was dissolved in 1 ml. of 0.5 *M* ammonium acetate buffer of *p*H 6.38 (20°) and placed on an XE-64 column (1.9 × 43.5 cm.) which had been equilibrated with the same buffer. The chromatogram was developed with the buffer at room temperature with a flow rate of 3.5–4.5 ml. per hour. The volume of the fractions collected was 3.0 ml. The eluates were analyzed by determination of the absorption at 275 mμ, Folin–Lowry color reaction and pressor activity. The various fractions were combined as indicated in Table II, lyophilized 3 times to remove the ammonium acetate[11] and tested for pressor activity. The results are summarized in Table II. The recovery of pressor activity in these fractions was 80%, with 73% of the weight of the original material being present.

TABLE II

ION-EXCHANGE CHROMATOGRAPHY OF SYNTHETIC LYSINE-VASOPRESSIN

Tube no.	Weight, mg.	Pressor activity, units/mg.
47, 48	4.4	290
49	5.7	260–270
50–54	30.8	260–300
55–64	24.4	270
65–75	7.6	220

As much as 800 mg. of the material could be chromatographed on the same column without overloading it, with 81% of the pressor activity being recovered in material representing 74% of the weight placed on the column. The main fraction was desalted on an XE-64 column rather than by repeated lyophilization, which is not convenient for large amounts of material.

Paper electrophoresis[12] on Whatman No. 3 MM paper with 0.1 *M* pyridine acetate buffer of *p*H 4.0 at 400 volts showed the material containing 245 pressor units per mg. as well as the chromatographically purified preparations to travel as single spots. Paper chromatography was applied with the solvent system butanol–acetic acid–water (4:1:5) on Whatman No. 1 paper and again single spots were obtained. Starch column analysis[13] of hydrolysates showed the following amino acid content (with the ratio for glycine arbitrarily taken as 1 and the values of the material before chromatography given in parenthesis): phenylalanine 0.9 (1.0), tyrosine 0.7 (0.8), proline 0.8 (0.7), glutamic acid 1.0 (0.9), aspartic acid 1.0 (0.9), glycine 1.0, lysine 1.0 (0.8), cystine 0.9 (0.9) and ammonia 3.5 (3.0).

The specific rotation was $[\alpha]^{22}D$ −23.8° (*c* 0.5, 1 *N* acetic acid). For analysis, the compound was dried at 100° for 8 hr. over P_2O_5.

Anal. Calcd. for $C_{46}H_{65}O_{12}N_{13}S_2 \cdot C_2H_4O_2$: C, 51.6; H, 6.23; N, 16.3. Found: C, 51.9; H, 6.25; N, 16.4.

Acknowledgments.—The authors wish to thank Mr. Joseph Albert for carrying out the microanalyses reported herein, Miss Dade Tull and Miss Maureen O'Connell for the pressor assays, Mrs. Lorraine S. Abrash for the amino acid analyses and Mr. David N. Reifsnyder for his assistance.

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[CONTRIBUTION FROM THE LABORATORIES OF THE DIVISION OF NUCLEOPROTEIN CHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH AND THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK 21, NEW YORK]

Physicochemical Effects of High-speed Mixing on Deoxyribonucleic Acid[1]

BY HERBERT S. ROSENKRANZ[2] AND AARON BENDICH

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Brief high-speed mixing of DNA solutions results in a decrease of the sedimentation coefficient of the sample as well as a narrowing of the distribution of sedimentation coefficients. This is interpreted as being due to scission of those DNA macromolecules above a certain size ($S > 26$). This behavior is not accompanied by significant denaturation.

Introduction

It is known that changes in macromolecular configuration occur when deoxyribonucleic acid (DNA) is subjected to a variety of physical treatments such as X-irradiation,[3–6] ultrasonic[7,8] and sonic vibrations[8–12] and shear.[13,13a] Since high-speed mixing or mincing is very frequently part of the several procedures employed in the isolation of DNA (see ref. 14 for review on methods for DNA isolation), it was considered of interest to determine whether or not such a treatment would affect the DNA.

There are reports which indicate that degradation may result when proteins are subjected to high-speed mixing.[15–17] Chargaff[18] has cautioned against the use of high-speed mixing in the preparation of DNA, and it may be deduced from the work of several laboratories[19–21] that DNA is indeed affected

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(2) Taken from the thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Medical Sciences of Cornell University, New York, N. Y., February 1959.

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by such treatment; the nature of this effect has not been well documented. The present report deals with the effects of high-speed mixing on the sedimentation behavior of DNA.

Experimental

Nucleic Acid.—DNA samples were prepared from human leucocytes by the Dounce detergent procedure[22] modified in that salt-free solvents were avoided. Some of the properties of the preparations (designated as 3LD and 8LD in previous publications[23,24]) have been reported. Solutions of the DNA in 0.001 *M* NaCl (1 mg. per ml.) were used for the experiments reported here. The samples derived from 3LD (No. 1) by high-speed mixing were numbered 2 to 5.

High-speed Mixing.—A VirTis Model "45" Homogenizer[25] equipped with a stainless steel micro-homogenization cup (2.0 cm. × 1.2 cm. (inner diameter)) and micro-blades (1.1 cm. in diameter) was used. The speed of this instrument can be regulated from 100 to 45,000 r.p.m.

Aliquots (1.5 ml.) of the above DNA solution were pipetted into the homogenization cup and subjected to high-speed mixing. The temperature of the DNA solution was 4°. There was no significant increase in temperature due to this treatment. The treated solutions were diluted to a DNA concentration of 0.0037%, with 0.20 *M* NaCl and analyzed in the ultracentrifuge. A KI test for free radical formation was negative.

Ultracentrifugal Analysis.—A Spinco Model E ultracentrifuge equipped with ultraviolet optics[26,27] was used. The specimens were centrifuged at 59,780 r.p.m. (259,700 × *g*) using 12 mm. cells with Kel-F centerpieces. Integral sedimentation distribution curves corrected to water and 20° were calculated by a modification[28] of the method of Schumaker and Schachman.[26] Average sedimentation coefficients ($S_{50\%}$) were estimated from the 0.5 intercept of the sedimentation distribution curves (see Fig. 1).

Hyperchromic Shifts.—It has been reported that on acidification[29] and alkalinization[30] "undenatured" DNA exhibits a hyperchromic effect at 260 mμ. The magnitude of this effect is taken to be[31] a measure of the intactness of the proposed twin-helical structure[32] of DNA. Any treatment which will cause a separation of the twin strands, *i.e.*, breakage of the hydrogen bonds holding them together, will result in a decrease in the magnitude of this hyperchromic effect. On the other hand, a treatment such as exposure to sonic vibrations which results in a scission of the double-chain with retention of the helical structure but diminution in length[8-11] does not lead to a change in the hyperchromic shift.[8,33]

(a) **Addition of Alkali.**—To 2.0 ml. of the 0.0037% DNA solution in 0.20 *M* NaCl were added 0.04 ml. of 19 *N* NaOH (final *p*H *ca.* 13.5). The hyperchromic effect (per cent. increase in optical density at 260 mμ, read against the appropriate blank) was corrected for the dilution (2%) caused by the addition of the alkali. The data are given in Table I. The reproducibility of the method is ± 1.5.

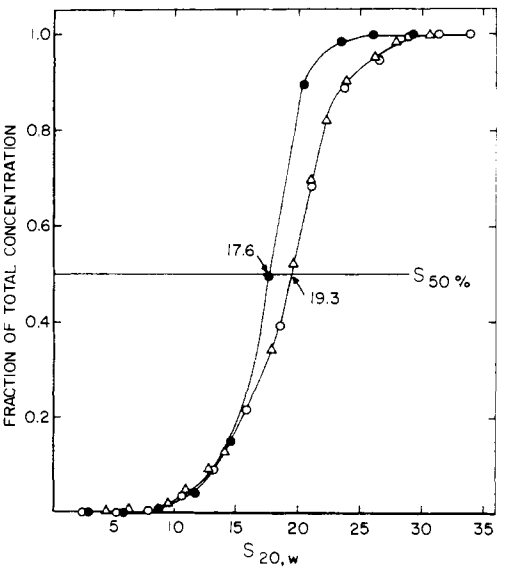

Fig. 1.—Effect of high-speed mixing on the sedimentation behavior of DNA. Sedimentation coefficient distribution curves: O—O—O and △—△—△, duplicate measurements on control sample No. 1 (3LD); ●—●—● DNA after high-speed mixing (sample No. 5).

(b) **Addition of Acid.**—1 *M* HCl was added gradually with continuous stirring to 5 ml. of the 0.0037% DNA solution in 0.20 *M* NaCl to *p*H 3.0.

Sonically Degraded DNA.—A solution (1 mg. per ml. of 0.2 *M* NaCl) of DNA prepared from fresh calf thymus by the method of Schwander and Signer[34] was exposed to the vibrations of a 9Kc Sonic Oscillator (Raytheon Mg. Co., Model S102A). A detailed description of the effects of sonic vibrations is in preparation.

TABLE I

PROPERTIES OF DNA SAMPLES SUBJECTED TO HIGH-SPEED MIXING

Sample	Speed mixing, r.p.m. (× 10^{-3})	Time mixing, min.	Alkaline hyperchromic shift (%)[a]	$S_{50\%}$, S
1 (3LD-control)	0	..	28	19.4
2	22.4	1	25	18.7
3	31.4	1	28	18.0
4	40.4	1	29	17.6
5	40.4	2	25	17.6
Sonicated (control)	0	..	34	11.2
Sonicated and "homogenized"	40.4	1	34	11.2
8LD (Control)	0	..	33	29.0
8LD "Homogenized"	40.4	1	33	16.3

[a] The acid hyperchromic shifts for samples No. 1 and 5 were 31 and 27%, respectively.

Viscosity.—Reduced specific viscosities were determined on solutions of 8LD, before and after high-speed mixing, within a concentration range of 0.002 to 0.008%. These measurements were carried out at 25° with a capillary viscometer according to the design of Schachman.[35] The average flow rate for the solvent (0.2 *M* NaCl) was about 170 sec. The reduced specific viscosities were extrapolated to zero concentration to yield the intrinsic viscosity. The reduced specific viscosities did not differ one from another at the DNA concentrations used.

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Results and Discussion

It can be seen from the data of Table I that the average sedimentation coefficient of DNA decreased when subjected to high-speed mixing. This change was dependent upon the speed of mixing. A comparison of samples No. 1, 4 and 5 shows that the alteration in average sedimentation coefficient ($S_{50\%}$) had taken place within the first minute of mixing. The observed decrease of $S_{50\%}$, from 19.4 to 17.6 S, corresponds to a drop in molecular weights from 5.1×10^6 to 4.0×10^6 as calculated using the formula of Doty, McGill and Rice.[11]

An analysis of the sedimentation distribution curves of the samples (as exemplified in Fig. 1 by samples No. 1 and No. 5) reveals that the shift to lower values of sedimentation coefficient is accompanied by a concurrent narrowing of the distribution of sedimentation coefficients. The extent of the heterogeneity as revealed by these distributions can be estimated from the ratios of number to weight-average molecular weights (M_w/M_n) estimated from the weight (S_w) and number average (S_n) sedimentation coefficients[28] which is 2.20 for sample No. 1 and 1.67 for sample No. 5. This is considered to be an appreciable change in distribution. Another estimate of the degree of heterogeneity is obtained when the data for the middle two-thirds (*i.e.*, 2σ) of the sedimenting material are plotted on probability paper (see Fig. 2). From the

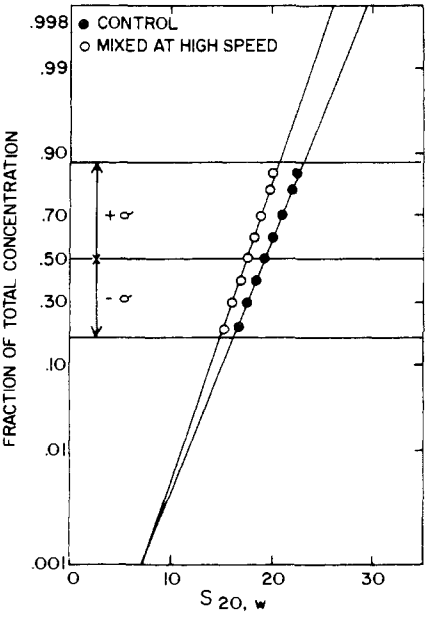

Fig. 2.—Effect of high-speed mixing on the spread of sedimentation coefficients of DNA. Integral distribution of sedimentation coefficients plotted on probability paper: ●—●—●, sample No. 1 (3LD-control); O—O—O, sample No. 5.

plot, the value $\frac{\Delta S\sigma}{S_{50\%}}$, where $\Delta S\sigma$ is equal to the difference between the S value at the 0.82 intercept ($+\sigma$) and $S_{50\%}$, is calculated. This is equal to 0.155 for both solutions. This is taken to mean (*cf.*[28]) that the change in sedimentation coefficient which is brought about by the high-speed mixing is due to an effect only on those fractions of DNA the sedimentation coefficients of which lie outside the 66% range.

In order to determine whether the changes observed upon the high-speed mixing of DNA were real and significant, the extent of reproducibility of the ultracentrifugal technique was assessed. In Fig. 1 are shown distributions of sedimentation coefficients of sample No. 1, determined at different times and using different ultracentrifuge cells. One curve can be drawn through the experimental points obtained from the two runs. Another example of reproducibility is shown in Fig. 3.

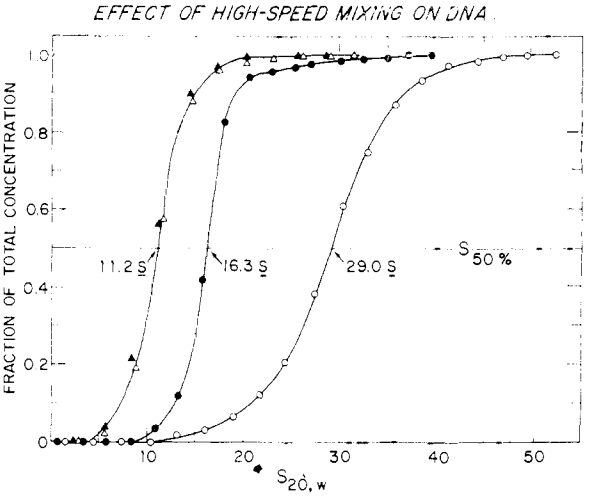

Fig. 3.—Sedimentation coefficient distribution curves: ▲—▲—▲, DNA exposed to sonic vibrations (control); △—△—△, same sample but exposed to high-speed mixing after sonication; O—O—O, sample 8LD (control); ●—●—●, same sample (8LD) but after high-speed mixing for one minute.

The results in this study are consistent with the interpretation that high-speed mixing introduces double-chain scissions in those molecules of DNA with sedimentation coefficient larger than 26 S corresponding to those with molecular weights in excess of *ca.* 11.6×10^6. This cleavage does not seem to affect the integrity of the presumed double helical structure as the treated samples exhibited hyperchromic effects very similar to those of the control (Table I). Those macromolecules with sedimentation coefficients below 26 S are probably not affected by the high-speed mixing under the conditions employed in these experiments. If this interpretation is a correct one, a DNA preparation in which molecules with sedimentation coefficients in excess of 26 S are absent should not be affected by high-speed mixing. Such a DNA sample was obtained by exposing DNA to the vibrations of a sonic oscillator. When this sample was subjected to high-speed mixing it showed the same behavior in the ultracentrifuge (Fig. 3) as did the sonicated sample that had not been exposed to high-speed mixing. On the other hand, a DNA specimen in which a large proportion of molecules possess sedimentation coefficients in excess of 26 S should exhibit a drastically different distribution of sedimentation coefficients following its exposure to

high-speed mixing. Indeed, an examination of Fig. 3 reveals that when a DNA sample (8LD) with an average sedimentation coefficient of 29 *S* was homogenized, those molecules with sedimentation coefficients above 26 *S* were almost quantitatively degraded. This decrease in sedimentation coefficients was accompanied by a drop in the intrinsic viscosity from 103 to 15 in units of 100 cc./g. This treatment did not however lead to a lowering of the alkali hyperchromic shift (Table I).[36]

The effect on DNA of high-speed mixing is reminiscent of the effects of sonic treatment; in that case there also occurs a scission of the twin-helical chain which is not accompanied by a separation of the twin strand (denaturation).[8-11]

It should be borne in mind that these studies were carried out using a small volume of DNA solution in a small homogenization cup. It may be that the extent of the alteration of the size of the DNA is dependent upon the design of the apparatus.

The question can be asked whether the scission of the DNA fibers was perhaps accomplished by the cutting action of the blades. It would seem that the effect of high-speed mixing is similar to that of sonication, hence the major factor leading to the degradation might be cavitation. This method of degradation lends itself well to the preparation from DNA specimens with very high sedimentation coefficients of specimens of smaller size with sedimentation coefficients below 26 *S*. An advantage of this method of degradation is the extremely short period of time necessary to bring about these alterations. In view of the experiments reported here, it should be possible to prepare a graded series of DNA preparations from sedimentation coefficients in excess of 30 *S* down to 26 *S* in less than a minute. Under these circumstances minimal denaturation is to be expected.

The data suggest that the widely-used process of high-speed mixing in the course of the isolation of DNA might give a product which is appreciably smaller than that obtained when such a step is not included in the isolation procedure. However, high-speed mixing has frequently been used to speed up the dissolution of fibers of DNA and the present data indicate that such a procedure leads to degradation.

High-speed mixing could be used in the preparation of DNA samples with high values of sedimentation coefficients and a narrower distribution of sedimentation coefficients than untreated samples. Such DNA samples might prove to be useful specimens for physical studies such as the effects of a decreased M_w/M_n ratio on the molecular weight obtained by light-scattering (see discussion in ref.[28]).

Acknowledgments.—The authors are very grateful to Dr. G. B. Brown for his interest and advice in the course of this study and to Dr. G. di Mayorca for a gift of the DNA samples used in this study as well as for helpful suggestions.

(36) When these sedimentation and viscosity data are substituted in the relationship of Doty, *et al.*,[11] or in the equation of Mandelkern and Flory (*J. Chem. Phys.*, **20**, 209 (1952)) values of *ca.* 16 and 3 × 10^6 are obtained for the molecular weights of the control and homogenized samples, respectively.

[CONTRIBUTION FROM THE COLLIP MEDICAL RESEARCH LABORATORY, UNIVERSITY OF WESTERN ONTARIO, AND FROM THE DEPARTMENT OF CHEMISTRY, LAVAL UNIVERSITY]

Steroids and Related Products. XII.[1] The Synthesis of 17α-Bromo-11-dehydrocorticosterone[2]

BY R. DEGHENGHI AND CH. R. ENGEL[3]

RECEIVED NOVEMBER 2, 1959

The synthesis of 17α-bromo-11-dehydrocorticosterone, the first 17-halogenated derivative of a glucocorticoid, from 3α-acetoxy-11,20-dioxopregnane, is described.

A comparison of the glucocorticoid activity of 11-dehydro-17α-methylcorticosterone, 11-dehydrocorticosterone and cortisone shows an apparent parallelism of this activity with the electronegativity of the 17α-substituent.[4] It seemed attractive to investigate whether or not this parallelism was merely coincidental, and therefore to extend the series by synthesizing glucocorticoid derivatives with 17α-substituents more electronegative than a hydroxy group. The preparation of 17α-halogenated products appeared to merit particular attention because in a number of cases halogen substitution in positions vicinal to carbonyl and carbinol groupings results in a marked increase in biological activity.[5] We decided to include in our study parent compounds of the related progesterone and mineralocorticoid series; recently we reported the synthesis and high progestational activity of 17α-bromoprogesterone.[6a] Now we wish to record the synthesis of the first 17-halogenated glucocorticoid, 17α-bromo-11-dehydrocorticosterone (XVII).

Since 17α-bromo-21-methyl-20-ketones and the corresponding 17,21-dibromo and 17-bromo-21-iodo derivatives are relatively easy to prepare, it would seem attractive to convert such compounds directly to 21-hydroxy- or 21-acyloxy-17α-bromo-20-ketones. In a subsequent paper[6b] we show that this approach is not practical in the 17α-bromo

(1) Paper XI of this series: Ch. R. Engel and W. W. Huculak, *Can. J. Chem.*, **37**, 2031 (1959).

(2) The main results reported in this paper were included in a communication presented before the 4th International Congress of Biochemistry in Vienna, September, 1958.

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