of molecular dimensions the procedure of comparing the preparations at the same value of χ was followed, rather than at the same gradient; this would more nearly represent a comparison under similar states of orientation. The angle $\chi = 25°$ was chosen as requiring the least extrapolation of data on preparations V and BG (see Fig. 3).

In Table II, column II gives the molecular weights determined in this Laboratory by light scattering. The values were obtained by recalculation of previously published data on the basis of recently determined values for refractive index increment, dn/dc, and extinction coefficient, ϵ.[8] Column III gives the apparent rotary diffusion constant θ, calculated for all samples at that gradient at which χ is 25°. Column IV gives the axial

TABLE II

I Prepn.	II $M \times 10^{-6}$	III θ, sec.$^{-1}$	IV a/b	V $2a$, Å.	VI L, Å.	VII $\overline{R^2}^{1/2}$
V	6.87	4.64	755	19,000	7100	5030
S VII	5.92	21.2	354	10,900	7650	5400
NS	5.86	18.2	370	11,200	7000	4950
BG	2.50	282	140	4,400	5650	4000
Steiner	4.4			4,000	6710	4730

(27) Tables of the experimental data on χ and δ at various concentrations and gradients have been deposited as Document number 4236 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the document number and by remitting $1.25 for photoprints, or $1.25 for 35 mm. microfilm in advance by check or money order payable to Chief, Photoduplication Service, Library of Congress.

ratio, a/b, and V the major axis $2a$ of the ellipsoid having the diffusion constant θ given in Column III. Columns VI and VII give the rod length L and the coil radius $\overline{R^2}^{1/2}$ obtained from the limiting slope of the $P^{-1}(\theta)$ plot obtained from the light scattering measurements.

Formula 1 was used for calculations; ρ was assumed to be 1.8,[28] and η_0 at 25° is 0.00916 poise. Data obtained by Steiner[29] on another nucleic acid preparation, are also listed in Table II.

The curves of birefringence *versus* gradient rise more rapidly with gradient than is to be expected from a solution of monodisperse rigid particles. Unlike the behavior of χ, this can be explained equally well by stretching of the molecule or by polydispersity. There is an element of arbitrariness in the calculation of $(g_1 - g_2)$ from these data in view of their failure to fit the theoretical curve. If we use the experimental value of δ/c at $\chi = 25°$, we find the values of $(g_1 - g_2)$ given in Table III. The relative values would not be greatly changed by using the initial slopes of the δ/c plots.

TABLE III

Prepn.	V	S-VII	NS	BG
$(g_1 - g_2) \times 10^3$	1.0	1.0	1.5	4.1

The outstanding feature of these results is the contrast between the essential agreement between light scattering and flow birefringence in the lower molecular weight preparations and the discrepancy that develops at high molecular weight. For V preparation the disagreement is by a factor of 3.

Both the flow birefringence and light scattering experiments have been performed repeatedly in this Laboratory and found to be reproducible. Because of the extreme disagreement on V preparation a check was performed by measuring flow birefringence parameters on a solution transferred from the light scattering cell. The concentration ranges for the two series of measurements are the same, so the possibility of different states of aggregation may be ruled out. Preliminary measurements on a virus DNA sample prepared by an entirely different and much less drastic method showed the same discrepancy: the molecular weight was 10 million; and the flow birefringence length, about the same as in V preparation, also exceeded the light scattering length by a factor of three.

Before we concern ourselves with attempts to explain the discrepancy, we would like to state in summary form what can be said from the flow birefringence results considered by themselves.

These results are consistent with a picture of DNA solutions as containing elongated rigid particles, in a distribution of sizes, having an average length as indicated in Table II, column V, the type of average being one weighted strongly toward the longer particles. In the absence of a firm theoretical basis for interpreting the data in terms of a flexible particle, we are unable to say if they are consistent equally with such a model, or what the characteristic dimension would be.

We are forced, therefore, in considering explanations for the apparent conflict between flow birefringence and light scattering, to rely on arguments on a qualitative nature. We have considered two possibilities: polydispersity, and the inadequacy of the rigid ellipsoid model. The possibility that DNA preparations contain a small amount of very long molecules, along with degradation products of these molecules, has been raised above. The failure of the treatment based on the Montroll–Simha distribution to account for the flow birefringence data does not rule this out, so many restrictive assumptions having been made in order to make the calculations tractable.

However, attempts to account for the difficulty by assuming a distribution composed of two rod-like components, the weight fractions and length ratios being variable, were made without success. The calculations in fact suggested that no such distribution can be found and made it further unlikely that a system composed of more components could do better.

The results of light scattering measurements have suggested the possibility that DNA in solution is a moderately flexible or kinked rod. If this picture is correct, it is to be expected that the longer the molecule the greater the degree of bending or kinking. Peterlin[30] has developed a theory of the light scattering of such molecules and has interpreted some of the data of this Laboratory on the basis of it. He is able to calculate from the scattering envelope both the root mean square end-to-end distance and the contour length of the molecule. He finds for S-VII, $\overline{R^2}^{1/2} = 4950$ Å., contour length 43,000 Å.; for BG, $\overline{R^2}^{1/2} = 3750$ Å., and contour length 26,000 Å. We note that the contour length increases faster than the characteristic dimension obtained from light scattering.

As mentioned previously, there is unfortunately no theory for the flow birefringence of non-rigid molecules valid to the same degree of approximation as the theory of Peterlin and Stuart. It seems plausible, as a speculation, that flow birefringence may measure an extreme dimension of such a molecule, such as the distance between the two elements farthest apart at a given time, rather than the radius of gyration obtained from light scattering.

If this is to account for the discrepancy, we have to explain the fact that the contour length of the low molecular weight BG exceeds considerably the light scattering dimension, yet the two methods are in fair agreement.

The dependence of apparent dimension on shear might vary with flexibility in one of two ways. If the molecules are only moderately kinked, and are not readily bent by the shear, the system might show up as polydisperse in a flow birefringence measurement, since there is a range of effective hydrodynamic lengths due to different degrees of kinking. On the other hand, high coiled, flexible molecules could be deformed by the shear; and the apparent θ would decrease with shear, rather than increase, as in a polydisperse system. Curves having this second character have been observed by

(28) C. F. Vilbrandt and H. G. Tennent, THIS JOURNAL, **65**, 1806 (1943).

(29) R. F. Steiner, *Trans. Faraday Soc.*, **48**, 1185 (1952).

(30) A. Peterlin, *J. Polymer Sci.*, **10**, 425 (1953).

Schoenberg, Riseman and Eirich[31] in high molecular weight polystyrene. The data are not consistent with the second possibility.

It does not seem likely that a theoretical solution of this problem will be achieved in the near future. Some light would be shed on the matter if an experimental study similar to this one could be carried out on some quite different molecule also behaving as a flexible rod, such as cellulose or cellulose derivatives.

In further support of the kinked rod model, it may be seen from Table III that the optical factor $(g_1 - g_2)$ decreases as the size of the molecule increases. This decrease with size, if it is not an inherent optical property of rods of dimension greater than the wave length of the light used, is plausibly attributable to the greater bending of the longer molecules.

When the limitation of flow birefringence calculation to the case of a rigid particle is considered, one must feel that the theoretical basis of light scattering as a tool for macromolecular studies is better founded. When the two methods are in disagreement, a reflection on flow birefringence is implied. However, this should not blind one to the much greater sensitivity of flow birefringence for the study of high molecular weight DNA preparations. For example, the differences between preparations V and S-VII appear to be tremendous by the standards of flow birefringence; by light scattering they are barely beyond experimental error.

An analysis by Doty and co-workers[8] of the flow birefringence and light scattering results discussed here, as well as viscosity and sedimentation data and electron microscope photographs, has led them to the conclusion that a model somewhere between a random coil and a kinked rod is capable of accounting for all the observed properties, while a rigid rod or ellipsoid leads to striking inconsistencies. Readers are referred to their paper for details.

Acknowledgments.—Though all the data reported here was obtained at Harvard University, a considerable amount of exploratory work on flow birefringence of DNA was performed by M. G. during the tenure of a National Institutes of Health Postdoctoral Fellowship at Brooklyn Polytechnic Institute. Particular thanks go to Dr. K. G. Stern for his help and advice at this time. Both of us would like to express our gratitude to Professor Paul M. Doty for his interest, help and encouragement. Part of the financial support of this work was provided by U. S. Public Health Service Grant G-3286. NOTE ADDED IN PROOF: We have been informed that Dr. N. S. Simmons, who provided the DNA sample referred to as N.S., designates his preparations by the letters A, B, C, ... and that the preparation used in this work was preparation B.

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NOTES

Ultracentrifugal Determination of Molecular Weights of Small Molecules by the Archibald Procedure[1]

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Recent advances in analytical techniques have made it possible to carry on detailed structural studies of increasingly complex molecules. Substances whose sizes are intermediate to the macromolecules and the simple organic molecules are becoming the subject of intensive study. In these studies the molecular weight and homogeneity are often of considerable importance. Although there have been several methods devised for the determination of molecular weights of small molecules,[2a,b] there is at present no convenient established method for determining both the molecular weight and homogeneity of substances whose molecular weights are under 10,000.

Archibald[3] has derived a procedure for determining these parameters which is both convenient and applicable to substances of low molecular weight. Porath[4] has used the procedure to determine the molecular weight of bacitracin. However, the procedure has not been applied to any well defined substance and its reliability has not yet been evaluated.

A standard substance has been analyzed in the Spinco model E ultracentrifuge by means of the theory developed by Archibald, and the results are the subject of this communication. In order to check the results obtained by the Archibald procedure the sedimentation constant of the substance has also been determined by velocity sedimentation in the synthetic boundary ultracentrifuge cell of Pickels, *et al.*[2] These results have been supplemented by diffusion measurements.

Experimental

Material.—The saponin, digitonin, has been chosen as the standard substance. This material is a solid, colorless, non-ionizing substance whose formula weight has been reliably established as 1229.3.[5] The digitonin used in this work was the product available commercially from Hofmann-LaRoche. The melting point, 240° (S.236), corresponds to that given in the literature for the pure sub-

(1) Presented at the 1953 Fall Meeting of the American Chemical Society, Chicago, Ill., on September 6, 1953.

(2) (a) H. Gutfreund and A. G. Ogston, *Biochem. J.*, **44**, 163 (1949); (b) E. G. Pickels, W. F. Harrington and H. K Schachman, *Proc. Natl. Acad. Sci.*, **38**, 943 (1952).

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(5) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, p. 580.