SEDIMENTATION STUDY OF SODIUM DEOXYPENTOSENUCLEATE PREPARATIONS FROM HERRING SPERM AND CALF THYMUS

by

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Velocity ultracentrifuge has been one of the most powerful agents in characterizing sodium deoxypentosenucleate (DNA) in solution[1–8]. Previous sedimentation measurements have been concerned mainly with calf thymus DNA. In this laboratory, studies on preparation and properties of herring sperm DNA have been carried out[9], and the results of the sedimentation measurements of some of the preparations which have been known to have different degrees of polymerization are presented in the present report. Data on a preparation from calf thymus are also included. Viscosity of these preparations was also measured using capillary viscometers of relatively low velocity gradient. From the results of these measurements the configuration of DNA molecule in solution is discussed.

Most reported values of the sedimentation coefficient at zero concentration (s_0) lie between 12–15 S (Svedberg unit) and the molecular weight calculated by combining it with diffusion data is definitely lower than that recently obtained from light-scattering experiments[10–14]. In this study, however, one of the herring DNA samples and the calf thymus preparation were found to have s_0 value of 20 S or more, and it seems highly probable that the previous sedimentation studies were carried out on preparations which had suffered some degree of degradation. After the completion of the experiments described here, a paper by PEACOCKE AND SCHACHMAN[8] appeared; they also found high s_0 value for calf thymus DNA, in agreement with our results.

MATERIALS AND METHODS

DNA. (i) Herring sperm DNA. Soft roes of herring (*Clupea pallasii*) were extracted with 2 *M* sodium chloride* and DNA was precipitated from the clarified extract by adding ethanol. The precipitate was purified by treatment with chloroform-octanol and reprecipitation by ethanol. Details of preparation have been reported previously[9]. In this study five batches of DNA, H-1b, -6a, -7a, -8a, and -9a were measured. H-1b, -6a, and -9a were prepared from soft roes stored frozen for not less than several weeks. H-7a was obtained from salted herrings on sale at a fish store. H-8a was obtained from the same batch of soft roes as H-9a but the preparation was initiated about a week after the harvest. These preparations were stored in a desiccator over calcium-chloride. Electrophoretic homogeneity has been found to be good, in almost every case[16].

(ii) Calf thymus DNA. The sample, T-2a, was prepared by a method essentially similar to that of SIGNER AND SCHWANDER[17]. Thymus glands were frozen with solid carbon dioxide soon

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* H-1b was prepared by extraction of soft roes with 1 *M* NaCl. Nucleoclupein was found to be incompletely extractable with 1 *M* NaCl. 2 *M* NaCl was used in later experiments since it was found to be more suitable[15].

after they were excised, and preparation was initiated the next day. The purified DNA sample was stored over saturated sodium chloride solution in a cold room.

(iii) Analysis. H-8a, -9a and T-2a were analyzed for nitrogen by micro-Kjeldahl method and for phosphorus by KING's method[18] after prolonged dialysis against distilled water followed by lyophilization. The results agreed well with each other, giving N 15.6%, P 9.1%, N/P 1.71.

Methods. Sedimentation velocity was measured by a Spinco model E ultracentrifuge, equipped with a Philpot-Svensson schlieren cylindrical lens system. Speed of the rotor was set always at 59,780 r.p.m. Observed sedimentation coefficient was corrected to that in water at 20°C ($s_{20,w}$) in a conventional manner[19]. The reported lowering of the rotor temperature at high speed[20] was not taken into account.

Viscosity was measured at 20°C with two capillary viscometers with coiled capillaries of about 0.5 mm in diameter and 120 cm in length[21]. The average velocity gradients are fairly low, namely 80 and 95 sec^{-1} for water at 20°C.

Concentration of DNA in a solution was determined by its maximum ultraviolet absorption measured with a Beckman model DU spectrophotometer, after appropriate dilution with the solvent. The ratio of ultraviolet extinction coefficient at 259 mμ to nitrogen content of the solution (mg/ml) was found to be 125 on the average, which is in good agreement with the value by FRICK[22].

RESULTS

Sedimentation velocity

Sedimentation coefficients were measured in a concentration range between about 0.5% and 0.02%, in sodium phosphate buffer of ionic strength 0.2 at pH 7.7 (herring sperm DNA) or in 0.2 *M* sodium chloride (calf thymus DNA). Primary charge effect on sedimentation rate will be absent in these solvents[7]. Typical sedimentation patterns are shown in Fig. 1. No peak other than that of DNA was found in all cases and the "insoluble fraction"[23] was not detectable in any appreciable amount.

At higher concentrations, the sedimentation boundary was very sharp, but the degree of sharpness varied from preparation to preparation (see Fig. 1). H-8a and T-2a showed hypersharp peaks, H-1b and -6a slight broadening with the progress of sedimentation, and H-9a was intermediate between them. Boundary of H-7a at high concentration was markedly abnormal in shape, being steeper in the advancing side (Fig. 1e); the same kind of anomaly was seen also with H-1b and -6a at higher concentrations though far less clearly. At low concentrations boundary spreading was evident, especially for low molecular weight samples, and this limited the lowest

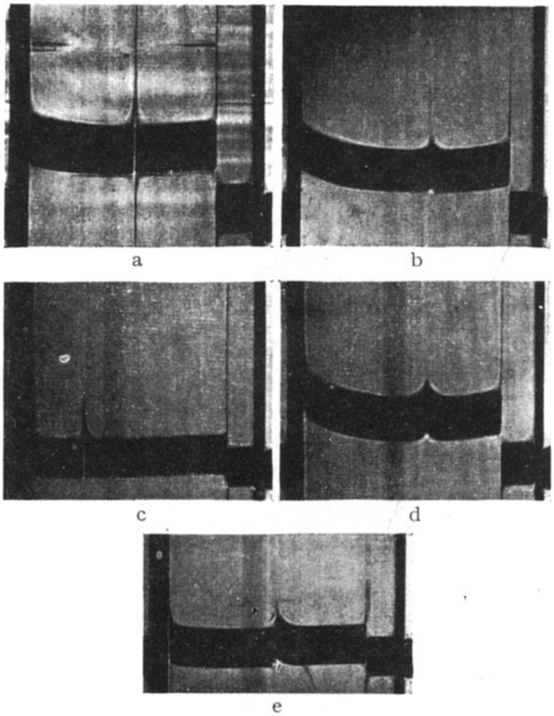

Fig. 1. Typical sedimentation patterns. (a) T-2a, 0.33%, bar angle 50°; (b) H-8a, 0.028%, 60°; (c) H-6a, 0.38%, 70°; (d) H-6a, 0.074%, 60°; (e) H-7a, 0.30%, 70°. The patterns of T-2a and H-8a, and of H-6a and H-1b, are very similar with each other.

concentration which could be measured with any accuracy. Boundary spreading of H-7a at a low concentration was much more rapid than other preparations and high accuracy of sedimentation coefficient was not obtained for this sample.

In Fig. 2 are shown the sedimentation coefficients ($s_{20, w}$) of the six preparations plotted against concentration. Sedimentation coefficient was found to differ markedly from preparation to preparation. It also changed greatly with concentration, especially in the dilute region. Table I shows values of $s_{20, w}$ of two preparations at low concentrations for which concentration dependence was most pronounced. The higher the sedimentation coefficient at infinite dilution (s_0), the steeper was the initial slope of the $s_{20, w}$ *vs.* c curve, and the curves crossed with each other at some medium concentration. At a high concentration the sedimentation coefficient of a high molecular weight sample (high s_0) was slightly smaller than that of a low molecular weight sample.

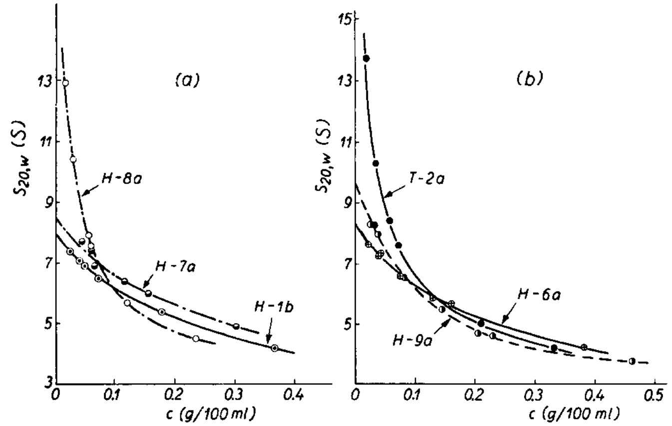

Fig. 2. Variation of $s_{20, w}$ with concentration. Concentration is corrected for the dilution due to the sector shape of the cell and to the changing field.

TABLE I

SEDIMENTATION COEFFICIENTS OF H-8a AND T-2a AT CONCENTRATIONS BELOW 0.1%

Sample	Concn. (g/100 ml)	$s_{22, w}$ (S)
H-8a	0.059	7.6
(in phosphate buffer,	0.055	7.9
pH 7.7, μ 0.2)	0.028	10.4
	0.014	12.9
T-2a	0.071	7.6
(in 0.2 *M* NaCl)	0.055	8.4
	0.034	10.3
	0.018	13.7
	0.009	(17)

Most of the data were collected at temperatures between 20 and 30°C but a few measurements were made at 10–15°C. They fall on a smooth curve and the temperature variation of $s_{20, w}$ reported by KOENIG AND PERRINGS[5] was not significant.

Viscosity

Results of viscosity measurements are shown in Fig. 3 in which viscosity number (specific viscosity divided by concentration, η_{sp}/c) is plotted against concentration. The concentration range covered was varied with preparation so that the values of viscosity ratio (ratio of solution viscosity to solvent viscosity) of the solutions fall between 1.05 and 2.5. Limiting viscosity numbers, $[\eta]$, obtained by extrapolation of the lines of Fig. 3 are listed in Table II.

In cases of the most viscous samples H-8a and T-2a, dependence of viscosity upon velocity gradient was evident since the two viscometers used gave slightly different values; the specific viscosity determined with the lower gradient viscometer was consistently higher by 3–4% than that with the higher gradient one. It was found that the viscosity values reported by Reichmann *et al.*[14] at a similar velocity gradient are very near to the values for H-8a and T-2a. If the relation of viscosity ratio *vs.* velocity gradient reported by Reichmann *et al.* is assumed to apply also to these preparations, the limiting viscosity number at zero gradient will be about 20% higher than that of Table II. Values obtained for the other preparations will be equal, without serious error, to the ones at zero gradient.

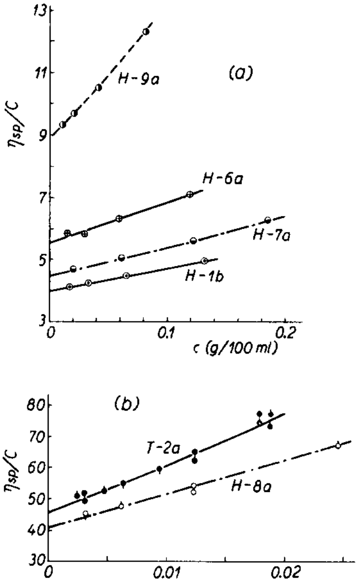

Fig. 3. Viscosity number (η_{sp}/c) plotted against concentration. In (b), the points with a short line upward are obtained by the higher velocity gradient viscometer, and those with a short line downward by the lower gradient viscometer.

Extrapolation of sedimentation coefficient to zero concentration

Since the sedimentation coefficient varies markedly in the dilute concentration range, a suitable method of extrapolation was necessary. The plot of $1/s_{20,w}$ against concentration (Fig. 4) gives curves concave downward when taken over the whole concentration range measured. At low concentrations, the curves become nearly straight, but they do not seem adequate for extrapolation in cases of T-2a, H-8a and -9a. When $s_{20,w}$ is multiplied by the solution viscosity[24], resulting curves are strongly concave upward just as was described by Jullander[25] for nitrocellulose samples, though this plot has the advantage of giving an upper limit to the sedimentation

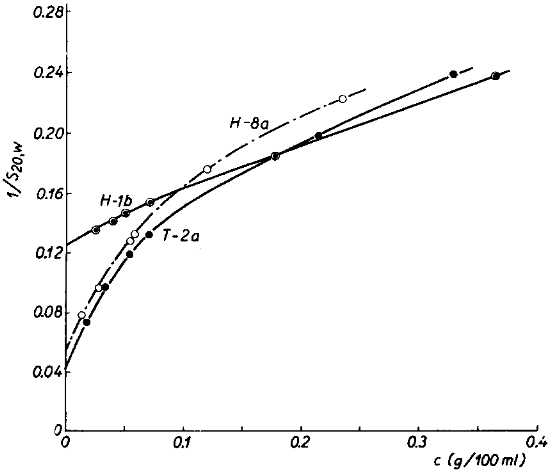

Fig. 4. $1/s_{20,w}$ *vs.* *c* for H-1b, H-8a and T-2a.

coefficient at zero concentration. However, when $s_{20,w}$ is multiplied by $\eta' = 1 + [\eta]c$, where $[\eta]$ is the limiting viscosity number and c the concentration, the plot of $\eta' \times s_{20,w}$ vs. c gives fairly straight lines* as can be seen in Fig. 5. Sedimentation coefficients at zero concentration, s_0, were determined principally in this manner, taking also the $1/s_{20,w}$ plot into consideration. They are listed in Table II.

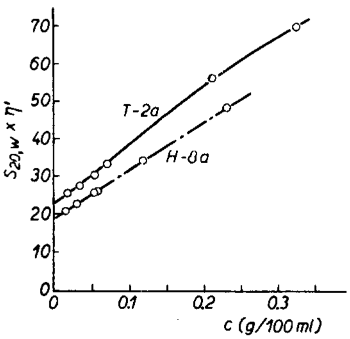

Fig. 5. Extrapolation of the sedimentation coefficients of H-8a and T-2a by plotting $s_{20,w} \times \eta' = s_{20,w}(1 + [\eta]c)$ against c.

In Table II are also listed the values of K_s in the equation

$$\frac{s_0}{s_{20,w}} = 1 + K_s c + A c^2 + \ldots$$

which were estimated from the initial slope of the $1/s_{20,w}$ vs. c plot (Fig. 4). The ratio $K_s/[\eta]$ shown in the last column of the Table II was found to be fairly constant, averaging to about 0.8.

Recently a few measurements have been made to compare the sedimentation coefficient of a given preparation in 0.2 M NaCl and in the phosphate buffer, and an unexpected difference of about 10% was found. Since the viscosities of H-8a as well as T-2a coincide exactly in these two solvents, this discrepancy seems to be primarily due to the secondary charge effect[19] which acts as a retarding force in sodium phosphate buffer. This point is now being examined in some detail.

TABLE II

SEDIMENTATION COEFFICIENT AT ZERO CONCENTRATION (s_0), LIMITING VISCOSITY NUMBER ($[\eta]$), MOLECULAR WEIGHT (M_r AND M_c), K_s, AND $K_s/[\eta]$

Sample	s_0 (*S*)	$[\eta]$ (*dl/g*)	M_r (*10⁶*)	M_c (*10⁶*)	K_s (*dl/g*)	$K_s/[\eta]$
H-1b	8.0	4.0	0.39	0.57	3.4	0.85
H-6a	8.4	5.6	0.47	0.73	4.1	0.73
H-7a	8.6	4.5	0.45	0.68	3.7	0.82
H-9a	10.0	8.9	0.75	1.19	8.4	0.94
H-8a	19	41	3.7	6.7	34	0.83
T-2a	23	46	5.2	9.4	42	0.91

Note: Values of s_0 for herring sperm samples are probably about 10% smaller than true ones because of the secondary charge effect. Limiting viscosity numbers for H-8a and T-2a will probably be about 20% higher at zero velocity gradient than the values listed above. Molecular weights and $K_s/[\eta]$ were calculated without correcting for these factors.

Estimation of molecular weight

Though molecular weight cannot definitely be determined by sedimentation constant s_0 and limiting viscosity number $[\eta]$, it will be pertinent to estimate it using some model for DNA molecule. Calculation was performed by the following two methods.

(1) An unhydrated, rigid prolate ellipsoid molecule was assumed and its axial

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* This finding is coincident with the mode of expression adopted by KOENIG AND PERRINGS[5]

$$s_{20,w} = \frac{s_0 + Bc}{1 + Dc}$$

provided the constant D equals to $[\eta]$.

ratio was computed from the limiting viscosity number by SIMHA's equation[26]. Frictional ratio f/f_0 was then obtained by PERRIN's equation [27] and the molecular weight was calculated by the following equation

$$M_r = \sqrt{162}\,\pi N \left[\frac{s_0 (f/f_0) V^{1/3} \eta_0}{1 - V \varrho_0} \right]^{3/2} \qquad (1)$$

where N is the Avogadro's number, V the partial specific volume, and η_0 and ϱ_0 are the viscosity and density of the solvent, respectively.

(2) A random coil model was assumed and MANDELKERN-FLORY equation[28] was applied.

$$M_c = \left[\frac{s_0 [\eta]^{1/3} \eta_0 N}{2.5 \cdot 10^6 \, (1 - V \varrho_0)} \right]^{3/2} \qquad (2)$$

The results are shown in Table II. Partial specific volume was assumed to be 0.55[1].

DISCUSSION

Large differences exist between the sedimentation constants as well as the viscosity numbers of different preparations, in sharp contrast to the electrophoretic mobility which varies little from sample to sample[8, 16]. As was described in the section "MATERIALS", preparations H-1b, -6a, -7a and -9a were obtained from soft roes which were not very fresh, while H-8a and T-2a were purified from materials as fresh as possible. Since the method of preparation of these samples was essentially the same, the differences in s_0 and $[\eta]$ are probably due primarily to the difference in molecular weight of DNA in the starting material. The low s_0 values obtained from the samples prepared from stored soft herring roes are therefore indicative of some degradation of DNA during preservation of soft roes*.

On the other hand, the sedimentation constants (s_0) of H-8a and T-2a exceed all values hitherto reported (12–15 S)** except that of PEACOCKE AND SCHACHMAN[8]. Since H-8a and T-2a were obtained from fresh sources, they are apparently in the least degraded state among the preparations studied here. Our recent measurements on DNA obtained from fresh sperm of rainbow trout showed sedimentation coefficients and viscosity numbers very similar to T-2a. Thus it is highly probable that most of the preparations studied by previous investigators had suffered some degradation. Although the possibility that the preparations with high s_0 are products of unnatural aggregation may not be definitely excluded at present, it can be concluded that the preparations with a sedimentation constant of 20 S or higher are nearer to the native state than the one with s_0 of 12–15 S.

It must be noted here that a fairly long period of time had elapsed after the preparation of herring DNA samples when the measurements reported here were done, and some change of their properties by aging was possible. H-1b, the oldest sample, was prepared about four years before the first measurements in this study and H-9a,

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* This conclusion does not exclude the possibility of any degradation of DNA during purification. It is shown by ZAMENHOF *et al.*[29] that injured DNA is more sensitive to physical and chemical agents than uninjured. Also our unpublished experiments showed that viscosities of low molecular weight samples H-1b and H-9a were more sensitive to change of pH than H-8a or T-2a. Thus, it is possible that degraded DNA would more readily suffer further deterioration during purification.

** One of the preparations measured by TENNENT AND VILBRANDT (STN-1)[1] has similarly high sedimentation coefficient but no extrapolated value was reported.

the newest among the herring DNA samples, about a year and a half before. However, the effect of aging of DNA on the physical properties studied here seems to be negligible, since the sedimentation coefficients and viscosity numbers of T-2a soon after preparation and after about a year and, furthermore, of the solution before the final ethanol precipitation of T-2a, were found to fall on identical curves. With herring preparations no detectable variation of sedimentation coefficient was observed during the period of the experiments covering more than a year. In addition, results of the previous measurements of viscosity and diffusion on "H-I" and "H-VI" which were done not so long after their preparation[9] are consistent in general with the present results (H-1b and H-6a are derived from H-I and H-VI, respectively). Thus the fact that the measurements have been made with preparations of different degrees of aging does not affect the conclusion mentioned in the preceding paragraphs.

DOTY and others[10–14] reported that light-scattering measurements on DNA prepared very carefully from fresh materials yield molecular weight value (about $6 \cdot 10^6$) which is definitely higher than the values determined by sedimentation and diffusion (of the order of $1 \cdot 10^6$). It must be remembered that the latter values were based on definitely lower s_0 values than those obtained by PEACOCKE AND SCHACHMAN[8] who measured a sample offered by DOTY, and also than those for H-8a and T-2a. The close similarity between the latter two preparations and those studied by REICHMANN *et al*[14] is evident from the similarity of their viscosities as noted previously. Thus one of the most important reasons of the discrepancy between the value of molecular weight from sedimentation-diffusion and that from light-scattering lies probably in that the previous sedimentation values refer to less polymerized samples than those used in the light-scattering experiments. Several arguments invoked in an attempt to explain the discrepancy, such as the effect of impurity contributing much scattering, heavily weighted nature of the light-scattering molecular weight, or the failure of elimination of intermolecular interactions in the determination of s_0, must therefore be critically reexamined.

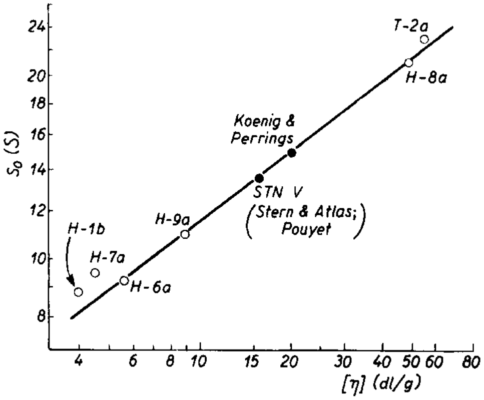

Fig. 6. Relation between log s_0 and log $[\eta]$. (s_0 of herring DNA was corrected for the secondary charge effect (1.10 times the observed value.); $[\eta]$ of H–8a and T–2a were assumed to be 20% higher than those listed in Table II). Data by STERN AND ATLAS[7] and POUYET[40] on "STN V" and by KOENIG AND PERRINGS[5] are also included.

The two sets of values of molecular weight listed in Table II are only of relative significance since the molecular configuration of DNA in solution has not yet been elucidated. The thin, rigid rod model has been widely accepted without fully convincing evidence for it. DEKKER AND SCHACHMAN[30] have presented some evidence in favor of considerable stiffness of the DNA molecule, and suggested that s_0 will change little on change of $[\eta]$. To test this point, log s_0 was plotted against log $[\eta]$ in Fig. 6. The data by KOENIG AND PERRINGS[5] is included as well as that on "STN V" studied by STERN AND ATLAS[7] and by POUYET[30]. The points fall on a nearly straight line, and the tendency for the curve to become horizontal cannot be seen. The slope of this line (0.38) is definitely larger than the theoretical value (0.123) for prolate ellipsoids of very large axial ratio with constant minor axis*. Thus such a model cannot be adequate for these preparations. In addition, if the length of the minor axis is calculated for our preparations by the Perrin and the Simha relationships, it shows variation well beyond the experimental error[8] from 25 A for the high s_0 sample to 17 A for the low s_0 sample, though these figures are in broad agreement with the diameter of the two-stranded model of DNA[31]. Since the ultraviolet absorption of all our preparations was found to show normal increase in an alkaline medium, the characteristic internal structure of DNA is possibly well reserved. The results of calculation of the diameter are then contrary to the expectation that the polynucleotide chain should have constant diameter irrespective of molecular weight. Thus the rough agreement of the diameter calculated on the stiff rod model with that expected from the chemical structure of polynucleotide chain is only fortuitous.

A rod model has become much less convincing also from the results of light-scattering experiments and random coil (or stiff coil) model is reported to be more consistent. If, then, a relation of the type of MANDELKERN AND FLORY holds between the molecular weight M, s^0 and $[\eta]$, namely,

$$M \propto (s_0[\eta]^{1/3})^{3/2} \tag{7}$$

and if the modified Staudinger rule, familiar in the linear polymer field,

$$[\eta] \propto M^a \tag{8}$$

is assumed to hold, the following relation is readily derived.

$$\log s_0 = \frac{1}{3}\left(\frac{2}{a} - 1\right) \log [\eta] + \text{const.} \tag{9}$$

The exponent a of eqn. (8) can thus be determined from the slope of log s_0 and log $[\eta]$ plot, without knowledge of molecular weights. From the plot in Fig. 6, a is found to be 0.93. This figure is slightly higher than that found for randomly coiled polymers (between 0.50 and 0.80)[33], and in notable agreement with that found for cellulose derivatives[34]. Thus, if one accepts the coil model as well as the validity of eqns. (7) and (8), it is concluded that the coil is fairly stiff, in agreement with the deduction from the light-scattering experiments[14].

Besides the experimental difficulty in accurate determination of s_0, the above discussions suffer some uncertainly since the values of s_0 and $[\eta]$ refer to unfractionated samples. However, the possible polydispersity of them (see below) will not so seriously affect the relation between s_0 and $[\eta]$ that it is necessary to revise the qualitative conclusions mentioned above.

The presence of a wide variety of molecular species in a DNA preparation has recently been shown by chromatographic separation and other techniques[35–37] but

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* This can be derived as follows: For prolate ellipsoids of very large axial ratio (a/b), SIMHA's and PERRIN's equations can be approximated by [32]

$$[\eta] \propto (a/b)^{1.732} \tag{3}$$

$$f/f_0 \propto (a/b)^{0.454} \tag{4}$$

If the minor axis is constant, the molecular weight is proportional to a/b,

$$M \propto (a/b) \tag{5}$$

Combining eqns. (1), (3), (4) and (5), we find

$$\log s_0 = 0.123 \log [\eta] + \text{const.} \tag{6}$$

little is known about the heterogeneity with respect to molecular weight. Sedimentation pattern is, as a guide of the heterogeneity, not so useful for DNA as for protein solutions because, as was pointed out by PEACOCKE AND SCHACHMAN[8], sedimentation rate is relatively insensitive to changes in molecular weight and boundary sharpening effect predominates. However, it should be pointed out that the sedimentation patterns for H-7a and, to a much lesser extent, H-1b and -6a, have some resemblance to those reported by PEACOCKE AND SCHACHMAN for a mixture of sonic degraded and undegraded DNA. This, together with the marked boundary spreading at low concentrations strongly suggests that these lower molecular weight samples are considerably heterogeneous. On the other hand, for the highest molecular weight samples, boundary spreading which may serve as a measure of heterogeneity occurs only when the concentration is too low for a quantitative treatment to be justified, and much effort will be necessary to gain information about the degree of homogeneity of such preparations.

The problem of heterogeneity of a DNA preparation has another aspect; it is the presence or absence of rapidly sedimenting component such as reported by CECIL AND OGSTON[2] and by BENDICH *et al*[23]. If such components were present in our preparations, the concentration scale of the $s_{20,w}$ *vs.* c relation as well as the value of $K_s/[\eta]$ had to be changed, as the solutions used for measurements had not been subjected to preliminary centrifugation; the actual concentration at which sedimentation is being measured would be lower than the concentration of the original solution, and the viscosity number of the solution the sedimentation of which was measured might be less than the value described here. However, rapidly sedimenting components have not been observed in our sedimentation measurements. Furthermore, in a preliminary experiment in which solutions of T-2a and H-1b were spun in an angle rotor at about 20,000 times gravity for one hour, it was found that the viscosity numbers of the supernatants did not change, and the amount of sedimented material was not greater than was expected from the optically observed sedimentation rate. Thus the presence of a rapidly sedimenting component is excluded in our case.

The values of $K_s/[\eta]$ in Table II can therefore be considered as true, and their constancy suggests a close relationship between the concentration dependence of sedimentation coefficient and the limiting viscosity number as was also found for many linear polymers[38, 39].

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SUMMARY

Sedimentation coefficients $s_{20,w}$ of five preparations of sodium deoxypentosenucleate (DNA) purified from herring sperm and one from calf thymus were measured by a Spinco ultracentrifuge at concentrations below 0.5 %. Limiting viscosity numbers $[\eta]$ of these preparations were determined using capillary viscometers of relatively low velocity gradient.

Marked variation of $s_{20,w}$ and viscosity from preparation to preparation was observed. Sedimentation coefficients at zero concentration, s_0 (in Svedbergs), were found to be 8.0, 8.4, 8.6, 10.0 and 19 for herring sperm DNA in sodium phosphate buffer of ionic strength 0.2 at pH 7.7, and 23 for calf thymus preparation in 0.2 M sodium chloride (s_0 values of herring DNA preparations are probably about 10 % lower than true ones because of the secondary charge effect).

Among the herring DNA samples, four were obtained from soft roes stored frozen for not less than several weeks, and one from fresh material. The calf thymus preparation was obtained from very fresh material. The latter two preparations, which will be in the least degraded state, possess the highest s_0 values, which exceed all values hitherto reported for DNA (12–15*S*) except those of PEACOCKE AND SCHACHMAN.

Plot of log s_0 *vs.* log [η] gives a straight line with a slope of 0.38. This is definitely higher than the slope expected for unhydrated, rigid, prolate ellipsoids of revolution with constant minor axis, and therefore such a model is inadequate for DNA. If, instead, a coil model is assumed, it should be fairly stiff judging from the value of the exponent of the modified Staudinger rule, estimated to be 0.9 from the slope of log s_0 *vs.* log [η] plot.

The samples with lower s_0 and [η] are probably considerably heterogeneous with respect to molecular weight, judging from the sedimentation patterns. Rapidly sedimenting "gel component" or "insoluble fraction" was not detected in any case.

When $1/s_{20,w}$ is plotted against concentration, the resulting curve is concave toward the concentration axis. The ratio of K_s, a constant characterizing the slope of this curve at infinite dilution, to [η] was found to be fairly constant with these preparations, averaging about 0.8.

RÉSUMÉ

Les coefficients de sédimentation $s_{20,w}$ de cinq préparations de désoxypentosenucléate de sodium (DNA) purifiés à partir du sperme de harreng et d'une préparation purifiée à partir du thymus de veau ont été mesurés dans une ultracentrifugeuse Spinco à des concentrations inférieures à 0.5 %. Les nombres de viscosité limite [η] de ces préparations ont été déterminés à l'aide de viscosimètres capillaires de gradient de vélocité relativement bas.

Des variations importantes du $s_{20,w}$ et de la viscosité ont été observées d'une préparation à l'autre. Les coefficients de sédimentation à la concentration zéro, s_0 (en unités Svedberg) sont de 8.0; 8.4; 8.6; 10.0 et 19 pour le DNA du sperme de harreng en tampon phosphate de sodium de force ionique 0.2 et de pH 7.7, et de 23 pour la préparation du thymus de veau dans le chlorure de sodium 0.2 *M* (les valeurs de s_0 pour le DNA de harreng sont probablement d'environ 10% trop faibles en raison de l'effet secondaire de charge).

Parmi les échantillons de DNA de harreng, quatre ont été obtenus à partir de laitances conservées à l'état congelé pendant au moins plusieurs semaines, et une à partir d'un matériel frais. La préparation de thymus de veau a été obtenue à partir d'un matériel très frais. Les deux dernières préparations, qui doivent être dans l'état le moins dégradé, possèdent les valeurs les plus élevées de s_0, valeurs qui dépassent toutes les valeurs publiées jusqu'à présent pour le DNA (12–15*S*), à l'exception de celles de PEACOCKE ET SCHACHMAN.

En portant log s_0 en fonction de log [η], on obtient une droite de pente 0.38. Cette pente est sensiblement plus élevée que celle qu'on attendrait pour des ellipsoïdes allongées de révolution à petit axe constant, non-hydratées, rigides, et, par conséquent, un tel modèle ne rend pas compte des propriétés du DNA. Si, au contraire, on suppose un modèle enroulé, il faudrait qu'il soit très rigide étant donnée la valeur de l'exposant dans la formule de Staudinger modifiée, valeur estimée à 0.9 à partir de la pente de la droite reliant log s_0 à log [η].

Les échantillons qui possèdent les s_0 et les [η] les plus faibles sont probablement très hétérogènes du point de vue poids moléculaire, si l'on en juge par les diagrammes de sédimentation. Dans aucun cas les auteurs n'ont décelé de "constituant géliforme" ou de "fraction insoluble" à sédimentation rapide.

Quand on porte $1/s_{20,w}$ en fonction de la concentration, la courbe résultante est concave vers l'axe des concentrations. Le rapport entre K_s, une constante caractérisant la pente de cette courbe à dilution infinie, et [η] est pratiquement constant pour ces préparations, sa valeur moyenne étant de 0.8.

ZUSAMMENFASSUNG

Die Sedimentierungskoeffizienten $s_{20,w}$ von Natriumdesoxypentosenukleat-(DNS)-präparaten wurden mit Hilfe einer Spinco-Ultrazentrifuge in Konzentrationen unter 0.5 % gemessen; eines dieser gereinigten Präparate stammte aus Kalbthymus, fünf andere aus Heringsperma. Die limitierenden Viskositätszahlen [η] dieser Präparate wurden an Hand von kapillaren Viskosimetern mit verhältnismässig geringem Geschwindigkeitsgradienten bestimmt.

Die Präparate zeigten sehr verschiedene Sedimentierungskoeffizienten und Viskositäten. Im Falle von Heringsperma-DNS in Natriumphosphat-Puffer, Ionenstärke 0.2 bei pH 7.7, wurden die folgenden Sedimentierungskoeffizienten bei Konzentration gleich Null, s_0 (in Svedbergeinheiten) gefunden: 8.0, 8.4, 8.6, 10.0, 19. Im Falle des Kalbthymuspräparates in Natriumchloride 0.2 *M* war der s_0-Wert 23. (Wegen des sekundären Ladungseffektes sind die gefundenen s_0-Werte für die Hering-DNS-Präparate wahrscheinlich um ungefähr 10 % zu niedrig.)

Vier der Hering-DNS-Muster wurden aus während mindestens einige Wochen lang in gefrorenem Zustande aufgehobener Fischmilch gewonnen. Ein Muster stammte aus frischem Material. Das Kalbthymuspräparat wurde aus sehr frischem Material hergestellt. Die letzteren beiden Präparate, welche sich im besten Zustande befinden, weisen die höchsten s_0-Werte auf,

welche alle bis jetzt für DNS erhaltenen Werte (12–15 S), ausgenommen diejenigen von PEACOCKE UND SCHACHMAN, übertreffen.

Wenn man log s_0 im Verhältnis zu log $[\eta]$ aufträgt, erhält man eine Gerade mit einer Neigung von 0.38. Dieser Wert ist bedeutend höher als die für anhydrische, starre, längliche Revolutionsellipsoide mit konstanter Kurzachse zu erwartende Neigung, und daher ist solch ein Modell für DNS ungeeignet. Falls man statt dessen ein Spulenmodell annimmt, so sollte dieses ziemlich steif sein, wenn man sich nach dem Exponentwert der geänderten Staudingerschen Regel richtet, welcher, der Neigung der log s_0 gegenüber log $[\eta]$-Kurve gemäss, auf 0.9 geschätzt wird.

Niedrigere s_0- und $[\eta]$-Werte aufweisende Muster sind wahrscheinlich hinsichtlich des Molekulargewichtes viel heterogener, wenn man sich nach dem Sedimentierungs-"pattern" richtet. In keinem Falle wurde eine schnell sedimentierende "Gelkomponente" oder "unlösliche Fraktion" entdeckt.

Falls man $1/s_{20,w}$ im Verhältnis zu der Konzentration aufträgt, erhält man eine, zur Konzentrationsachse hin, konkave Kurve. Der Quotient von K_s (eine bei unendlicher Verdünnung für die Neigung dieser Kurve charakteristische Konstante) und $[\eta]$ war bei diesen Präparaten ziemlich beständig, und betrug durchschnittlich ungefähr 0.8.

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