This could cause a vastly different array of charges on the surface of the molecule thereby altering the isoelectric point. Hartman *et al.*,[19] postulate such a phenomenon in connection with the formation of an inactive form of trypsin upon adsorption onto Pyrex glass. Inactivation is accompanied with a change of the isoelectric point from 10.8 for the dissolved protein to 7. However, ribonuclease is active in the adsorbed state.[20]

(19) R. S. Hartman, J. B. Bateman and H. E. Edelhoch, THIS JOURNAL, **75**, 5748 (1953).

(20) H. B. Bull and L. B. Barnett, in preparation.

It is concluded that certain combinations of phenomena such as those described, perhaps coupled with other similar effects, might well explain the entire array of microelectrophoretic data. That in some instances the isoelectric point of a dissolved and adsorbed protein is the same is quite plausible.

Acknowledgments.—We wish to express our thanks to the United States Public Health Service for their Research Fellowship and to the National Science Foundation for financial support.

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[CONTRIBUTION FROM THE DIVISION OF NUCLEOPROTEIN CHEMISTRY OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Shear Degradation of Deoxyribonucleic Acid[1]

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The shear forces developed by a simple glass atomizer have been used to cleave DNA molecules. Light scattering, sedimentation and viscosity measurements show that the cleaved molecules are stiff chains and that no detectable denaturation has occurred. The results show that the fragments result from double-chain scissions. The extrapolation of the zero concentration lines of the light scattering curves is discussed and it is suggested that the error of the weight-average molecular weight obtained is small.

In physical and biological studies on deoxyribonucleic acid (DNA) it is frequently desirable to have at hand a means of decreasing its molecular weight in a known and simple manner. While enzymatic digestion, ionizing radiation and sonication[2] appear to be useful tools for this purpose, the first two have serious disadvantages, namely, the relatively large number of single-chain breaks as opposed to double-chain scissions, and the occurrence of denaturation.

In this paper, we describe a simple method for degrading DNA by mechanical shear.[3] No free radicals are formed and double-chain scission is the only observable type of fracture. No detectable denaturation occurs. A unique characteristic of this method is that the cleavage is non-random; larger molecules are more readily cut than smaller ones, resulting in decreased polydispersity.

Experimental

Apparatus and Procedure.—The apparatus consists of an all-glass atomizer of the type used for spraying paper chromatograms.[4] The diameter of the central capillary and the width of the annular space were 0.15 mm. each. The results were found to depend slightly on these dimensions as well as on the solid angle of the air cone, which was about 20°. The atomizer was modified so that the velocity of the liquid stream issuing from the central capillary and the velocity of the surrounding air stream were controlled by separate air (or nitrogen) pressures; it was convenient to use for all runs a nitrogen pressure of 5 lb./in.^{2} to produce the liquid stream. The applied pressure for the air stream ranged from 7 to 117 cm. of mercury. The spray was collected in a Hoffman bulb, which was in turn connected to the liquid chamber of the atomizer. After a spraying, the nitrogen pressure was released and the stopcock connecting the Hoffman bulb to the liquid chamber of the atomizer was opened, thus permitting recycling in simple fashion.

Sedimentation.—Sedimentation velocity measurements were carried out using a Spinco Model E Ultracentrifuge equipped with ultraviolet optics. The DNA concentration was 0.03 mg./ml., in 0.2 *M* salt. Sedimentation distribution curves were calculated using the method of Schumaker and Schachman.[5]

Light Scattering and Viscosity.—The details of these measurements have been given elsewhere.[6]

DNA Samples.—The DNA used in this study was extracted from *Diplococcus pneumoniae* and deproteinized by the method of Kay, Simmons and Dounce,[7] Emmanuel and Chaikoff[8] or Sevag. In spite of the fact that the initial sedimentation distributions differ somewhat depending on the method of isolation of the DNA, the sheared DNA's showed essentially the same distributions for comparable shear rates. The data given in this paper were obtained with DNA isolated according to Kay, *et al.* Calf thymus DNA behaved similarly.

Results and Discussion

Variables.—The DNA and salt concentrations, temperature, liquid velocity and air pressure were all examined for their effects on the cleavage products. The range of DNA concentration was 0.03–0.8 mg./ml., the salt concentration range was 0.002–0.2 *M*, the temperature range was 0–60° and the liquid velocity was varied 50-fold. In no case did these variations change the nature of the products, as shown by physical measurements. However, the pressure of the air stream is critical. The higher the air pressure, the greater the shear gradient and the lower the molecular weight of the cleaved DNA; furthermore, fewer passages through

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant CY-3190) and from the Atomic Energy Commission (Contract No. AT(30-1)-910).

(2) P. Doty, B. Bunce McGill and S. Rice, *Proc. Natl. Acad.*, **44**, 432 (1958).

(3) L. F. Cavalieri, THIS JOURNAL, **79**, 5319 (1957).

(4) Purchased from Kopp and Co., New York, N. Y.

(5) V. N. Schumaker and H. K. Schachman, *Biochim. Biophys. Acta*, **23**, 628 (1956).

(6) L. F. Cavalieri, M. Rosoff and B. Rosenberg, THIS JOURNAL, **78**, 5239 (1956).

(7) E. R. M. Kay, N. S. Simmons and A. L. Dounce, *ibid.*, **74**, 1724 (1952).

(8) C. F. Emmanuel and I. L. Chaikoff, *Biochim et Biophys. Acta*, **24**, 261 (1957).

the atomizer are then required to complete the cleavage. After a certain number of passages, the exact number being a function of the air pressure and atomizer design, no further changes in the product can be discerned upon continued cycling. An increase in pressure, however, initiates the cleavage process once again so that the molecular weight of the product can be decreased in a stepwise manner until a lower limit is reached beyond which further increase in pressure has no effect. For our apparatus the minimum number-average molecular weight attainable was about 1×10^6.

The Cleavage Process.—Cleavage of the DNA doubtlessly occurs by means of the mechanical shear generated at the air–liquid interface, where the shear gradient is estimated to be of the order of 10^5 sec.$^{-1}$ for the higher air pressures used. Oxygen plays no role, since substitution of a nitrogen stream for the air stream had no effect on the products. The presence of a scavenger (1 M fructose)[9] in large excess also had no effect. Furthermore, free radicals do not act non-randomly, nor would they cease to act after a certain number of passages. We deduce that the sugar-phosphate backbone of the DNA is cleaved mechanically.

Since the mass distributions decrease simultaneously in dispersion and molecular weight (Table I, Fig. 1), it would appear that only molecules above a certain size are cleaved in a given shear gradient, while the smaller ones escape fragmentation. One may infer that if a cleaved molecule is still above the minimum size, it will be cleaved again on subsequent passage, and so on. It can be predicted, then, that the narrowest possible distribution will have a twofold spread in size assuming that cleavage occurs in the middle of a molecule; that is, the highest molecular-weight material should have a little less than twice the lowest molecular weight. This appears to be the case for the narrowest peak (Fig. 1), where about 85% of the DNA lies in a twofold molecular weight range.

TABLE I

LIGHT SCATTERING, SEDIMENTATION AND VISCOSITY RESULTS

Sample[a]	M_w' $\times 10^{-6}$[b]	Obsd. $S_{20,w}$[c] (max)	Calcd. $[\eta]$[d]	$\overline{\sigma^2}$[e] $\times 10^{-12}$	Z[f]	M_n[g]	M_w[h]
1	3.50	24.0	30	2.80	(2)	2.42	3.30
2	2.86	14.2	20	1.61	4.0	2.32	2.86
3	2.50	14.0	18	1.19	4.5	2.13	2.56
4	1.25	11.0	8	0.12	12	1.13	1.22

[a] Sample 1 is the original DNA; 2, 3 and 4 were sprayed at air pressures 17, 25 and 100 cm. of mercury, respectively. [b] M_w' is the apparent weight-average molecular weight obtained from the linear intercept of the zero concentration line of the light scattering plot. [c] These are the values of the sedimentation constant at the peak of the sedimentation distribution. [d] $[\eta]$ is the reduced specific viscosity (deciliters/g.) at zero concentration and zero shear gradient. [e] $\overline{\sigma^2}$ is the dispersion, defined as

$$\overline{\sigma^2} = \sum_i (M_i - M_w)^2 w_i$$

where w_i is the weight fraction of material of molecular weight M_i, and M_w is the weight-average molecular weight. [f] $Z = ((M_w)^2/\overline{\sigma^2}) - 1$. The value in parentheses for sample 1 is in doubt because the exact value of the exponent here is unknown. [g] This is the number-average molecular weight calculated from the mass distributions, by graphical integration. [h] Calculated from the normalized mass distribution, which was obtained from the sedimentation distribution.

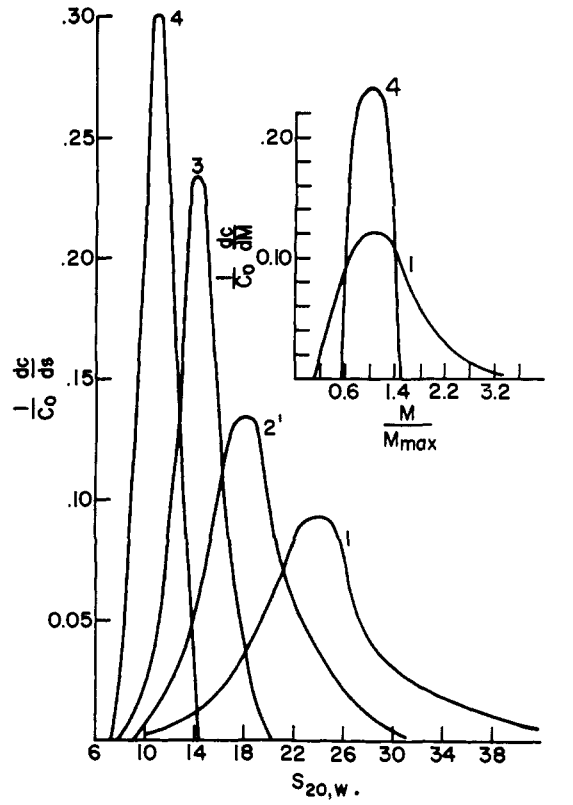

Fig. 1.—Sedimentation distributions: curves 1–4 are typical distributions. Curves 1, 3 and 4 correspond to samples 1, 3 and 4. (Curve 2 was omitted because it lies too close to 3. The curve shown, 2', represents a sample obtained at 13 cm. of Hg.) In the inset, mass distributions (see text) are compared for samples 1 and 4; the curves are arbitrarily placed on the same axis by the normalizing factor $1/M_{max}$.

Mass and Configuration Parameters.—In light scattering work it has generally been assumed that the intercept of the initial tangent in the Zimm plot yields the reciprocal of the weight-average molecular weight for DNA. The basis of this is the assumption that the mass distribution of the DNA is the most probable one, *i.e.*, $M_w/M_n = 2$; in this case there is no difficulty in extrapolating the initial tangent since the zero concentration line is linear. While it may be true that some DNA samples have this distribution, it is, of course, not generally so; in fact evidence obtained on our laboratory and elsewhere[10] suggests rather strongly that the distribution depends on the method of isolation of the DNA. Consequently, the molecular weight values appearing in the literature must be viewed with some reserve, as has already been pointed out by Sadron and Pouyet,[11] who simply that downward curvature of the zero concentra-

(9) D. E. Lea, "Actions of Radiations on Living Cells," Cambridge, 1947, p. 56.

(10) K. V. Shooter, "Progress in Biophysics," Vol. 8, Pergamon Press, New York, N. Y., 1957, p. 309.

(11) C. Sadron and J. Pouyet, "Symposium on Biological Polymers," IV International Congress of Biochemistry, Vienna, 1958.

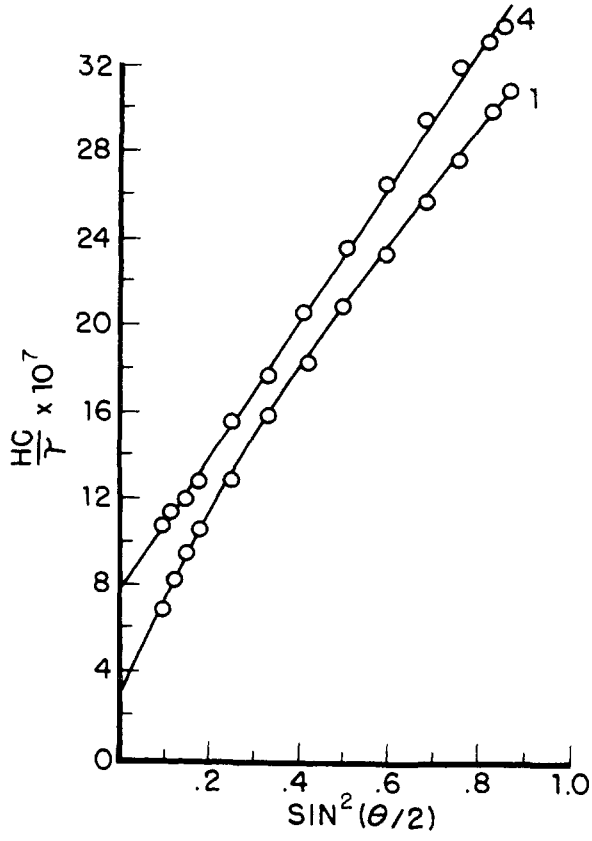

Fig. 2.—Light scattering: these curves are the zero concentration lines of Zimm plots. Curve 1 represents sample 1 (unsprayed DNA); curve 4, sample 4. The apparent weight-average molecular weights (M_w') given in Table I were obtained from the intercepts as shown in the figure. The curves for samples 2 and 3 are linear, like that for sample 4.

tion line may occur at low angles, beyond the experimental range.

The problem at hand, then, is to interpret the intercept for the various sheared samples. More specifically, we wish to know whether any downward curvature below 35° is being overlooked, thus resulting in low M_w values. The fact that sample 4 has a low degree of polydispersity has permitted the use of a simple empirical approach, involving the Peterlin theory,[12] enabling estimation of the weight-average weight. It will be recalled that this theory permits the evaluation of the stiffness of monodisperse molecular chains in terms of a worm-like model.[13] The stiffness is specified by $x(= L/a$, where L is the contour length and a, the persistence length, varies from 0 for flexible coils to ∞ for rods). The experimental reciprocal scattering factor $P(\theta)^{-1}$ is plotted *vs.* $\sin^2 \theta/2$ to determine the initial slope; $P(\theta)^{-1}$ is then plotted against 3 × initial slope × $\sin^2 \theta/2$ and compared to the family of theoretical curves for $x = \infty$ to $x = 0$. From the best fit, one then determines the value of x.

In the experimental light-scattering plot for sample 4 (Table I, Fig. 2) several downward extrapolations of the zero-concentration line were made, in addition to the linear one. These were then transformed in the manner just described and were compared to the theoretical curves. For the linear extrapolation the curve corresponded to $x = 10$. For an intercept 15% lower than the linear one, the $P(\theta)^{-1}$ curve fell below that for a rod ($x = 0$), thus showing that this assumed amount of downward curvature was too great. It was found by trial that an assumed intercept about 7% lower than the one obtained from linear extrapolation was the lower limit. In other words, the $P(\theta)^{-1}$ curve drops rather abruptly to that for a rod in a very narrow range of assumed intercepts. Assuming that DNA is a stiff chain rather than a rod, we interpret these calculations as indicating that the linear intercept (within about 7%) is that of the initial tangent, *i.e.*, it equals $1/M_w$.[14]

The degree of polydispersity of samples 1, 2 and 3 is not negligible; however, the experimental curves are similar to that of sample 4 and the same situation obtains regarding assumed intercepts. Namely, for samples 2 and 3 the zero-concentration line is linear and downward extrapolation leads to abnormal $P(\theta)^{-1}$ curves, as above. For sample 1, the light scattering curve is gently curved (Fig. 2), but the same results are obtained on calculating the assumed $P(\theta)^{-1}$ curves. The fact is that lowering the intercept more than about 7% causes kinking of the curve in the region between 0 and 35°, making its shape inconsistent with the theoretical $P(\theta)^{-1}$ curves for monodisperse chains of any degree of stiffness or for coils or rods of any degree of polydispersity (for $Z \geqslant 1$).[15]

We suggest, then, that although the critique of Sadron and Pouyet[11] is valid, the error for these DNA samples is rather small (<10%) if extrapolations below 35° are carried out in such manner that the general contour of the experimental curve is followed to 0°. This conclusion has been independently verified by scattering a solution of sample 4 before and after (heat) denaturation. Since measurements are made at lower values of h^2p^2 ($h = (4\pi/\lambda) \sin \theta/2$, p = radius of gyration) for the denatured DNA, the equal intercepts (and hence M_w's) which are obtained for denatured and undenatured DNA indicate that the extrapolation is correct (ref. 14, p. 476).

In Fig. 3 is plotted log $[\eta]$ *vs.* log M_w', where M_w' is the (minimum) weight-average molecular weight, obtained by linear extrapolation. The empirical equation for this relationship is $[\eta] = KM^{\alpha}$. The fact that a straight line is obtained for the sheared samples suggests that the stiffness does not change greatly over the molecular weight range examined. The value of unity for the slope suggests that the sheared DNA samples are moderately stiff. If they are deliberately denatured by lowering the *p*H the viscosity drops markedly, just as it does with

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

(13) O. Kratky and G. Porod, *Rec. trav. chim.*, **68**, 1106 (1949).

(14) Recently P. Geiduschek and A. Holtzer in "Advances in Biological and Medical Physics," Vol. VI, Academic Press, Inc., New York, N. Y., 1958, p. 482, suggested a similar procedure for determining M_w from the intercept. HC/τ *vs.* log $\sin^2 \theta/2$ is compared to the Peterlin curves for various values of x. The value of x is determined for the best fit, and the extrapolation to $\theta = 0$ is carried out again.

(15) The same type of situation obtains if $P(\theta)$ is plotted against $\sin \theta/2$, again using assumed intercepts. It can be shown that for low intercepts the theoretical curve for a rod is produced.

unsprayed DNA.[6] The denaturation is also accompanied by the usual hyperchromic effect of 35–40% at 260 mμ. These results show that shearing of the DNA does not result in perceptible denaturation. Further experimental verification that denaturation does not occur on shearing is afforded by observing the hyperchromic effect as a function of temperature. With undenatured DNA a sigmoid-like curve is obtained when the optical density is plotted against the temperature. On the other hand, samples which are known to be denatured show skewed curves with the (reduced) hyperchromic effect occurring at lower temperatures. Sheared DNA samples yield curves which are identical to those for undenatured DNA. The hyperchromic effect occurs over about a 10° range for undenatured material. In 10^{-3} M sodium chloride the range is 65–75°; in 0.2 M salt the range is 80–90°.

Typical sedimentation distributions are shown in Fig. 1. A plot of log S_{max} *vs.* log M_w' is shown in Fig. 3, where S_{max} is the sedimentation constant of the maximum of the distribution. The empirical equation relating the sedimentation constant to M_w' for the sheared samples is[16]

$$S_{max} = 0.141(M_w')^{0.31} \quad (1)$$

Sedimentation distributions were converted to normalized weight distributions by the use of this equation. While the procedure for obtaining the mass distributions is empirical, it is noteworthy that the M_w values calculated from them agree with those obtained from light scattering, which indicates that the constants of eq. 1 hold throughout each distribution for sheared DNA. Equation 1 does not apply to the unsprayed DNA since the point does not fall on the line shown in Fig. 3. From M_w' and S_{max} for this sample a new constant (= 0.24) before M_w' in eq. 1 was calculated, assuming the same exponent, 0.31. The M_w given in the table was calculated from these values. It is interesting to note that molecular weights calculated using the Flory–Mandelkern equation and the values for S_{max} and $[\eta]$ (samples 2, 3 and 4) are in excellent agreement with the M_w values in the table. However, while this equation holds for extended molecules,[17] it was derived for flexible chains, and its use for DNA must be regarded as empirical.

Dispersions, $\overline{\sigma^2}$, were also calculated for the mass distributions and are presented in the table.

(16) Doty, *et al.*, ref. 2, have obtained the equation $S = 0.063M^{0.37}$ for sonicated DNA samples. The difference between this equation and our eq. 1 is most probably due to the nature of the mass distributions. Our distributions become narrower on cleaving while those of Doty, *et al.*, are believed to remain the most probable.

(17) M. L. Hunt, S. Newman, H. A. Scheraga and P. J. Flory, *J. Phys. Chem.*, **60**, 1278 (1956).

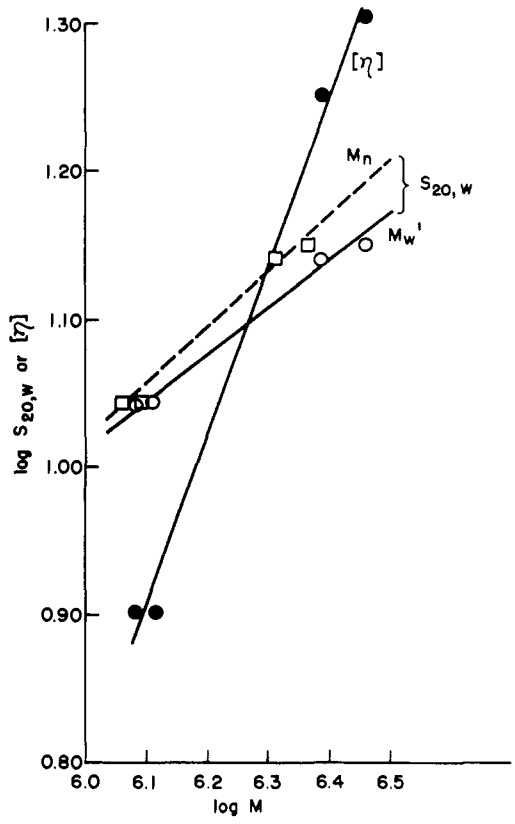

Fig. 3.—Viscosity–sedimentation–molecular weight relations: the ordinate refers to either log S_{max} or log $[\eta]$. M_w' is the reciprocal intercept of the light scattering curve. The broken line refers to the calculated number-average molecular weight, M_n (see text).

There is a decrease in polydispersity as the molecular weight decreases, as shown in the inset of Fig. 1, where the mass distributions for samples 1 and 4 are superimposed on the same axis; samples 2 and 3 also show progressive narrowing. This indicates preferential cleavage of the larger molecules. Number-average molecular weights were obtained from the mass distributions.

Type of Scission.—It seems safe to conclude that the DNA fragments are stiff chains and that the helical structure is intact. The results are consistent with the occurrence of double chain scissions accompanied by no detectable denaturation.

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