with 5 ml. of cation-exchange resin. The effluent was concentrated to a 2-ml. volume and then diluted with 200 ml. of glacial acetic acid. The solution was treated first with 1.475 g. of lead tetraacetate and, 15 min. later, with 0.4 g. of oxalic acid dissolved in glacial acetic acid.[15] The precipitate was filtered, the filtrate concentrated under reduced pressure, and the concentrate taken up in 10 ml. of 0.05 *N* hydrochloric acid and heated for 30 min. at 80° (for hydrolysis of formate ester groups). The solution was diluted with 50 ml. of water, and 1.5 g. of barium benzoate and 0.9 g. of bromine were introduced. After overnight reaction, the non-oxidized sugar was separated in the usual fashion (using cation- and anion-exchange resins for final purification). Paper chromatography using butanol–ethanol–water (4:1:5) showed the presence of a ketose with an R_f value only slightly less than that of glucose. Compound IX has not as yet been obtained in crystalline form. Compound IX, dissolved in water, was treated with a threefold excess of sodium borohydride. After 18 hr., cation-exchange resin was added to the stirred solution. When gaseous evolution had ceased, the mixture was filtered. Boric acid was removed from the filtrate by repeated reconcentrations with methanol. An aqueous ethanolic solution of the concentrate spontaneously gave small separate crops of two kinds of crystalline material. These were (a) D-*glycero*-D-*ido*-heptitol in the form of platelets with m.p. 125–127° and an undepressed mixed m.p. (using authentic heptitol[19] prepared by reduction of D-*glycero*-D-*ido*-heptose) and (b) D-*glycero*-D-*manno*-heptitol in the form of needles with m.p. 152–153° and an undepressed mixed m.p. (with authentic heptitol[20] prepared from D-*glycero*-D-*manno*-heptose).

The infrared spectra of the compounds considered in this paper will be presented in a forthcoming publication. Measurements in the 2 to 15 μ region were made with a Beckman IR-4 spectrometer using crystalline samples pressed into pellets of potassium chloride or iodide.

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[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING INSTITUTE AND THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Studies on the Sedimentation Behavior of Artificial Mixtures of Deoxyribonucleic Acid[1]

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The sedimentation behavior of two artificial mixtures of degraded and undegraded DNA were examined in the analytical ultracentrifuge equipped with ultraviolet optics. The components of the mixtures were found to sediment independently of each other with no pronounced interaction. The data confirm that very little experimental error is involved in the ultracentrifugal technique when applied to dilute solutions (*ca.* 0.003%) of DNA.

Johnston and Ogston observed[3] that an interaction occurred between proteins when they were subjected to ultracentrifugation in solution at a total concentration of solute between 3 and 8%. It was found that the material with lower sedimentation coefficient was enriched with material of higher sedimentation coefficient. This interaction, which has become known as the "Johnston–Ogston effect," was followed using an ultracentrifuge equipped with a schlieren optical system.

In a study of the ultracentrifugal heterogeneity of deoxyribonucleic acid (DNA), Peacocke and Schachman[4] prepared an artificial mixture composed of DNA of 20 *S* and a sonically degraded sample of sedimentation coefficient 8*S*. Again a schlieren optical system was used to follow the centrifugation and this necessitated concentrations of DNA of 0.12 to 0.17%. They stated that the interpretation of their study was made difficult due to the Johnston–Ogston effect observed at these relatively high concentrations.

The commercial availability of an ultraviolet optical attachment to the analytical ultracentrifuge[5–7] has made it possible to study the sedimentation behavior of DNA at much lower concentrations. Accordingly, the problem of the sedimentation of artificial mixtures was re-investigated to see whether or not, in dilute solutions, DNA preparations of different sedimentation coefficients would sediment separately from each other. This study was considered of special interest since it has been demonstrated, with many different preparations, that DNA at low concentrations (0.003%) exhibits a heterogeneity when examined in the ultracentrifuge equipped with ultraviolet optics.[5–8] Such a study would provide information on the interaction of the different molecular species as well as on the sensitivity of the ultracentrifugal technique.[9]

For this purpose in two separate experiments, a DNA preparation obtained by conventional methods was mixed with a DNA sample obtained therefrom by sonic degradation. It has been shown[10–12] that the sonic treatment of DNA leads to a diminution in length of the twin-helical chain[13] which is not accompanied by any denaturation, *i.e.*, separation of the twin strands.

These studies afforded an opportunity to determine the error in the quantitative assessment

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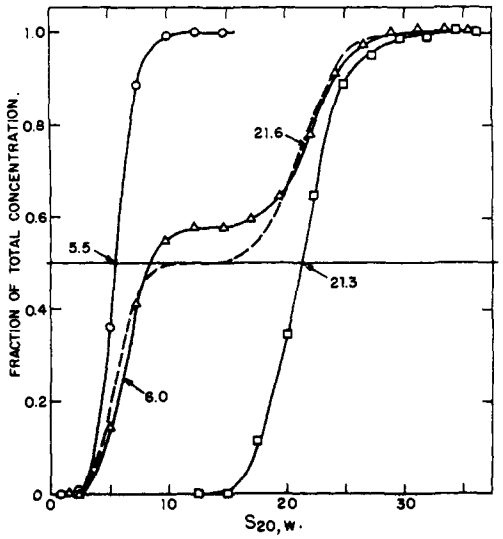

Fig. 1.—Integral sedimentation distribution coefficient curves: O, sample D; Δ, mixture I (A + D); ---, calculated for mixture I; □, sample A.

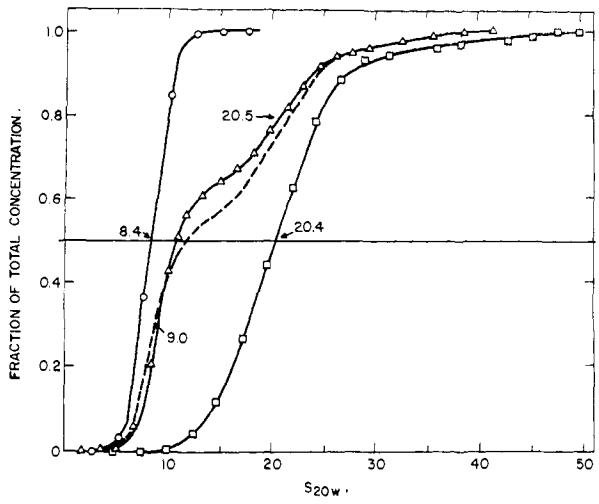

Fig. 2.—Integral sedimentation distribution coefficient curves: O, sample C; Δ, mixture II (B + C); ---, calculated for mixture II; □, sample B.

of sedimentation coefficients (see also ref. 14) and distribution of sedimentation coefficients and also furnished data concerning the problem of the heterogeneity of nucleic acids.

Materials and Methods

Nucleic Acid.—Sample A was prepared from fresh calf thymus by the method of Schwander and Signer.[15] Sample B was prepared from human leucocytes by the Dounce detergent procedure[16] modified in that salt-free solvents were avoided. Both samples were free of RNA as determined by the orcinol reaction.[17] Upon alkalinization to *p*H *ca.* 13.5[18] and acidification to *p*H 2.7[19] both DNA samples exhibited the hyperchromic shift at 260 mμ expected of undenatured DNA. We are greatly indebted to Drs. G. di Mayorca and M. Rosoff for making these samples available to us.

Sonic Degradation.—Aliquots of 0.4 mg. per ml. solutions of the calf thymus DNA (sample A) in 0.2 *M* NaCl were exposed to the vibrations of a 9 Kc. Sonic Oscillator (Raytheon Mfg. Co. Model S102A) for 2.5 minutes (sample C) and 30 minutes (sample D), respectively. Cold water was circulated through the jacket surrounding the cup in which the degradations were carried out.

Characterization of the Sonically Degraded DNA Samples.—The absorption at 260 mμ remained unchanged following exposure of the DNA solutions to the sonic treatment. Upon alkalinization and acidification the samples still exhibited the hyperchromic shift expected of undenatured DNA. A more complete study of the effect of sonic vibrations on DNA is in preparation.[12]

Preparation of Mixtures.—1:1 Mixtures of undegraded and degraded samples were obtained by mixing solutions of equal 260 mμ absorbancies as follows: Mixture I, samples A + D; mixture II, samples B + C. The final concentrations were 0.003% of total DNA in 0.20*N* NaCl.

Ultracentrifugal Analysis.—A Spinco Model E ultracentrifuge equipped with ultraviolet optics[6,7] was used. The experiments were carried out at 59780 r.p.m. (259700 × *g*) on 0.003% solutions of DNA in 0.20 *N* NaCl. Pictures were taken at 4 minute intervals and the photographic negatives were scanned with a Model R Analytrol photodensitometer (Spinco Division, Beckman Instruments, Inc.) equipped with a calibration filter. The integral distributions of sedimentation coefficients, corrected to 20° and water were calculated by a modification of the method of Schumaker and Schachman.[7] The distributions are reproduced in Figs. 1 and 2, in which values of $S_{20,w}$ on the abscissae are plotted against the fraction of total DNA concentration on the ordinates. The average sedimentation coefficients $s_{50\%}$, were estimated from the 0.5 intercepts of the curves for the unmixed samples and from the midpoints of the curves for the fast and slow components of the mixtures in Figs. 1 and 2.

Calculation of Theoretical Distribution Curves of the Mixtures.—To determine the distribution curves that would have been obtained if the two components in each artificial mixture had sedimented independently of each other, the following calculation was carried out. For each interval of 2 Svedberg units on the abscissa (Figs. 1 and 2) the corresponding value of the fractions of the total DNA for each component of the mixture was read from the curves. These were summed and divided by 2 to give weighted averages for the theoretical curves shown as the dashed line in the figures.

Results and Discussions

In Figs. 1 and 2 are shown the sedimentation distribution curves of the degraded and undegraded DNA samples as well as of the 1:1 mixture of the two. Included in each figure are theoretical curves calculated on the assumption that under ideal conditions (no interactions) a mixture would sediment as the weighted average of its components. An examination of these figures reveals that the components of the mixture are clearly resolvable and that there is no significant interaction between the deliberately mixed macromolecular species. This is also seen from the data in Table I. It can be observed that in the mixtures 50% of the material has sedimentation coefficients extremely close to that of the degraded DNA while the remaining half has a sedimentation behavior which is almost indistinguishable from the undegraded DNA. Had there been pronounced interaction of the Johnston–Ogston type, the slower (degraded) component would have shown a considerably enhanced concentration due to the retardation of the faster sedimenting component.[4]

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A comparison of the experimental curves with the theoretically calculated curves for the mixture shows that at the "interphase" of the two components they do not coincide exactly. This slight discrepancy is ascribed to the experimental error involved due to the following (see ref. 7): (a) the sensitivity response of the Analytrol photodensitometer: (b) the error involved in retracing manually the diagrams obtained with the photodensitometer; (c) the error introduced when reading the coördinates of the tracing obtained above. If the total error in each of these operations in addition to that of the ultracentrifugal run[20] itself is 2%, the nonsuperimposibility of the two curves can be understood. Considering all of the possible experimental errors, the two curves are quite similar. This would indicate that no significant molecular interactions are involved at the concentrations employed. It can also be concluded from these results that ultracentrifugal analyses employing ultraviolet optics afford a sensitive and accurate method for the determination of the sedimentation properties of DNA, in confirmation of the results of other investigations.[6,7]

Several techniques have revealed that DNA is a mixture of polynucleotides.[21-24] These and many ultracentrifugal analyses[5-8,25] have shown that most DNA preparations are composed of polynucleotides which differ from each other in size and shape. These polynucleotides constitute a family of very closely related homologs which are extremely elongated and could possibly be intertwined. It would therefore be expected that certain analytical procedures might not tend to distinguish among these sharply, as their physical chemical properties would presumably overlap severely. Thus for the most part, sedimentation studies reveal nucleic acids to be composed of molecules of a broad spectrum of sedimentation coefficients, and it might have been anticipated under these circumstances that interactions would occur. The current data indicate that such interaction, if it exists at all, is negligible at concentrations of 0.003%. This conclusion can also be made from a reexamination of sedimentation data obtained on fractions of DNA. In that study,[8] a sample of DNA exhibiting the usual wide spread in sedimentation coefficients was separated by anion-exchange chromatography[23] into samples of increasing sedimentation coefficients as determined on 0.003% solutions. When the sedimentation data on these fractions were averaged, a calculated distribution of sedimentation coefficients was obtained which was nearly the same as that of the original unfractionated DNA. Such a result would not have been anticipated if there were severe interactions among these components or if the Johnston–Ogston effect were significant.

TABLE I[a]

	Sample	$s_{50\%}$ Separately, S	$s_{50\%}$ of components of mixture, S
Mixture I	D	5.5	5.8
	A	21.3	21.6
Mixture II	C	8.4	9.0
	B	20.4	20.5

[a] $s_{50\%}$ is the average sedimentation coefficient estimated from the 0.5 intercepts for the curves on the unmixed samples and from the mid-points of the curves for the fast and slow components of the mixtures in Figs. 1 and 2. Samples D and C were obtained from sample A (calf thymus) by exposure to sonic vibrations. Sample B was obtained from human leucocytes.

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