Sedimentation Equilibrium and other Physicochemical Studies on the Lysine-Rich Fraction of Calf Thymus Histones

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1. The lysine-rich fraction (Ia + Ib, or f1) of calf thymus histones was isolated as the sulphate by acid extraction. 2. Sedimentation-equilibrium measurements with interference optics showed that this fraction was monodisperse with a molecular weight of 19500 ± 2000 . 3. The 'apparent molecular weight' calculated from the sedimentation-equilibrium studies varied markedly with concentration. The large second virial coefficient implied by such variation was attributed to the very high charge/mass ratio of this relatively small protein. Estimates of the charge were made from the values of this virial coefficient. 4. The very large value of the virial coefficient explains anomalies in the earlier reports of the molecular weight of this histone and also why the z-average molecular weight can appear to be lower than the weight-average molecular weight. 5. The differences of the specific refractive increments, and the partial specific volumes, between dialysed and undialysed solutions of this histone fraction could also be attributed to its high molecular charge, which was estimated from these differences and agreed, within the expected limits, with the value deduced from the second virial coefficient. 6. Sedimentation-velocity measurements combined with the known molecular weight imply that lysine-rich histone has a high frictional ratio and an extended shape. Optical-rotatory-dispersion measurements indicated that it had a low helical content.

The group of nuclear proteins classified as histones have provided a considerable problem in protein fractionation; the number of individual histone fractions is still far from clear (Murray, 1965; Fambrough & Bonner, 1966). However, sufficient progress has been made over the last few years to merit a detailed examination of some of these fractions with physicochemical techniques. The lysine-rich fraction of calf thymus gland was chosen for investigation as this could be easily extracted and the available evidence suggested that this fraction did not exhibit the marked aggregation which is typical of the arginine-rich histones (Davison & Shooter, 1956; Trautmann & Crampton, 1959). There was, moreover, considerable disagreement in the literature about the molecular weight of this protein, for the previously published values range from 8400 to 21 000 (Ui, 1957; Trautmann & Crampton, 1959; Satake, Rasmussen & Luck, 1960; Teller, Kinkade & Cole, 1965), and it seemed important to clarify this situation.

The nomenclature of histones remains in a confused state (see, e.g., the survey by Murray, 1964). The lysine-rich fraction from calf thymus used in this work corresponds to a mixture of fractions Ia and Ib described by Murray (1965), or to fraction f1 described by Johns (1964). In the remainder of this paper it is referred to simply as 'fraction 1'.

MATERIALS AND METHODS

Fraction 1 was extracted from calf thymus gland by an acid extraction method very similar to the procedure devised by Murray (1966). Thymus glands were dropped into liquid N₂ immediately after removal from the calves. All subsequent operations were carried out at 4°. Approx. 200 g. of thymus gland was homogenized with 400 ml. of a solution containing 0.14 M-NaCl and 0.01 M-sodium citrate and the suspension was then centrifuged at 1500g for 30 min. in an MSE preparative centrifuge. Fatty debris and the supernatant liquid were discarded. The sediment was homogenized again in 400 ml. of the same saline-citrate medium and the suspension was then centrifuged at 1500g for 30 min. Washing of the residue by homogenization and

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centrifugation was repeated six more times or until the supernatant was clear after centrifugation.

The residue was then placed in a blender and homogenized briefly with 200 ml. of water. Then 3.5 N-H₂SO₄ was added slowly with stirring until the pH of the suspension had fallen to 2.7. The mixture was then stirred at this pH for 20 min. and centrifuged; the supernatant was discarded. The residue was homogenized again and the suspension titrated to pH 1.9. After stirring at this pH, the suspension was centrifuged and the supernatant was collected and poured into a volume of ethanol about three times its own volume. The precipitated histone sulphate was allowed to settle overnight and then collected by centrifugation. The precipitate was washed three times by dispersion in ethanol followed by centrifugation and was finally dried in a vacuum desiccator. At intervals during the drying process, the desiccator was opened and the lumps of histone sulphate were broken up so that the final product was obtained in the form of a finely divided white powder.

Determination of dry weight. The proportion of moisture in each fraction was determined by drying a known weight of a sample at 110° under vacuum over P₂O₅ for 24 hr.

Estimation of histone solutions. Samples (0.1 ml.) of solutions of fraction 1 were added to 2 ml. of water and the resulting solutions analysed by the micro-buret method of Itzhaki & Gill (1964). The method was calibrated in terms of the dry weight of histone sulphate by making up solutions by direct weighing of the moist solid and correcting for moisture content.

Sedimentation analysis. A Spinco model E ultracentrifuge fitted with schlieren and interference optical systems was used. The temperature was maintained at 20° in all experiments. For the measurement of sedimentation coefficients, a valve-type synthetic-boundary cell was used with a rotor speed of 59 780 rev./min., and the schlieren photographs were recorded at 8 min. intervals. Preliminary sedimentation-equilibrium experiments were made with the multichannel short-column cell of Yphantis (1960); equilibrium was attained in under 2 hr. More detailed sedimentation-equilibrium investigations were made with a double-sector cell fitted with an interference mask, by filling it with a 2–3 mm. column of solution. A small volume of a fluoro-carbon oil (FC43, supplied by Beckman Ltd., Glenrothes, Fife) was placed in the solution sector, and a slight excess of solvent in the other sector (van Holde & Baldwin, 1958). This enabled the positions of the bottom and meniscus of the solution column to be measured accurately. The rotor speed in these equilibrium experiments was 14 290 or 19 160 rev./min. and each run lasted about 16 hr. At equilibrium both schlieren and interference photographs were taken on either Kodak metallographic or Ilford R 30 plates. During the approach to equilibrium, photographs of a small portion of the interference image ('slit photographs') were taken by placing a shutter with a slit in it and a ruled glass plate in front of the camera. In some cases, after the attainment of equilibrium, the rotor speed was increased to 42 040 rev./min. and slit photographs were also taken during this period. By these means it was possible to calculate the absolute concentration of the solution at equilibrium at any one point in the centrifugal field in terms of interference fringes (Charlwood, 1965). After completion of the equilibrium run, a blank run was performed in the manner suggested by Richards & Schachman (1959). The concentration in interference fringes of the solution with solute

evenly distributed throughout, i.e. before the commencement of a sedimentation experiment, was found either by a synthetic-boundary experiment with interference optics or by measurement in a differential refractometer. This quantity will be denoted $\mathcal{J}_0$.

A Projectorscope (Precision Grinding Co., Mitcham Junction, Surrey) was used for the measurement of all the photographic plates. Sedimentation coefficients were evaluated in the usual way. On the schlieren photograph of the cell at equilibrium the height of the schlieren trace, $\Delta y(r)$, was measured at values of the radius, r , that increased by equal increments. The interference photographs were measured by a method described by Creeth (1962). The vertical position of any one fringe was measured at constant horizontal increments. By subtracting the vertical position of a fringe at the meniscus, and dividing by the vertical distance between the centres of two adjacent fringes, the vertical displacement of a fringe at any horizontal co-ordinate, r , with respect to its position at the meniscus can be calculated in units of 'fringes'. This was then corrected for the fringe slope due to window distortion etc. by using the results of the blank run. The resultant fringe displacement with respect to the meniscus could be combined with the absolute fringe displacement at any one point to give the absolute fringe displacements at all points in the centrifugal field. The absolute fringe displacement at a point r in the field is referred to below as $\mathcal{J}(r)$. The calculations involved $\mathcal{J}(r)$, $\Delta y(r)$ and r , and are discussed below.

Solutions. In all sedimentation experiments, solutions of the approximate concentration required were made up by dissolving weighed quantities of histone sulphate in 2 ml. of 0.1 M- or 0.5 M-NaCl and then dialysing against 1 l. of solvent for 24 hr. For experiments at different pH values, the buffer solutions described by Miller & Golder (1950) were used. The diffusate was used for layering over solution in all synthetic-boundary experiments, and also in the sector opposite the solution sector in the equilibrium experiments.

Partial specific volumes. The partial specific volumes of the two fractions were calculated from the measurement with a 5 ml. pycnometer of the densities of solutions of known concentrations. As pointed out by Casassa & Eisenberg (1964), there are three ways of measuring apparent partial specific volumes and in this work two of these were employed. In the first, histone sulphate was dissolved in 0.1 M- or 0.5 M-NaCl and the density of the solution was measured. The density of the relevant salt solution and the concentration of the protein solution were also measured. If ρ_{soln} is the density of the salt solution (g./ml.), $\Delta\rho$ the difference in density between histone sulphate solution and salt solution, and c the concentration of histone sulphate in solution (g./ml.), then the partial specific volume is:

$$\bar{v} = \frac{1}{\rho_{\text{soln}}} \left(1 - \frac{\Delta\rho}{c} \right) \quad (1)$$

In the second procedure, histone sulphate was dissolved in salt solution and then dialysed against a large volume of the same salt solution. The density of diffusate ($\rho_{\text{diff.}}$) and of dialysed solution were measured; the difference between these two will be denoted $\Delta\rho^*$. The concentration of the histone solution was also measured. Even though many sulphate ions will have diffused through the dialysis membrane, the resulting measured concentration will be in

terms of dry histone sulphate because the methods for measuring concentration in solution were calibrated with this material. Another partial specific volume, $\bar{v}'$, is then given by:

$$\bar{v}' = \frac{1}{\rho_{\text{diff}}} \left(1 - \frac{\Delta\rho^*}{c} \right) \quad (2)$$

The relevance of the distinction between $\bar{v}$ and $\bar{v}'$ is discussed below.

Refractive-index increments. The difference between the refractive indices of a protein solution and solvent or a protein solution and its diffusate was measured in a Brice-Phoenix differential refractometer that had previously been calibrated by means of KCl solutions. The measurements were made at room temperature (approx. 25°). As with the partial specific volumes, two refractive-index increments were measured, and these are defined by the corresponding expressions:

$$\psi = \frac{\Delta n}{c} \quad (3)$$

$$\psi' = \frac{\Delta n^*}{c} \quad (4)$$

where Δn is the difference in refractive index between protein solution and solvent, Δn^* is the difference in refractive index between a dialysed protein solution and diffusate, and c is the concentration of protein in terms of histone sulphate.

Optical rotatory dispersion. The optical rotatory dispersion of protein solutions was measured from 220 to 550 m μ by means of a Bellingham and Stanley (Bendix) Polaromatic 62 spectropolarimeter. The histone sulphate was dissolved in 2 ml. of 0.1 M-NaCl or in a buffer solution of I 0.1 and dialysed against 1 l. of this solvent for 24 hr. The concentration of protein was estimated by the biuret method, and the specific-rotation values, which were those of histone sulphate, were converted into mean residue rotation, $[\alpha]_{\lambda}$. The constants a_0 and b_0 of Moffitt & Yang (1956) were evaluated by the usual plot of $[\alpha]_{\lambda}(\lambda^2 - \lambda_0^2)/\lambda_0^2$ against $\lambda_0^2/(\lambda^2 - \lambda_0^2)$ where $\lambda_0 = 212 \text{ m}\mu$.

THEORY

The problem of defining the thermodynamic components in multicomponent systems containing the three components water-charged protein-diffusible salt has been discussed by Scatchard (1946) and by Casassa & Eisenberg (1964), and the formulation discussed by the last named is followed here. The macromolecular component is defined as histone sulphate associated with such a number of moles of sodium sulphate and sodium chloride that the activity of the remaining diffusible salt is equal to the activity of salt in the diffusate. The macromolecular component defined in this way is referred to below as component 2*, and quantities associated with this component will be denoted by a star (*). The water is referred to as component 1 and the diffusible salt as component 3. The advantage of defining the macromolecular component in this way is that all terms in the sedimentation-equilibrium equation that describe

interaction between the macromolecular component and diffusible salt are eliminated, and the equation assumes a form similar to that for a simple two-component system, which is, if the macromolecule is monodisperse:

$$\frac{1}{c_{(r)}^*} \cdot \frac{dc^*}{dr} = M^* L^* (1 + B M^* c_{(r)}^*)^{-1} \quad (5)$$

$c_{(r)}^*$ is the concentration of component 2* at a distance r from the centre of rotation, M^* is the molecular weight of component 2*, B is a second virial coefficient (higher terms in the virial equation have been ignored) and L^* is given by:

$$L^* = \frac{(1 - \bar{v}^* \rho) \omega^2}{RT} \quad (6)$$

Casassa & Eisenberg (1961) have shown that:

$$M^* L^* = M L' \quad (7)$$

where:

$$L' = \frac{(1 - \bar{v}' \rho) \omega^2}{RT}$$

and M is the molecular weight, in this case, of dry histone sulphate. Thus the molecular weight can be evaluated from:

$$\frac{1}{c_{(r)}^*} \cdot \frac{dc^*}{dr} = M L' (1 + B M^* c_{(r)}^*)^{-1} \quad (8)$$

If component 2* is polydisperse, the relevant equation is:

$$\frac{1}{c_{(r)}^*} \cdot \frac{dc^*}{dr} = M_{w(r)} L' (1 + B'' M_{w(r)}^* c_{(r)}^*)^{-1} \quad (9)$$

where $M_{w(r)}$ and $M_{w(r)}^*$ are now weight-average molecular weights at r and correspond to M and M^* above (Casassa & Eisenberg, 1964). Definition of an apparent molecular weight as:

$$M_{w(r)}^{\text{app.}} = M_{w(r)} (1 + B'' M_{w(r)}^* c_{(r)}^*)^{-1} \quad (10)$$

insertion of the relation between absolute fringe displacement, $\mathcal{J}_{(r)}$ and concentration, and rearrangement of the equation gives:

$$\frac{d \ln \mathcal{J}_{(r)}}{d(r^2)} = M_{w(r)}^{\text{app.}} \cdot \frac{L'}{2} \quad (11)$$

$M_{w(r)}^{\text{app.}}$ can then be evaluated by plotting $\ln \mathcal{J}_{(r)}$ against r^2 . As is well known, a linear plot is not unequivocal evidence for the monodispersity of the solute; if $B'' M_{w(r)}^* c_{(r)}^* \ll 1$ and if $c_{(r)}^*$ increases considerably with r , downward curvature may result and may cancel out any upward curvature resulting from solute polydispersity.

An alternative arrangement of the differential

equilibrium-sedimentation equation (Lamm, 1929) is:

$$\frac{d \ln \left(\frac{1}{r} \frac{dc^*}{dr} \right)}{d(r^2)} = M_{z(r)}^{app} \cdot \frac{L'}{2} \quad (12)$$

where $M_{z(r)}^{app}$ is a complex function of $M_{w(r)}$ and of $c_{(r)}$. In terms of experimental quantities, this may be written:

$$\frac{d \ln \left(\frac{\Delta y_{(r)}}{r} \right)}{d(r^2)} = M_{z(r)}^{app} \cdot \frac{L'}{2} \quad (13)$$

This simplifies to two important limiting forms. When the solute is monodisperse, it follows by appropriate differentiation of eqn. (5) that Lamm's eqn. (12) yields a molecular weight (still conveniently denoted as if a z -average) given by:

$$M_{z(r)}^{app} = M(1 + BM^*c_{(r)}^*)^{-2} \quad (14)$$

However, when the solute is polydisperse and $B^*M_{w(r)}c_{(r)}^* \ll 1$, differentiation of eqn. (9) shows that the molecular weight yielded by Lamm's eqn. (12) is:

$$M_{z(r)}^{app} = M_{z(r)} \quad (15)$$

Of particular interest is the comparison between eqns. (8) and (14), which both refer to a monodisperse solute when $BM^*c_{(r)} \ll 1$; it is clear that in this instance:

$$M_{z(r)}^{app} < M_{w(r)}^{app}$$

Both the differential forms of the equilibrium equation are expressed in terms of the average molecular weights at a particular point in the centrifugal field. To evaluate the weight-average molecular weight of all the solute material present, M_w , the equations must be integrated, a procedure which has been described by Fujita (1962) and which results in the following:

$$\frac{1}{M_w^{app}} = \frac{1}{M_w} + B'c_0^* + \text{higher } c_0^* \text{ terms} \quad (16)$$

where M_w^{app} is calculated from:

$$M_w^{app} = \frac{c_b^* - c_m^*}{c_0^*} \cdot \frac{2}{r_b^2 - r_m^2} \cdot \frac{1}{L'}$$

Subscripts b and m refer to the bottom and meniscus of the solution column respectively, and c_0^* is the concentration of solution when solute is evenly distributed throughout. B' is a complex function, which includes the centrifugal field. M_w can be evaluated by performing experiments at different initial solute concentrations.

Table 1. *Apparent and extrapolated molecular-weight values of histone fraction 1*

Details of the buffers are given by Miller & Golder (1950).

$\mathcal{J}_0$	$M_w^{app.}$	$M_z^{app.}$
Preparation 1		
In 0.5 M.-NaCl, pH 4.5; rotor speed 19160 rev./min.		
10.21	16010	14260
5.18	16720	17480
2.94	18300	17020
$M_w = 20090^*$		
In glycine buffer (0.04 M.-glycine-0.006 M.-NaOH-0.54 M.-NaCl), pH 8.6 and 10.5; rotor speed 19160 rev./min.		
30.05	13070	
9.50	16000	
6.35	16000	
$M_w = 18110^*$		
Preparation 2		
In 0.1 M.-NaCl, pH 4.5; rotor speed 14290 rev./min.		
22.51	12466	
17.75	13060	7300
16.98	13160	8020
16.00		9020
14.83	13970	10560
14.04	14800	
11.36	15600	11560
11.04	16050	
6.30	17400	
3.83	17700	
$M_w = 20400^*$		
In 0.5 M.-NaCl, pH 4.5; rotor speed 19160 rev./min.		
16.00	14770	
8.99	17390	
6.14	18500	
2.90	18690	
$M_w = 20620^*$		
Preparation 3 (short-column experiments)		
In 0.1 M.-NaCl, pH 4.5; rotor speed 19160 rev./min.		
35.04	9350	
26.85	11290	
18.95	12560	
9.31	13970	
$M_w = 17700^*$		

*By extrapolation of $(M_w^{app})^{-1}$ to $\mathcal{J}_0 = 0$.

RESULTS

For each equilibrium sedimentation experiment, M_w^{app} was calculated, $\ln \mathcal{J}_{(r)}$ was plotted against r^2 , and $\ln(\Delta y_{(r)}/r)$ was plotted against r^2 . The latter plots, derived from the schlieren measurements, were always scattered and it was difficult to detect any curvature, so a straight line was drawn throughout the points and a value of M^{app} evaluated from the slope. The results of measurements on three separate preparations of fraction 1 are given in Table 1. The M_w values were obtained by a least-mean-squares plot of $(M_w^{app})^{-1}$ versus $\mathcal{J}_0$ and extrapolation to zero concentration. The measure-

Fig. 1. Equilibrium sedimentation of histone fraction 1 in 0.1 M-NaCl, pH 4.5, $s_0=22.51$. •, Experimental values in a plot of $\log s(r)$ versus r^2 . A straight line is drawn between the two extreme points to show deviations from linearity.

Fig. 2. Equilibrium sedimentation of histone fraction 1 in 0.5 M-NaCl, pH 4.5, $s_0=2.94$. •, Experimental values in a plot of $\log s(r)$ versus r^2 .

Fig. 3. Equilibrium sedimentation of histone fraction 1 in 0.1 M-NaCl, pH 4.5, $s_0=3.83$. •, Experimental values in a plot of $\log s(r)$ versus r^2 .

Table 2. Sedimentation coefficients of histone fraction 1 in 0.1 M-sodium chloride, pH 4.5

Preparation 2		Preparation 3	
s_0	$S_{20,w}$ (s)	s_0	$S_{20,w}$ (s)
22.51	0.84	26.85	0.87
16.98	0.88	18.95	0.87
13.40	0.89	9.31	0.88

$$S_{20,w}^0=0.90\text{ s}$$

Table 3. Specific refractive-index increments and partial specific volumes of histone fraction 1

10^3c (g./ml.)	ψ (ml./g.)	10^3c (g./ml.)	ψ' (ml./g.)
Fraction 1 in 0.1 M-NaCl			
5.39	0.171 ± 0.001	4.92	0.162 ± 0.006
4.17	0.168 ± 0.004	4.01	0.159 ± 0.005
4.05	0.170 ± 0.002	4.16	0.164 ± 0.006
3.45	0.168 ± 0.004	3.10	0.162 ± 0.004
2.72	0.166 ± 0.003	2.50	0.159 ± 0.006
1.66	0.169 ± 0.004	1.50	0.166 ± 0.006
Fraction 1 in 0.5 M-NaCl			
5.13	0.162 ± 0.002	4.47	0.153 ± 0.006
3.03	0.162 ± 0.002	2.80	0.146 ± 0.006
1.98	0.155 ± 0.005		
0.97	0.166 ± 0.004		
10^3c (g./ml.)	$\bar{v}$	$\bar{v}'$	
Fraction 1 in 0.1 M-NaCl			
8.20	0.71 ± 0.1		
4.59	0.72 ± 0.1		
7.76		0.75 ± 0.1	
4.65		0.74 ± 0.1	
Fraction 1 in 0.5 M-NaCl			
5.81		0.75 ± 0.1	

Table 4. *Parameters of the Moffitt-Yang equation for solutions of histone fraction 1*

Solvent	pH	$10^3 c$ (g./ml.)	b_0	a_0
0.08 M-NaCl-0.0053 M-glycine-0.0147 M-HCl	2	6.03	0	-967
0.08 M-NaCl-0.02 M-sodium acetate-0.059 M-acetic acid	4	6.42	0	-834
0.08 M-NaCl-0.0023 M- Na_2HPO_4 -0.0066 M- NaH_2PO_4	6	6.11	0	-881
0.08 M-NaCl-0.01 M-HCl-0.002 M-veronal	8.2	8.68	-68	-567
0.08 M-NaCl-0.002 M-HCl-0.002 M-veronal	9.2	7.13	-21	-739

ments on preparation 3 were made with the Yphantis multichannel short-column cell and are less accurate than the other measurements. Plots of $\log \mathcal{J}_{(r)}$ against r^2 for solutions containing a high concentration of fraction 1 in 0.1 M-sodium chloride show a slight downward curvature (Fig. 1), which disappears when the salt concentration is increased to 0.5 M and the histone concentration is much decreased (Fig. 2). For very dilute solutions in 0.1 M-sodium chloride, the points are more scattered, but there is no evidence of upward curvature (Fig. 3).

Table 2 gives values of $S_{20,w}$ for preparations 2 and 3 in 0.1 M-sodium chloride solution. A least-mean-squares extrapolation of $S_{20,w}$ against $\mathcal{J}_0$ gave a $S_{20,w}^0$ value of 0.90 s. The results of measurements of refractive-index increments and partial specific volumes are given in Table 3. The value of $\bar{v}$ calculated from the amino acid composition of the polypeptide chain alone (Cohn & Edsall, 1943) is 0.77. The values of the parameters of the Moffitt equation that were evaluated from optical-rotatory-dispersion measurements are given in Table 4.

DISCUSSION

Molecular weights and monodispersity. The extrapolated values for the molecular weight, M_w , of fraction 1 indicate a value of 19500 with an uncertainty of 2000 over different preparations. This is in agreement with the value of Teller *et al.* (1965) and establishes that the molecular weight of this histone fraction is close to 20000 and not near to 10000 as previously thought. [A similar value has also been obtained by osmotic-pressure measurements, which gave a number-average molecular weight for preparation 3 of 21500 ± 1500 (J. Diggle & A. R. Peacocke, unpublished work).] This quantity is the molecular weight of the salt, histone sulphate. From the amino acid composition of the protein, the net positive charge on a polypeptide chain of molecular weight 20000 at pH 2.0 (the pH of extraction) may be calculated to be about ± 50 so that the molecular weight of sulphate ions will be about 2500. The molecular weight of the polypeptide chain is therefore about 17000, which is in agreement with the observations of Phillips (1963), who concluded that the weight of fraction 1

that contains one acetyl residue (the *N*-terminal group) was between 15000 and 18000.

The plots of $\log \mathcal{J}_{(r)}$ versus r^2 indicate that fraction 1 is monodisperse with respect to molecular weight within the limits discussed by Fujita & Williams (1966). This conclusion is broadly confirmed by the agreement between the M_w value and M_n from osmotic pressure (J. Diggle & A. R. Peacocke, unpublished work) and also accords with the conclusions of Teller *et al.* (1965). However, the agreement between M_n and M_w is only within the range of the error in each M value (about ± 1500 –2000) and could not exclude the presence of, for example, 10% of dimer aggregates. The best evidence for the monodispersity remains the linearity of the plot of $\log \mathcal{J}_{(r)}$ versus r^2 .

Kinkade & Cole (1966) have separated fraction 1 into four subfractions by chromatography on Amberlite IRC-50. These fractions differ in amino acid composition, but cannot differ greatly in molecular weight, in view of the monodispersity of this fraction now established, and are possibly an example of microheterogeneity in these proteins.

Virial coefficient and charge from sedimentation equilibrium. Table 1 shows that the apparent molecular weights of fraction 1 vary quite markedly with concentration. The variation is of such a magnitude that it encompasses the complete range of previously published molecular weights, and it is clear that in some of these earlier papers (e.g., Trautmann & Crampton, 1959) the conditions of pH and concentration under which those measurements were made were such that the apparent molecular weight calculated would be far removed from the true molecular weight. As it has been established that this histone fraction is monodisperse, the variation of apparent molecular weight with concentration can now be considered in relation to eqn. (8) and its integrated form (eqn. 16) and becomes:

$$\frac{1}{M^{\text{app.}}} = \frac{1}{M} + B' c_0^* + \text{higher } c_0^* \text{ terms} \quad (18)$$

where:

$$B' = B^* \cdot \frac{A}{2} \cdot \frac{(e^4 + 1)}{(e^4 - 1)}$$

Table 5. *Second virial coefficients derived from equilibrium-sedimentation experiments on histone fraction 1*

	$10^3 B'$	$10^3 B^*$
Preparation 1 in 0.5 M-NaCl	3.7	2.4
Preparation 1 in buffer, pH 8.6 and I 0.5	2.9	1.9
Preparation 2 in 0.1 M-NaCl	5.4	4.5
Preparation 2 in 0.5 M-NaCl	4.6	2.9
Preparation 3 in 0.1 M-NaCl	5.0	4.1

and

$$A = \frac{M^*(1 - \bar{v}_p)(r_b^2 - r_m^2)\omega^2}{2RT}$$

(Fujita, 1962). From the slopes of the plots of $1/M^{app}$ versus $\mathcal{J}_0$, the quantity $B'\lambda/a\psi^*$ can be evaluated where λ is the wavelength of the light used in the optical system of the centrifuge, a is the path length through the solution and ψ^* is a differential refractive increment defined as $\Delta n^*/c^*$. The value of ψ^* was not measured, but the approximation $\psi = \psi^*$ is probably not greatly in error for this purpose, and, by using this approximation together with known values of a and λ , B' and hence B^* can be calculated. These values are given in Table 5.

It has been shown (cf. Casassa & Eisenberg, 1964) that:

$$B^* = \left(\beta_{22}^* + \sum_d \frac{(\nu_d^*)^2}{m_d} \right) \frac{V_s}{(M^*)^2} \quad (19)$$

where ν_d^* is the number of diffusible ions of type d that must be associated with one molecule of

of +18 is calculated. This is smaller than the value of +50 expected on the basis of the amino acid composition of histone fraction 1 and suggests considerable binding of anions to the protein.

Eqn. (20) suggests that B^* should be inversely proportional to the molarity of the salt. This was not found to be the case; B^* was lower in 0.5 M than in 0.1 M-sodium chloride, but not by the factor 5. B^* was also lower at pH 8.6 than at pH 4.5, as would be expected if some of the lysine groups had been titrated at this pH, so lowering the net charge.

Another consequence of the high value of B^* is that the apparent M_z values are less than the apparent M_w values. As pointed out in the discussion of the theory, this occurs because B^* is present in a squared term in Lamm's differential eqns. (12) and (14), but in a first-power term in eqn. (8) for $M_{w(r)}$.

Virial coefficients and charge from refractive-index increments and partial specific volumes. The value of z can also be deduced approximately from equations relating ψ to ψ' and $\bar{v}$ to $\bar{v}'$, which are given in the review by Casassa & Eisenberg (1964). Making the same assumptions about the composition of component 2* as were made in the derivation of an expression for B^* , these equations are:

$$\psi' - \psi = \frac{1}{M_2} \cdot \frac{z}{2} (\psi_{NaCl} M_{NaCl} - \psi_{Na_2SO_4} M_{Na_2SO_4}) \quad (21)$$

and

$$\bar{v}' - \bar{v} = -\frac{z}{2} \cdot \frac{1}{M_2} \left[M_{NaCl} \left(\frac{1}{\rho_{soln.}} - \bar{v}_{NaCl} \right) - M_{Na_2SO_4} \left(\frac{1}{\rho_{soln.}} - \bar{v}_{Na_2SO_4} \right) \right] \quad (22)$$

histone sulphate to produce component 2*, m_d is the molal concentration of that ion in solution and V_s is the volume of solution containing 1000g. of water. β_{22}^* is defined as:

$$\beta_{22}^* = \left(\frac{\partial \ln \gamma_2^*}{\partial m_2} \right)_{T,P}$$

where γ_2^* is the activity coefficient of component 2*.

If we assume that the ions associated with histone sulphate after it has been dialysed are only those that are displaced by the ideal Donnan effect, than at such a limiting dilution the ν^* are related to the charge, z , on the protein cation by $\nu_{Na^+}^* = -z/2$ and $\nu_{Cl^-} = +z/2$ and, ignoring β_{22}^* :

$$B^* = \frac{z^2}{2M_{NaCl}} \cdot \frac{V_s}{(M^*)^2} \quad (20)$$

By substituting the observed value of B^* for solutions in 0.1 M-sodium chloride together with $M^* = 2 \times 10^4$ and $V_s = 10^3$, an approximate z value

The differences in the average values of these quantities for 0.1 M-sodium chloride yield $z = +18$ and $z = +25$ from eqns. (21) and (22) respectively. These are of the same order as the value of +18 deduced from the sedimentation equilibrium, via B^* , although the errors of the differences $\psi' - \psi$ and $\bar{v}' - \bar{v}$ are relatively large, so the agreement is better than the accuracy of the differences really merits.

The optical-rotatory-dispersion measurements agree with those of Bradbury, Crane-Robinson, Phillips, Johns & Murray (1966) and indicate that fraction 1 has a low helical content at low pH and only slightly more at higher pH. The sedimentation-velocity measurements are consistent with an extended structure for this fraction, since combination of the sedimentation coefficient with the molecular weight of the polypeptide chain gives the relatively high frictional ratio, f/f_0 , 1.40 for a molecule of molecular weight 20000.

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