VISCOSITY BEHAVIOR OF NON-FIBROUS DESOXYRIBONUCLEOHISTONE IN MEDIA OF DIFFERENT IONIC STRENGTHS

by

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INTRODUCTION

Since a procedure has been established for the preparation of a water soluble, non-fibrous form of desoxyribonucleohistone (DNH) from the cell nuclei of rat liver and other tissues[1], considerable interest has arisen as to the nature of this protein and how it differs from the fibrous form which has been studied for some time[2,3,4]. KUPKE, ELDREDGE AND LUCK[5] have reported on the sedimentation and electrophoretic properties of the non-fibrous DNH after purification of the protein to constant chemical composition. Comparisons of the intrinsic viscosities of the two forms were also made to indicate that the fibrous form is much more asymmetric in the media studied, although the difference is less at higher ionic strengths.

To characterize the non-fibrous protein further, the present series of viscosity studies, at different velocity gradients, made on DNH in water, 0.005 *M* NaCl, 1 *M* NaCl, and 2 *M* NaCl was undertaken. In order that the axial ratios of the highly asymmetric particles could be estimated by use of the SIMHA equation[6] it is necessary to study a given protein as a function of velocity gradient, in order that an extrapolation to zero shear rate can be carried out. Some anomalous effects, to be described, became apparent by comparing the viscosity curves for the different velocity gradients. A consideration will be made of these effects in terms of degradative and aggregation reactions which are suggested. In addition, more accurate extrapolations to obtain reduced sedimentation constants are made possible by combining this viscosity data with the sedimentation data of KUPKE *et al*[5].

EXPERIMENTAL

The type of capillary viscometer used was that described by DESREUX AND BISCHOFF[7]. The first three viscometers which were constructed were designed to give velocity gradients of approximately 200, 100, and 50 $\sec^{-1}$. The exact velocity gradients were calculated by means of the formula $dV/dr = hdgr/2\eta l$, where h is the pressure head, r is the radius, and l is the capillary length in centimeters; d is the density and g is the acceleration of gravity; η is the coefficient of viscosity in poises. The capillary radius of 0.014 cm at first employed was found to be too small for efficient operation, at the lowest velocity gradients, since the flow times were so long and capillary became so readily clogged. Therefore, a fourth viscometer was constructed for which the capillary radius

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was 0.0247 cm, designed to have about the same velocity gradient as viscometer W3. The dimensions, flow times for water, and calculated velocity gradients are summarized in Table I.

TABLE I

DESCRIPTION OF THE CAPILLARY VISCOMETERS

	Pressure head cm	*Capillary radius cm*	*Capillary length cm*	*Velocity gradient* sec^{-1}	*Flow time water sec*
1	6.1	0.014	25.4	186	1142.5
2	5.6	0.014	50.8	85	1807.4
3	3.5	0.014	50.8	53	2586.4
4	3.1	0.0247	101.6	42	471.8

The viscometers were immersed in a constant temperature water bath, regulated at 25.0° ± 0.01° C. Time measurements were made by use of a Cenco electric timer, reading to 0.01 sec, or by stopwatches which were calibrated against this timer. Precautions of course were taken to insure maximum cleanliness of the viscometers, before measuring.

All preparations of DNH studied were made according to the procedure of LUCK *et al.*[1]. For some of the studies, further terminal purification[5] of the lyophilized material was carried out, but in most cases the lyophilized powder was dissolved directly in either water or 1 *M* NaCl, then clarified by centrifuging in the Spinco Model L, head #40, at 20,000 R.P.M. for 15 minutes, and the pH adjusted to the desired value. Concentrations in mg of dry protein per ml were determined by phosphorus (KING-CARPENTER method) analyses or the U.V. absorption at 260 millicrons, the results of which were referred back to dry weight determinations. The extinction coefficients and analytical results obtained for the preparations used are given in Table II. It should be especially noted that when the lyophilized material is initially dissolved in 1 *M* NaCl rather than water a higher phosphorus content (4.5 %) is observed.

TABLE II

COMPOSITION AND ULTRA-VIOLET ABSORPTION OF VARIOUS SOLUTIONS OBTAINED FROM SEVERAL DESOXYNUCLEOHISTONE PREPARATIONS

Prepn. No.	*Solvent*	*Treatment*	*Ext. coeff.* $\frac{O.D.}{mg\ P/m}$	*Lyoph wt mg*	*Dry wt mg*	*%P*	*%N*	*N/P*
I		Lyophilized material heat dried		33.5	22.8	4.29	16.1	3.76
I	1 *M* NaCl	Lyophilized material treated with 1 *M* NaCl and clarified	225					3.77
I	Water	Same; salt dialyzed off	258		3.71	4.66	17.4	3.69
II	Water	Lyophilized material treated with water, residue extracted with 1 *M* NaCl, dialyzed, pooled, clarified	258		1.92	4.08*	16.9	4.15
I	Water	Terminally purified: water solution dialyzed and clarified		13.1	8.19	3.67**		
III	Water	Terminally purified	251	6.6	2.68	3.65**		
A	Water (D.W.K.)	Terminally purified				4.08	16.4	4.01

* The procedure for preparations II and III had been modified slightly from that used in preparations A and I (1), to be described in a future publication. Accordingly, the solubility properties were somewhat altered, *i.e.* a larger residue was observed from the initial water treatment, which could be almost completely dissolved by a subsequent treatment with 1 *M* NaCl.

** A somewhat lower concentration of protein during the final dialysis was employed than by D.W.K., with the result that considerable precipitation occurred during this dialysis, causing a drop in the phosphorus composition.

For the most extensive viscosity studies, at different velocity gradients, on DNH in 1 *M* NaCl, the stock solution used was prepared by dissolving the lyophilized material in 1 *M* NaCl, clarifying, and adjusting to pH 5.4. This pH was chosen, as it is at this pH that the ultracentrifuge photographs of KUPKE show that the smallest diffuse peak near the meniscus (the histone-rich component), and it was desired to study the principle component only. In addition, further viscosity determinations were carried out at a single velocity gradient, on DNH preparations which had been further purified to various extents, in order to determine the effect of purification. For the partial repurification, the lyophilized powder was treated first with water rather than 1 *M* NaCl, the resulting suspension then clarified in the Spinco L. Sodium chloride was added to the supernate to give a molar solution, and the pH adjusted to 6.5. For the complete terminal purification[5], the procedure was the same as just described, only in addition a dialysis at 4° C against 18 l of water was carried out, followed by a second clarification in the Spinco L, and then addition of salt.

Some separations of light and heavy components existing in 1 *M* NaCl solutions of DNH were made by means of prolonged runs in the Spinco Model L preparative centrifuge. Solutions at three different total concentrations, and two pH's, 5.4 and 6.5, were studied. The supernate and pellet fractions were each analyzed for nitrogen and phosphorus, and the U.V. absorptions at 260 millimicrons were taken. Wherever possible, the intrinsic viscosities and sedimentation constants were also determined. For run I, a solution at pH 5.4 was taken which had been used for viscosity measurement, and centrifuged in head #40 for 200 min at 40,000 R.P.M. To test whether all of the heavy components had been sedimented, the supernate was run again for three hours and the resulting supernate analyzed. Two more solutions of different concentrations were then run together for 24 hours. A surprising phenomenon was observed in the centrifugation of the 0.48% solution: the material in the large, hard pellet formed was found to be almost completely insoluble in 1 *M* NaCl, and could only be dispersed by the addition of one tenth volume of saturated NaOH. It was therefore not possible to determine the intrinsic viscosity or the sedimentation constant for this fraction.

RESULTS

On comparing the curves of specific viscosity/c versus concentration for water and 0.005 *M* NaCl, shown in Fig. 1, with those obtained for DNH in 1 *M* NaCl at pH 5.4 and 6.5 (Fig. 2), it will be seen at once that all solutions of DNH in 1 *M* NaCl show much higher values of η_{sp}/c than for lower ionic strengths and the values of intrinsic viscosity are about four times as great. In addition, it is interesting to note the way in which viscosity varies with velocity gradient for the case of both 0.005 *M* NaCl and 1 *M* NaCl. For DNH in 0.005 *M* NaCl the viscosity increment curves are parallel and displaced upward for decreasing velocity gradients. This is the reverse of the situation expected when particle orientation alone is considered, as the viscosity in this case decreases with increasing shear gradient. The same sort of viscosity behavior is observed for DNH in 1 *M* NaCl in the higher concentration range, but when the protein concentration is reduced below 0.15%, a second unexpected effect makes its appearance. A sharp rise in magnitude of η_{sp}/c occurs in this low concentration range, indicating the formation of particles of greater asymmetry on dilution. Moreover, the curves are seen to cross each other, so

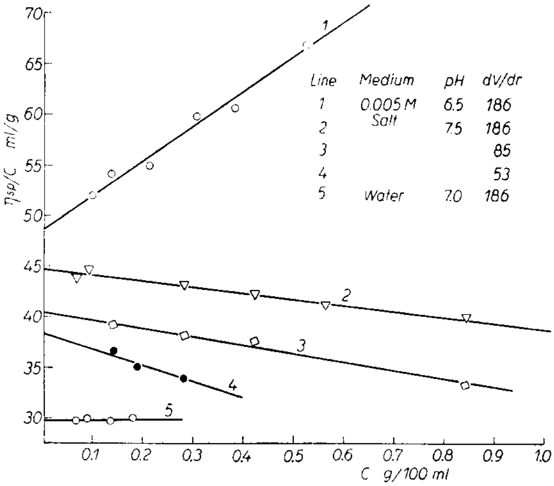

Fig. 1. Viscosity of solutions of non-fibrous DNH in media of low ionic strength.

that the values of intrinsic viscosity obtained by extrapolation vary with velocity gradient as expected by the effect of particle orientation along the stream lines.

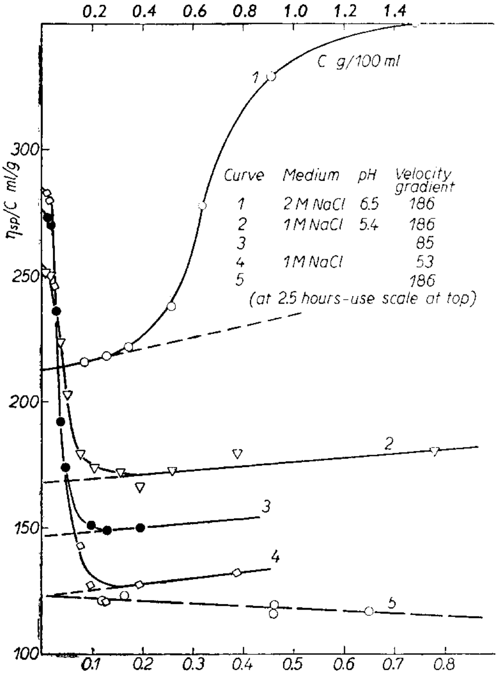

Fig. 2. Viscosity of solutions of non-fibrous DNH in media of high ionic strength. Variation of η_{sp}/c with velocity gradient shown.

When the values of intrinsic viscosity for DNH in 0.005 M NaCl, obtained by extrapolation of the straight lines (Fig. 1), are plotted as a function of the velocity gradient, a straight line is obtained which extrapolates to 36.2. Using 0.642 as the value of partial specific volume for non-fibrous DNH*, the reduced volume fraction intrinsic viscosity (ν_0) becomes 56.4, which corresponds to an axial ratio (b/a) of 25.9 by the SIMHA equation. One intrinsic viscosity determination was also made for DNH in water (pH 7.0) at 186 sec^{-1}, the line obtained being shown in Fig. 1. Since in this case determinations at other velocity gradients were not made, it is not possible to obtain a reduced intrinsic viscosity directly. However, if the effect of particle orientation is considered to be operative solely, a reduced intrinsic viscosity may be obtained by use of the SIMHA equation and a set of corrected curves for the various velocity gradients drawn up from results obtained on a number of protein preparations with varying asymmetries, each studied as a function of velocity gradient**. An axial ratio of 24.5 for DNH in water is in this way determined. The value is seen to agree closely with that obtained with DNH in 0.005 M NaCl, and with the value of 25 found by STEINER[8] for the non-fibrous form of DNH extracted from calf thymus gland by a procedure involving the use of solvents of low ionic strength only.

The axial ratios thus calculated do not necessarily describe single molecular species, since, especially at higher ionic strengths, several components are always present. The calculated axial ratios are, however, weighted square averages of the asymmetries of the components present.

In 1 M NaCl, in the concentration range above 0.15%, the curves level out and are reasonably close to straight lines which may be extrapolated to zero concentration. When this is done for the determination at pH 5.4, a reduced intrinisic viscosity may again be determined by plotting the intrinsic viscosities thus obtained as a function of velocity gradient and extrapolating to zero. The value of ν_0 obtained in this manner,

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* The partial specific volume was determined by Dr. N. T. ELDREDGE of this laboratory.

** The protein preparations used to establish these corrections to the SIMHA equation were fibrous thymus DNH, by the procedure of MIRSKY AND POLLISTER[2] a complex of methylene blue and tobacco mosaic virus (TMV) in the end-to-end dimeric state, some preparations of TMV, and non-fibrous DNH in 1 M NaCl, as described in this paper. These preparations have been characterized by sedimentation analysis and other physical studies, as reported in the literature or by work pursued in this laboratory to be published.

which should correspond to the component existing at higher concentrations, or as we shall see the principle component described from sedimentation analysis by KUPKE *et al*[5], is 178, which gives an axial ratio of 50.4, (see Table III). On the other hand, the more asymmetric component formed by dilution of the DNH below 0.15% is found to have a ν_0 of 460, which corresponds to an axial ratio of 87. An additional determination was made on DNH which had undergone the first stage of terminal purification, in that the lyophilized material was treated with water and the suspension clarified. The curve obtained (also shown in Fig. 2) extrapolates to an intrinsic viscosity of 202, which is somewhat lower than the value previously obtained. An intrinsic viscosity determination was also carried out on a DNH solution which had undergone complete terminal purification, at the lower velocity gradient of 42. The axial ratio obtained is seen to be exactly the same as that obtained for the solution which had not undergone the final dialysis, meaning that this purification step does not alter the particle asymmetry. A large difference in intrinsic viscosity does, however, result from varying the medium in which the lyophilized DNH is initially dissolved, much higher values being obtained if 1 *M* NaCl is the solvent rather than water. Moreover, the difference is not only physical, for, as mentioned, use of 1 *M* NaCl rather than water for the initial solvent leads to a higher percentage of phosphorus in the protein in solution.

TABLE III

INTRINSIC VISCOSITIES AND CORRECTED SEDIMENTATION CONSTANTS OBTAINED FOR DESOXYNUCLEOHISTONE IN VARIOUS MEDIA

Prepn. No.	*pH*	*Time of standing days*	*Solvent*	*Velocity gradient sec*$^{-1}$	$[\eta]$ *ml/g*	$[\eta]^0$ *ml/g*	ν^0	*b/a*	$S^0_{20,w}$ *by extrapol. of S*	$S^0_{20,w}$ *by extrapol. of* $S \cdot \eta r$
I	7.0		Water	186	29.7	33.4	52.0	24.5	10.05	10.21
A	6.5		0.005 *M* NaCl	186	47.0				20.0	19.7
I	7.5			186	44.7					
				85	40.4	36.2	56.4	25.9		
				53	38.3					
I	5.4	18	1 *M* NaCl	186	255					
				85	275	296	470	87		
				53	287					
Same: principle component				186	166					
(by extrapolation of				85	144	114	178	50.4		
lower portion of curves)				53	123					
I	6.5*	11	1 *M* NaCl	186	201		372	77	13.2	15.05
I	6.5**	18	1 *M* NaCl	42	231		375	77		
I	6.5	17	2 *M* NaCl	186	212		392	79	8.61	10.17

* Solution was prepared by treating lyophilized material with water, clarifying in Spinco L, and adding salt to make a molar solution.

** Solution was prepared by treating lyophilized material with water, clarifying in Spinco L, dialyzing against water, clarifying, and adding salt.

The seventh column gives values of weight intrinsic viscosity at zero velocity gradient and the eight values of volume fraction intrinsic viscosity at zero velocity gradient.

In order that a complete correlation might be made between the results of sedimentation analysis and viscosity, a viscosity determination at 186 sec^{-1} was also made for the remaining ionic strength included in the sedimentation studies[5], *i.e.* 2 *M* NaCl. The curve, shown at the top of Fig. 2, demonstrates somewhat opposite behavior from that observed for 1 M NaCl, that is, the sharp rise in magnitude of η_{sp}/c occurs as the DNH concentration is increased rather than decreased.

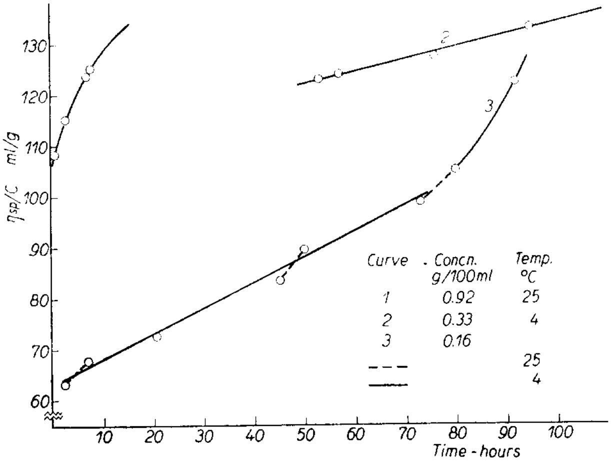

Fig. 3. Time dependence of η_{sp}/c for solutions of DNH in 1 M NaCl at three concentrations.

When some of the initial solutions at higher protein concentrations were measured, the viscosity was found to increase markedly with time, such that differences between successive measurements were significant. Therefore, the time dependence of viscosity was studied at several DNH concentrations. The results are shown in Fig. 3. The pairs of points joined by dotted lines represent the intervals during which the measurements were being made at 25°, while during the other longer intervals the solution had been stored in the cold room, at 4° C. The surprising result is that for a 0.16% DNH solution (undergone complete terminal purification), the initial value of η_{sp}/c observed right after dilution in 1 M NaCl is much lower than all values previously observed as a result of successive dilutions and several days of standing. The rise then occurs steadily over a period of days, to give a straight line, except at the very end, to reach finally a level corresponding to that previously found. It is interesting to note that it is at this concentration that the dip in the curves for η_{sp}/c occurs. The rate of viscosity increase is seen to be slowest at the 0.33% concentration and highest at 0.92%.

When the sedimentation rate determinations were made on DNH in the various media[5], it was not possible to extend the concentration range of the solutions studied to below 0.3%, because of the diffuseness of the peak resulting at low concentrations. It is quite possible that the accuracy of extrapolation might not be too great, because of the tendency of upward curving of the S vs. c curve as zero concentration is approached, which has been observed for such asymmetric molecules as fibrous DNH[3] and DNA[9]. A more accurate method of extrapolation, as suggested by SCHACHMAN AND LAUFFER[10], is to plot values of $\eta_r \cdot S_{20,w}$, where η_r is the relative viscosity of the solution at the same concentration as employed for the sedimentation rate determination, as function of concentration and extrapolate to zero. Since some of the 1 M NaCl solutions show considerable viscosity change with time, the viscosity values used were at 2½ hours standing after salt addition, (see dotted line in Fig. 2), which is about the average time interval for the ultracentrifuge runs. Similar time variation of η_{sp}/c was also observed for DNH in 2 M NaCl at the higher concentrations. The upper part of the sigmoid curve represents on the average about one week of standing. Extrapolation of some of the results back to 2½ hours gives a straight line, shown in dashes at the extended portion. This was used in the calculation of the relative viscosities used in the products plotted in Fig. 4. The best straight lines, fitted to the $\eta_r \cdot S_{20,w}$ vs. c data by the method of least squares, can be compared with the lines of $S_{20,w}$ vs. c. It is of interest to note that the two lines of each pair extrapolate to the same value of $S_{20,w}$ for the media water and 0.005 M NaCl, and that for 1 M and 2 M NaCl the values of $S_{20,w}$ obtained from the $\eta_r \cdot S_{20,w}$ plots are appreciably higher than those obtained by the plots of $S_{20,w}$ alone. This is not too surprising since the particles are of comparatively low asymmetry in the low ionic strength media, whereas they are highly asymmetric at the higher ionic strengths and the viscosity behavior is complex. In the case of the 0.005 M NaCl determinations, the solution used for the viscosity run at pH 6.5 (curve 1, Fig. 1) was prepared by KUPKE in the same manner and from the same DNH batch as used for the sedimentation runs.

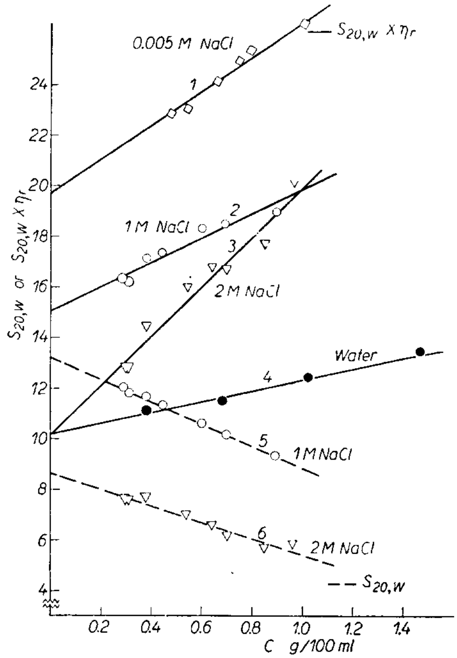

Fig. 4. Comparison of the plots of sedimentation constants times corresponding relative viscosities with those of the sedimentation constants as a function of concentration, for DNH in various media.

In order that a more complete understanding may be gained of the particle breakdown and dissociation reactions occurring in 1 M NaCl, which have already been suggested by the viscosity and sedimentation results, some fractionation experiments were carried out, by the centrifugation of 1 M NaCl solutions of DNH at three concentrations. The conditions used and the results obtained have been summarized in Table IV.

It was at first thought that the 200 minute period of centrifugation was not sufficiently long to cause complete sedimentation of the heavy component. Therefore, the supernate was run again for another three hours, with the result that a very small pellet was formed. However, there was very little decrease in average asymmetry as a result of the second centrifugation, meaning that very little of the more asymmetric component initially present could have been sedimented. The twenty-four hour period used in run II was actually too long for the desired separation, as ultracentrifugal analysis of the pellet showed the presence of some light fraction, the presence of which also explains the fact that the N/P ratio observed was higher than for the other heavy fractions analysed. The sedimentation constant calculated for the faster sedimenting of the two components has a value a little lower than that of the principle component[5], and for the lighter component a somewhat greater value than the one obtained for the diffuse or histone-rich component. The sedimentation constant calculated for the pellet fraction of run I roughly corresponds to the value for DNH in 2 M NaCl.

The results of chemical analyses show varying degrees of unequal distribution of nucleic acid and histone between the supernate and pellet fractions, indicating dissociation, but in no case is the splitting complete. As the initial DNH concentration is increased, the fraction of material going into the pellet is increased, and the supernates are found to be more nearly pure histone as indicated by the much higher N/P ratios.

TABLE IV

CHEMICAL ANALYSES AND PHYSICAL STUDIES ON THE FRACTIONS OBTAINED BY THE ULTRACENTRIFUGATION OF SOLUTIONS OF DNH IN 1 *M* NaCl

Run I: 0.0388 % DNH, pH 5.4. Time of standing since salt addition: 26 days.

Fraction	*Time of ultracent.*	*Vol. ml*	*Total mg P*	*N/P*	*% total N in fract.*	$S_{20,w}$	*dV/dr sec⁻¹*	$[\eta]$	ν^0	*b/a*
Supernate I	200 min	22.0	0.256	4.34	77.0*		186	139		
II	180						186	135	226	58
							53	140		
Pellet I	200	2.0	0.122	2.68	23.0	7.36				

Run II: pH 6.5. Time of standing since salt addition: 3 days. Time of ultracentrifugation: 24 hours.

Solution	*Fraction*	*Vol.*	*Total mg P*	*N/P*	*% of total N in fract.*	$S_{20,w}$	*dV/dr sec⁻¹*	$[\eta]$	ν^0	*b/a*
A. 0.0535 % DNH	Supernate	22.0	0.085	9.9	43.2*		85	105	180	51
	Pellet	2.2	0.416	2.93	56.8	fast 10.41 slow 1.63	53	164	271	64
B. 0.482 % DNH	Supernate									
	1. top	2.0	0.0075	63.5	29.9		85	28	46	23
	2. bottom	9.0	0.0702	30.4						
	Pellet**	4.0	2.25	2.60	66.7					

* These values were determined by difference.

** The pellet was first extracted with 4 ml of water, which was analyzed for phosphorus only and found to contain 0.012 mg P.

DISCUSSION

The reversed variation of magnitude of η_{sp}/c with velocity gradient from that expected for DNH in 0.005 *M* NaCl and in 1 *M* NaCl for higher protein concentration is a phenomenon worthy of further consideration. There is a possibility that the flexibility of the particles is a factor involved. However, KUHN AND KUHN[11] have shown that even for polymers having the maximum flexibility, that is zero shape resistance, the variation of specific viscosity with velocity gradient is a horizontal straight line, and that for all other particles there is a falling off of viscosity as the shear rate is increased. This explanation, therefore, is not sufficient. It would appear that orientation of the long axes of the various particles formed more or less along the stream lines by causing the solution to flow has resulted in a kind of reversible aggregation which increases the viscosity.

It might be asked why sharp peaks down to very low concentrations are not obtained for DNH in 1 *M* NaCl, when the particles formed are quite asymmetric and a higher $S^0_{20,w}$ is obtained by extrapolating $\eta_r \cdot S_{20,w}$ values to zero concentration. The answer is to be found in the fact that the extensive particle breakdown apparently occurs in the 0.1 to 0.2% concentration region, particularly as shown by the results of viscosity-time studies for the 0.16% solution. The time at which all analytical ultracentrifuge runs were made was shortly after salt addition, meaning that in this region the sub-particles formed were of minimum asymmetry, not having had a chance to reaggregate, since by Fig. 3 at least 5 days of standing are required to bring the value of η_{sp}/c up to the level found initially for higher DNH concentrations.

Studies on the supernate and residue fractions obtained by centrifugation reveal that in general there is a dissociation of DNH in 1 M NaCl, to yield a less asymmetric, more histone-rich component in the supernate than in the residues. However, additional effects were observed to result from the reduction of the total DNH concentration below 0.3%, indicating a very complex break-down of the particles at low concentrations. As the concentration is decreased, both the amount of material in the supernate and the percentage of DNA in the fraction increase, as evidenced by the decreasing N/P ratios. Furthermore, the asymmetry of the components of this fraction increases markedly, and there is also a well-defined lowering of the sedimentation constant of the main component in the pellet fraction, (Table IV). One possible interpretation which may be made of these results would be as follows: The DNH solution at the higher concentration of 0.48% in 1 M NaCl dissociates partially into a depolymerized, less asymmetric histone which remains in the supernate and a small amount of depolymerized nucleic acid of varying particle size, the presence of which accounts for the gradient of phosphorus concentration found in the supernate. On decreasing the DNH concentration to 0.0535%, without changing other conditions, more nucleic acid appears in the supernate, meaning that there is more dissociated nucleic acid in depolymerized form. The increased intrinsic viscosity observed could be explained by the increased amount of asymmetric DNA present. A comparison of the sedimentation constant of 10.4 found for the heavy component with the value of 13.0 obtained by KUPKE *et al.*[5] for the principle component at the same concentration (by extrapolation), reveals that a small portion of the original particle must have split off. Also, the fact that the $S_{20,w}$ observed for the slow moving boundary was over 1½ times his value for the diffuse component at higher concentrations can be explained by the occurrence of some aggregation of the light components.

This process of splitting off portions of the original particle by the action of salt is seen to be carried even further as a result of further dilution of the DNH and standing. The effect of dilution would appear to be the splitting off of a greater proportion of DNA, which can then undergo aggregation, explaining the viscosity increase with time. The $S_{20,w}$ was also determined for the principle component in a 0.16% solution of DNH in 1 M NaCl, pH 6.5, which had stood at 4° for 70 days, and found to have a value of 9.45 Svedbergs, which is lower still than the value for solution A, run II. This and the fact that the solution has lower initial asymmetry suggests that more histone as well as DNA splits off at this concentration. On reducing the concentration to zero, the steep viscosity rise can be explained in terms of further length-wise splitting, to give particles having a maximum axial ratio of 87, or 77 in the case of the terminally purified preparations, as determined from the values of intrinsic viscosity (Table III).

A consideration of the differences in the viscosity curves obtained for 1 M NaCl and for 2 M salt is also of interest. It will be noted that the intrinsic viscosities observed for the two media at pH 6.5 yield values of axial ratio of 77 and 79, which are the same within the experimental error. Thus, at low DNH concentrations in 1 M NaCl, the particles split to a more asymmetric form, just as they do in 2 M NaCl, only in this case the splitting apparently occurs over the entire concentration range. In addition, at the ionic strength of 2, by the rise in magnitude of η_{sp}/c occurring with time at concentrations above 0.2%, aggregation of the split fragments is seen to occur, which increases the asymmetry further. This type of splitting is similar to that suggested by BERNSTEIN AND MAZIA[12] for the dissociation reaction occurring in 1 M NaCl. However, the crosswise splitting of DNH in 1 M NaCl which appears to occur at 0.16% would be more analogous to BERNSTEIN AND MAZIA's degradation reaction.

Using the values of axial ratio and $S^0_{20,w}$ obtained as described, it is possible to calculate some rough values of molecular weight by use of the equation: $M^{2/3} = \frac{6\pi\eta S^0_{20,w} f/f_0 N^{2/3} (3V/4\pi)^{1/3}}{1 - V\varrho}$ [13]. The frictional ratio (f/f_0) is obtained from the axial ratio by use of the PERRIN equation. In the calculation, no hydration is assumed, so that in effect the molecular weight being calculated is that of the effective hydrodynamic ellipsoid, discussed by SCHERAGA AND MANDELKERN[14]. Although the values thus obtained are not true particle weights, the relative differences for the different media will at least be valid. The molecular weights calculated for DNH are 300,00 in water, 820,000 in 0.005 M NaCl, 965,000 for the principle component in 1 M NaCl, and finally 865,000 in 2 M NaCl. These results are entirely in accordance with the particle sizes suggested by the sedimentation results of KUPKE *et al.*[5]. They indicate the occurrence of aggregation to roughly a three-fold extent in all salt solutions, with increase of asymmetry at higher ionic strengths but not in 0.005 M NaCl. The molecular weight decrease in 2 M NaCl from the value in molar salt would correspond to splitting off of 10% of the principle component.

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SUMMARY

1. Complete viscosity-concentration determinations were carried out at three velocity gradients for non-fibrous desoxyribonucleohistone (DNH) in 0.005 M NaCl and 1 M NaCl. The variation of specific viscosity with velocity gradient at a given concentration was found to be the reverse of that expected, *i.e.* viscosity decreased with decreasing gradient, for DNH in 0.005 M NaCl and in 1 M NaCl in the concentration range above 0.16%.

2. An additional anomaly was observed for the behavior of DNH in 1 M NaCl: the viscosity curves rose steeply on decreasing the protein concentration below 0.16%, indicating the formation, on dilution, of a more asymmetric component. The values of intrinsic viscosity obtained were found to increase with decreasing velocity gradient, as expected by the result of particle orientation, according to the principle of structural viscosity.

3. In addition, the intrinsic viscosities were determined at one velocity gradient for DNH in water and in 2 M NaCl. Values of axial ratio for DNH thus obtained were as follows: 25 in water and 0.005 M NaCl, 50 for the principle component and 77 for the asymmetric component in 1 M NaCl, 79 in 2 M NaCl.

4. The values of $S^0_{20,w}$ determined by extrapolating $\eta_r \cdot S_{20,w}$ to zero were found to be higher than those resulting from extrapolation of $S_{20,w}$ *vs.* c, for molar and 2 M salt only, as expected for particles of high asymmetry.

5. To gain a more complete understanding of the manner in which the particles break down in 1 M NaCl, some mixtures were fractionated by centrifugation, and the supernate and residue fractions studied separately. It was found that as a result of dilution of DNH and increasing the length of time of standing, increasing amounts of material and especially of DNA were found in the supernate, and the average asymmetry of the supernate fraction also increased. In addition,

dilution and longer time of standing caused a decrease in the sedimentation constant of the main component in the pellet fraction. If the total concentration is 0.16%, the initial viscosity is much below the stable level and increases slowly with time as aggregation occurs, and the sedimentation constant of the principle component is at a minimum.

RÉSUMÉ

1. La relation entre viscosité et concentration pour des solutions de désoxyribonucléohistone (DNH) non fibreuse dans NaCl 0.005 *M* et NaCl 1 *M* a été étudiée sous trois gradients de vitesse. La viscosité spécifique à une concentration donnée varie en fonction du gradient de vitesse, en sens inverse de celui attendu, c'est-à-dire que la viscosité diminue quand le gradient diminue, dans le cas de DNH dans NaCl 0.005 *M* et 1 *M*, et pour une concentration supérieure à 0.16%.

2. Le comportement de DNH dans NaCl 1 *M* présente une anomalie supplémentaire: les courbes de viscosité s'élèvent rapidement quand la concentration en protéine tombe au dessous de 0.16%, trahissant la formation, par dilution, d'un composé plus asymétrique. Les valeurs de la viscosité intrinsèque augmentent quand le gradient de vitesse diminue, en raison de l'orientation des particules et conformément au principe de la viscosité structurale.

3. Les viscosités intrinsèques ont été de plus déterminées pour un gradient de vitesse dans l'eau et dans NaCl 2 *M*. Les valeurs du rapport axial ainsi obtenues sont les suivantes: 25 dans l'eau et dans NaCl 0.005 *M*; 50 pour le composé principal et 77 pour le composé asymétrique dans NaCl 1 *M*; 79 dans NaCl 2 *M*.

4. Les valeurs de $S^0_{20,w}$, déterminées en extrapolant $\eta_r \cdot S_{20,w}$ jusqu'à 0, sont plus élevées que celles qui résultent de l'extrapolation de $S_{20,w}$ en fonction de C, et ceci seulement en solution salines M et 2 *M*, résultat prévisible pour des particules très asymétriques.

5. Afin de mieux préciser la façon dont les particules se rompent dans NaCl 1 *M*, quelques mélanges ont été fractionnés par centrifugation et le surnageant et les fractions résiduelles étudiées séparément. En fonction de la dilution et du temps écoulé après celle-ci, des quantités croissantes de produits et particulièrement de DNA restent dans le surnageant, et l'asymétrie moyenne du surnageant augmente. En outre, après dilution et repos prolongé, la constante de sédimentation du composé principal du culot diminue. Si la concentration totale est égale à 0.016, la viscosité initiale est très en dessous du niveau stable et augmente lentement avec le temps au fur et à mesure que l'aggrégation progresse; la constante de sédimentation du constituant principal est à son minimum.

ZUSAMMENFASSUNG

1. Es wurden vollständige Viskositäts-Konzentrationsbestimmungen bei drei Geschwindigkeitsgradienten an nicht fibrillärem Desoxyribonukleohiston in 0.005 *M* NaCl und 1 *M* NaCl ausgeführt. Es wurde gefunden, dass sich die spezifische Viskosität bei einer gegebenen Konzentration mit den Geschwindigkeitsgradienten umgekehrt veränderte wie erwartet wurde, d.h. für Desoxyribonukleohiston (DNH) in 0.005 *M* NaCl und 1 *M* NaCl in einem Konzentrationsbereich über 0.16%, nahm die Viskosität mit abnehmendem Gradienten ab.

2. Für das Verhalten von DNH in 1 *M* NaCl wurde eine zusätzliche Anomalität beobachtet: Die Viskositätskurve steigt steil an, wenn die Proteinkonzentration unter 0.16% vermindert wird, dieses Verhalten zeigt die Bildung einer stärker asymmetrischen Komponenten bei der Verdünnung an. Die Werte für die "intrinsic" Viskosität nehmen mit abnehmenden Geschwindigkeits zu, wie es als Folge der Teilchenorientierung auf Grund der Strukturviskosität zu erwarten war.

3. Zusätzlich wurden die "intrinsic" Viskositäten für DNH in Wasser und 2 *M* NaCl bei einem Geschwindigkeitsgradienten bestimmt. Es wurden folgende Werte für das Achsenverhältnis des DNH gefunden: 25 in Wasser und 0.005 *M* NaCl, 50 für die Hauptkomponente und 77 für die asymmetrische Komponente in 1 *M* NaCl und 79 in 2 *M* NaCl.

4. Es wurde gefunden, dass die Werte für $s^0_{20,w}$ die durch Extrapolation von $\eta_r \times s_{20,w}$ gegen null erhalten wurden, höher liegen, als die durch Extrapolation von $s_{20,w}$ gegen c gewonnenen. Dies gilt nur für molare und 2 *M* Salzlösungen, wie es für Teilchen hoher Asymmetrie zu erwarten ist.

5. Um ein vollständigeres Bild über die Art und Weise, in der die Teilchen in 1 *M* NaCl abgebaut werden, zu erhalten, wurden einige Mischungen mit der Zentrifuge fraktioniert und die Überstands- und Rückstandsfraktionen getrennt untersucht. Es wurde gefunden, dass als Ergebnis der Verdünnung des DNH und längeren Stehenlassens grössere Materialmengen, insbesondere DNA, im Überstand gefunden wurden und die durchschnittliche Asymmetrie der Überstandfraktion ebenfalls grösser wurde. Darüberhinaus verursachen Verdünnung und längeres Stehenlassen eine Abnahme der Sedimentationskonstanten der Hauptkomponenten der Rückstandsfraktion. Wenn die Gesamtkonzentration 0.16 beträgt, liegt die Anfangsviskosität erheblich unter der beständigen Höhe und nimmt im Laufe der Zeit mit zunehmender Aggregation zu und die Sedimentationskonstante der Hauptkomponenten hat ein Minimum.

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