[CONTRIBUTION FROM THE DIVISION OF APPLIED BIOLOGY, NATIONAL RESEARCH COUNCIL OF CANADA]

Partial Specific Volumes of Proteins in Relation to Composition and Environment[1]

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RECEIVED SEPTEMBER 14, 1956

By means of the magnetic float method, the partial specific volumes of edestin and gelatin have been redetermined. The new values fit the general pattern of agreement between observed and calculated partial specific volumes of proteins. Since this correspondence depends on neglecting electrostriction, measurements were made at different values of *p*H, and also in urea solution, on gelatin, ovalbumin and serum albumins. The findings are consistent with the hypothesis that compact protein molecules contain an "excluded volume," which may alter in size under different conditions.

Accurate measurements of the partial specific volume $\bar{v}$ of a protein have hitherto been difficult to make.[3] They are important in the calculation of molecular weight M from ultracentrifuge data, because an error of 1% in $\bar{v}$ results in one of at least 3% in M. In the absence of complicating factors, it should be possible to calculate $\bar{v}$ from the amino acid composition of a protein.[4,5] When this was done, calculated and observed values were the same[6] with a few exceptions, notably those for edestin and gelatin. However, it was necessary to ignore electrostriction to reach this agreement.

The present study was designed to take advantage of the high accuracy to which density determinations may be made by the float method in exploring the relation between $\bar{v}$ and amino acid composition. The effect of environment (*p*H and solvent composition) was examined, and particular attention given to those proteins for which the calculated values did not agree with experimental ones available in the literature.

Materials

Two samples of high quality gelatin were used, both prepared by Eastman Kodak Co. One was derived from calfskin, the other from pigskin. The ash content was found to be less than 0.04% in both cases. A stock solution was prepared by dissolving gelatin in warm water, pouring the solution into a dialysis bag, and dialyzing for some days at 5° against a large volume of water. About 24 hours before measurements were begun, the flask was transferred to a room at 30° where subsequent weighing and adding operations were carried out.

Three preparations of edestin were made. Ultracentrifugal analysis of the first, I, which was made from hemp seed by the method of Bailey,[6] showed a main peak with two smaller peaks. Preparation II, made by a procedure similar to that of Goring and Johnson,[7] gave an ultracentrifuge pattern containing one major and one minor component. A sample of edestin from Nutritional Biochemicals Corp. (lot 1341) was purified by similar methods until the minor heavy component was almost eliminated (preparation III).

Lysozyme was obtained from Armour and Co. (lot 003 L1), and from Dr. L. R. Wetter (lot L.R.W.). It was dissolved in and dialyzed against phosphate buffer (*p*H 6.8, ionic strength 0.1), the small amount of insoluble material being filtered off before dialysis. Since some loss of lysozyme occurs through ordinary membranes, and only 300 mg. of lot L.R.W. was available, dialysis of this particular solution was restricted to 24 hours at room temperature. Samples of lot 003L1 were dialyzed for 24 or 48 hours. Electrophoresis of this lot in phosphate buffer (ionic strength 0.1, *p*H 7.0) gave rise to a single peak.

The majority of experiments on bovine serum albumin were done on lots N67210 and P67403 (Armour and Co.), but lot A1201 (Pentex Inc.) was used in two series. The usual small amounts of heavy component were detectable in the ultracentrifuge patterns. In the absence of added salt, albumin solutions became turbid at about *p*H 2, presumably due to separation of free fatty acid. Samples to be used at low *p*H were dialyzed to *p*H 2 (at about 7% concentration) against very dilute hydrochloric acid. After filtration through fine sintered glass, they were then dialyzed back to about *p*H 5 in water.

The human serum albumin was produced by Cutter Laboratories, according to method 6 of Cohn, *et al.*[8] Both sedimentation and electrophoresis revealed a small amount of contaminating globulin.

Horse serum albumin was prepared by the method of Kekwick.[9] A mixture of fractions A and B was finally made, as the yield was too low to enable the fractions to be studied separately. There was only a very small amount of globulin impurity, as shown by sedimentation and by electrophoresis.

Ovalbumin was obtained from Nutritional Biochemicals Corp. (lot 7030) and Pentex, Inc. (lot B4902). A small amount of insoluble material, apparently produced by surface denaturation, was removed by means of a Spinco preparative ultracentrifuge before the solutions were dialyzed.

Potassium chloride, used to check the calibration of the differential refractometer and the performance of the density apparatus, was purified in the way described by MacInnes and Dayhoff.[10] Otherwise, reagent grade chemicals were used.

Methods

Density Apparatus.—This closely resembled that described by MacInnes, *et al.*[11] The thermoregulator consisted of a 2.5 cm. diameter straight glass tube (containing about 120 ml. of mercury) surmounted by a capillary in which a nickel wire formed the upper contact. The regulator was mounted on the same bracket as the stirrer, since slight vibration improves the stability of regulation. Absolute temperature measurements were made with a platinum resistance thermometer and Mueller bridge. This thermometer was supplemented by a Beckmann, which provided a quick, although less accurate, check on fluctuations. Temperature was steady to ±0.001° during measurements, although slightly larger changes occurred over long time intervals. Readings were all made in the ranges 25.000 ± 0.005° or 30.000 ± 0.005°, henceforth referred to merely as 25 or 30°, respectively.

The Alnico magnets for the floats were reduced in strength by means of a solenoid carrying alternating current. The applied voltage was critical and slightly different for each magnet. Cells and floats were cleaned in cold chromic acid, which had no detectable effect on the float characteristics. Although the current used during measurements never exceeded 100 ma., up to 500 ma. could be passed (in the re-

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verse direction) as a guide to the final weights required. Weights lighter than 20 mg. were dropped through the neck of the cell on to the float, their fall guided by a glass tube. As larger weights tended to bounce off the float, a special glass device was constructed which enabled weights to be released only a short distance above the top of the float. It was not suitable, however, for the smaller weights, which required momentum to break through the liquid surface.

Since air bubbles on the float would vitiate accurate measurements, final dialysis was done slightly above the working temperature. This was not necessary when the dialysate was water alone, as excess dissolved air could be removed under vacuum without significant effect on the density of the water.[12]

The density apparatus, when tested with potassium chloride solutions, gave results which agreed to within 2 or 3 parts per million (p.p.m.) with figures calculated from eq. 4 and 6 of MacInnes and Dayhoff.[10] However, the results of preliminary experiments with bovine serum albumin and gelatin showed more spread than was expected. The positive correlation between the spread and the concentration of stock solution suggested that this scatter was due to two factors associated with the use of weight burets. These are the uncertainties due to solution splashing into inaccessible parts of the cell, and the difficulty of ensuring efficient mixing. Reduction in the concentration of stock solutions produced a marked improvement.

Comparative series were carried out as follows. Stock protein solution was dialyzed near the isoelectric point against water, and part of the solution was used for a set of measurements in this water. A second cell was filled either with water adjusted to just below *p*H 2 with hydrochloric acid or with a buffer solution. After the density of this had been determined, several additions of stock protein solution were made. The proportion of water thus added being known, the composition of the effective solvent at each stage could be calculated, and the density either measured in a separate experiment or deduced on justifiable assumptions. When buffer was absent, conditions were chosen so that the change of *p*H produced by each addition of stock solution involved alterations of charge on the proteins which were negligible so far as this investigation was concerned.

Normally, the weight of solvent (approx. 200 g.) initially in the cell had to be known to only 50 mg., but strong urea solutions had to be weighed on a balance capable of ten times this accuracy at the same load. For experiments with urea, mixtures were made up individually by weight from water and stock solutions of protein and urea. The effective solvent in each case was almost the same, but there were inevitably slight differences in the relative amounts of water and urea. Experiment showed that adequate allowance could be made by using the equations of Gucker, *et al.*,[13] to interpolate the variations in the density of urea solutions over the small ranges involved.

All calculations of apparent specific volume were made with the equation

$$\phi_p = 1/\rho_0 \{1 - (\rho - \rho_0)/n\rho\} \qquad (1)$$

where ρ = density of solution, ρ_0 = density of effective solvent, and n is the weight fraction of protein. Usually $\phi_p = \bar{v}$ for proteins.[3]

Protein Concentrations.—These were mainly determined directly by drying samples of stock protein solutions for 48 hr. in a convection oven at 105–110°. When buffer solutions were used, corrections were made, assuming that the mass of salts present was the same in equal volumes of dialysate and dialyzed protein solution.

Use of refractometer measurements either as a substitute for or as a check on the dry weights was originally intended. Since, however, there are discrepancies in published specific refractive increment values,[14,15] it was deemed necessary to make fresh determinations of these. The differential refractometer used[16] was calibrated by means of data for potassium chloride solutions at 5461 Å.[17] Refractometer measurements at 5780 Å., and parallel dry weight experiments, led to the figures shown in Table I. Since Halwer, *et al.*,[15] assessed the accuracy of their results as no better than ±0.4%, and the measurements in this work were done in a similar manner, the two sets agree within experimental error. There seems to be no obvious reason why Perlmann and Longsworth[14] should have obtained consistently higher results. The figures in the last column of Table I were used whenever protein concentrations were determined solely in the refractometer during the density work.

TABLE I

SPECIFIC REFRACTIVE INCREMENTS (× 10^6) AT 5780 Å. AND 25°

	Observers		
	Perlmann and Longsworth[14]	Halwer, *et al.*[15a]	This work
Lysozyme		1875	1882[b]
Bovine serum albumin	1867	1841	1850
Bovine serum albumin in 0.1 *M* NaCl or KCl	1887	1841	1850
Human serum albumin	1854		1840[b]
Horse serum albumin		1831	1810[b]
Ovalbumin	1851	1807	1809
β-Lactoglobulin	1842	1809	

[a] These measurements, made at 5461 Å., have been corrected to 5780 Å. by use of the dispersion results of Perlmann and Longsworth.[14] [b] Single measurement.

Consideration of Errors in $\bar{v}$.—The limits of error in the density measurements have been dealt with by MacInnes, *et al.*[11] Here attention will be directed to the uncertainties arising in the partial specific volume from errors in density and protein concentration measurements, and from the Donnan effect when dialysis is not done at the isoelectric point. The effect of deficiencies in the knowledge of amino acid composition and the volume contributions of particular amino acid residues on the "theoretical" values of $\bar{v}$ also will be dealt with.

Since the quantity actually measured is $(\rho - \rho_0) = \Delta\rho$, eq. 1 may be rewritten

$$\phi_p = 1/\rho_0 \{1 - \Delta\rho/n(\rho_0 + \Delta\rho)\} \qquad (2)$$

Differentiation of eq. 2 with respect to ρ_0 shows that ϕ_p is relatively insensitive to errors in ρ_0, which need not be considered further.

Equation 2 shows that if $\phi_p = \bar{v}$, the partial specific volume is most accurately assessed by calculating the regression of $\Delta\rho/(\rho_0 + \Delta\rho)$ on n. The slope of this line is equal to $(1 - \rho_0\phi_p)$, and the standard error of slope a measure of internal consistency of a series. As a slightly less informative quantity we can use the mean deviation of points from the regression line.

The effect of an error in $\Delta\rho$ on the value of ϕ_p calculated from a single measurement is obtained by differentiation of eq. 2

$$\partial\phi_p/\partial(\Delta\rho) = -1/n(\rho_0 + \Delta\rho)^2 \approx -1/n \qquad (3)$$

Thus, an error of 1 p.p.m. in $\Delta\rho$ will cause ϕ_p to be incorrect to the extent of 0.0001 and 0.001 at protein concentrations of 1.0 and 0.1%, respectively.

The effect of concentration errors is estimated as

$$\partial\phi_p/\partial n = \Delta\rho/n^2\rho_0(\rho_0 + \Delta\rho) = (1 - \rho_0\phi_p)/n\rho_0 \approx (1 - \phi_p)/n \qquad (4)$$

This relation shows that it is the relative error in the determination of n which is significant. Since errors in weighing the burets are negligible, it is essentially the errors in determining the concentration of stock solution that are effective, and create the same uncertainty independent of the absolute concentration in the cell. A relative error of 0.5% in concentration causes an error of about 0.0013 in ϕ_p in dilute salt solutions.

Experience has shown that the maximum errors in ϕ_p arising from density and concentration errors are generally about 0.001 and 0.002, respectively. Thus the maximum

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TABLE II

PARTIAL SPECIFIC VOLUMES OF GELATIN, EDESTIN AND LYSOZYME

Sample	Temp., °C.	Stock concn. (%)	Concn. range (%)	Solvent	*p*H	Mean $\bar{v}$	Av. dev. (p.p.m.)
Calfskin gelatin	30	2.368	0.11–1.06	Water	4.9	0.6935	1.4
Calfskin gelatin	30	2.450	.15–1.00	Water	4.9	.6956	2.2
Calfskin gelatin	30	2.450	.15–1.03	Water	1.9–2.7	.7047	3.3
Calfskin gelatin	30	3.888	.35–0.95	6.0 *M* urea	5.7–6.3	.6986	16.7
Calfskin gelatin	30	3.524	.12–0.91	Water	5.0	.6935	2.3
Calfskin gelatin	30	3.524	.30–0.86	6.0 *M* urea	5.5–7.4	.6994	19.1
Calfskin gelatin	30	3.819	.38–1.04	7.0 *M* urea	5.7–6.1	.6984	6.4
Calfskin gelatin	30	4.378	.19–1.25	Water	5.0	.6957	1.3
Calfskin gelatin	30	4.378	.31–1.01	7.5 *M* urea	6.0–6.4	.6996	5.3
Pigskin gelatin	30	2.557	.11–1.23	Water	5.5	.6963	2.8
Pigskin gelatin	30	2.089	.11–0.97	Water	5.4	.6961	4.2
Pigskin gelatin	30	2.089	.11– .95	Water	1.7–2.1	.7045	2.2
Edestin, batch I	25	4.026	.33– .86	0.75 *M* NaCl +	7.5	.7250	2.2
Edestin, batch II	25	1.570	.07– .38	0.25 ionic strength	7.5	.7207	1.2
Edestin, batch III	25	1.247	.10– .57	phosphate	7.1	.7274	1.3
Lysozyme (L.R.W.)	25		.10	0.1 ionic strength	6.8	.7234	
Lysozyme (lot 003L1)	25	3.913	.22– .84	phosphate	6.8	.7138	0.8

total from these causes is 0.003, but the standard error is usually about 0.002. However, when the same stock solution has been used to obtain comparative values under different conditions, the accuracy of the difference is limited almost entirely by errors in $\Delta\rho$.

Unless the protein has been dialyzed close to the isoelectric point, the Donnan effect may cause anomalies, since the electrolyte concentration will differ on the two sides of the membrane, whereas it is assumed to be the same for the purposes of determining protein concentration by dry weight or refractometry, and for the purpose of measuring $\Delta\rho$. In the present work edestin and lysozyme were not dialyzed at their isoelectric points. Approximate calculations of the Donnan effect indicated that there would be a partial cancellation of the various volume effects, so that the error would not be excessively large. One sample of bovine serum albumin dialyzed at *p*H 3.2 (Table III) gave results which showed that, in that case, errors arising from the Donnan effect were small.

The apparent specific volume of a protein may be calculated by the formula

$$\phi_p = \Sigma\phi_i W_i/\Sigma W_i \qquad (5)$$

where ϕ_i, W_i are, respectively, the apparent specific volume and weight percentage of a constituent amino acid residue.

$$\partial\phi_p/\partial\phi_i = W_i/\Sigma W_i \qquad (6)$$

and

$$\partial\phi_p/\partial W_i = (\phi_i\Sigma W_i - \Sigma\phi_i W_i)/\Sigma^2 W_i = (\phi_i - \phi_p)/\Sigma W_i \qquad (7)$$

Equations 6 and 7 were used to estimate errors in the "theoretical" value of ϕ_p, on the basis of composition figures given by Tristram,[18] probable errors of amino acid analysis,[18] and probable errors in ϕ_i.[4] A standard error of about 0.004 was arrived at. A small amount of protein impurity, when present, will contribute errors to the composition figures, but the effect on ϕ_p will clearly be small.

Results

The values of $\bar{v}$ obtained with the gelatins are shown in Table II. Both samples, at the isoelectric point, gave a value of about 0.695, substantially greater than 0.682 found by Krishnamurti and Svedberg,[19] and 0.680 given by Kraemer,[20] but still lower than the theoretical figure of 0.707.[5] (Kraemer used 2 *M* potassium thiocyanate as solvent, and reported concentration dependence of $\bar{v}$, the figure of 0.680 being obtained by extrapolation to infinite dilution.) At *p*H 2 the volume was about 1.2% greater than at *p*H 5. The scatter of measurements in urea, at first very great, was much reduced by use of the more accurate balance for weighing out the mixtures. However, the mean values of $\bar{v}$ were the same, all apparently slightly higher than in water at *p*H 5.

The figures obtained with edestin (Table II) showed a relatively large spread between preparations, although there was no correlation with the proportion of heavy components present. The spread is probably due to the difficulty of getting accurate dry weights in the presence of so much salt, but there are also slight uncertainties arising from the Donnan effect. The highest value (for batch III) is 1.2% greater than the theoretical value of 0.719,[5] whereas the only other figure available, 0.744,[21] is much higher, although measured under similar conditions.

Lysozyme (lot 003L1) gave $\bar{v} = 0.7138$ after dialysis for 48 hours (Table II), whereas Colvin[22] found 0.688 for this lot. Single measurements on samples dialyzed for only 24 hours gave values agreeing with 0.7138 within experimental error. The value for batch L.R.W., 0.723, refers to a single measurement at a low concentration, and is hence much less reliable. It is in good agreement with 0.722 which was obtained pycnometrically on the same lot.[23] All measurements made here were quite close to the calculated value of 0.717,[5] but, as with edestin, there are residual uncertainties in the dry weights and Donnan effects.

Thus, the values of $\bar{v}$ found here for edestin and gelatin are appreciably different from those reported previously, both being closer to the theoretical, and thus bringing these substances into line with the general pattern of approximate agreement between theoretical and experimental figures.[5] The new figures for lysozyme confirm that it is no exception either.

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TABLE III

PARTIAL SPECIFIC VOLUMES OF BOVINE SERUM ALBUMIN AT 25°

Lot no.	Stock concn. (%)	Concn. range (%)	Treatment	Mean $\bar{v}$	Av. dev. (p.p.m.)
N67210	4.155	0.14–1.07	Dialyzed in water, *p*H 4.9	0.7381	2.1
N67210	2.603	.13–0.85	Dialyzed in water, *p*H 5.1[a]	.7357	1.8
N67210	3.080	.11–1.06	Dialyzed in 0.1 *M* KCl, *p*H 5.0	.7357	4.6
N67210	3.080	.13–1.06	Dialyzed in 0.1 *M* KCl, *p*H 5.0 and measured at *p*H 2.0–2.6	.7448	2.7
N67210	1.611	.09–0.38	Dialyzed in 0.1 *M* KCl, *p*H 5.1[a]	.7325	1.2
N67210	1.611	.10– .39	Dialyzed in 0.1 *M* KCl, *p*H 5.1[a] and measured at *p*H 1.8–2.1	.7414	2.2
P67403	2.505	.13– .67	Dialyzed in water, *p*H 4.9	.7338	2.1
P67403	2.127	.18– .57	Dialyzed in 0.1 *M* KCl, *p*H 5.3	.7363	0.7
P67403	2.059	.18– .62	Dialyzed in water, *p*H 5.1[a]	.7302	0.6
P67403	2.897	.16– .62	Dialyzed in 0.1 *M* KCl, *p*H 5.2[a]	.7325	2.6
P67403	2.897	.22– .61	Dialyzed in 0.1 *M* KCl, *p*H 5.2[a] and measured at *p*H 1.9–2.4	.7390	2.0
P67403	2.847	.22– .68	Dialyzed in 0.1 *M* KCl, *p*H 3.2[a]	.7386	5.0
A1201	4.118	.15– .89	Dialyzed in water, *p*H 4.8	.7349	1.2
A1201	4.118	.17– .96	Dialyzed in water, *p*H 4.8 and measured in borate buffer (0.02–0.025 *M* borax adjusted to *p*H 10.0)	.7505	6.9
P67403	3.458	.10– .89	Dialyzed in water, *p*H 5.1	.7382	1.0
P67403	3.458	.11– .83	Dialyzed in water, *p*H 5.1 and measured in borate buffer (0.02–0.025 *M* borax adjusted to *p*H 10.6)	.7523	3.0
P67403		.5167	Dialyzed in water, *p*H 5.0 and measured in 8.0 *M* urea, 0.05 *M* borax and 0.005 *M* *p*-chloromercuribenzoate (*p*H 9.8–9.9)	.7460	
P67403		.9223		.7463	
P67403		1.332		.7459	

[a] These samples were pretreated at *p*H 2 (see text).

The results obtained with bovine serum albumin are given in Table III. They show good agreement with the figure of 0.7343 obtained near the isoelectric point by Dayhoff, *et al.*[3] There may be a slight difference between the batches, and also a small decrease in $\bar{v}$ after pretreatment at *p*H 2, but these apparent effects are of the order of experimental error. In lots N67210 and P67403 the partial specific volume near *p*H 2 was approximately 1% greater than near the isoelectric point. Lots P67403 and A1201 gave values near *p*H 10.5 and *p*H 10.0, respectively, which were 2% higher than at the isoelectric point. In 8 *M* urea, at *p*H 9.8–9.9, in the presence of *p*-chloromercuribenzoate, an apparent increase in $\bar{v}$ with time was found to be due entirely to a small decrease in the density of solvent with time. In this solution the protein possessed a $\bar{v}$ about 1% lower than it did at the same *p*H in the absence of urea.

The results of experiments on human serum albumin near the isoelectric point (Table IV) may be compared with 0.736,[24] 0.733[25] and 0.729.[26] The change of $\bar{v}$ between *p*H 5 and *p*H 2 was the same as for bovine serum albumin.

Horse serum albumin gave $\bar{v} = 0.734_5$ (Table IV), close to the values for the other albumins, but considerably lower than 0.748 often quoted.[27] The change of $\bar{v}$ between *p*H 5 and *p*H 2 was again almost identical with that observed for the other albumins.

The results of experiments on ovalbumin are given in Table IV. The mean value near the isoelectric point, 0.7461, was in good agreement with 0.7479 obtained by Dayhoff, *et al.*[3] At *p*H 2 there was an increase of about 1.5%, whereas in 8 *M* urea solution there was a decrease of about 1.3%.

Discussion

Isoelectric Conditions.—It has been shown that the "theoretical" values of $\bar{v}$ are probably fairly accurate, but experimental values quoted by McMeekin and Marshall[5] are more difficult to assess, as in many cases details of the methods used, and probable error, are inadequate, or lacking entirely. Nevertheless, the correspondence between "theoretical" and observed values is reasonably good. So far, however, the effects of electrostriction have been ignored in this comparison. This question has been discussed by Edsall[28] and Waugh.[29] Unless most of the carboxyl ions and charged amino groups are involved in the formation of internal salt linkages, normal electrostriction must occur. The evidence against such linkages has been discussed by Jacobsen and Linderstrøm-Lang.[30] Except for some groups in hemoglobin,[31] all the polar groups in proteins are so readily accessible for titration that they are not likely to be involved in strong internal bonds. Presumably, therefore, the effect of electrostriction is masked by a compensating factor. It seems most likely that this is a steric effect, due to the manner of the folding and packing of the peptide chains, whereby a small volume, the excluded volume, is rendered inaccessible to solvent.[28,29]

Table V shows estimates of the excluded volume, obtained by taking the observed $\bar{v}$ and subtracting the "theoretical" less electrostriction. This last was calculated from composition figures,[18] assum-

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TABLE IV

PARTIAL SPECIFIC VOLUMES OF HUMAN AND HORSE SERUM ALBUMINS AND OVALBUMIN, AT 25°

Sample	Stock concn. (%)	Concn. ranges (%)	Solvent	*p*H	Mean	Av. dev. (p.p.m.)
Human serum albumin	3.173	0.13–0.88	Water	5.4	0.7362	1.9
Human serum albumin	3.173	.14– .90	Water	1.8–2.3	.7435	3.0
Horse serum albumin	1.747	.09– .46	Water	4.8	.7345	0.5
Horse serum albumin	1.747	.08– .40	Water	1.8–2.0	.7406	0.7
Ovalbumin, lot 7030	3.766	.12–1.00	Water	4.8	.7461	3.2
Ovalbumin, lot 7030	3.766	.13–0.91	Water	1.9–2.3	.7540	1.9
Ovalbumin, lot 7030	3.766	.22– .63	8 *M* urea	6.6–6.9	.7375[a]	6.4
Ovalbumin, lot B4902	2.695	.12– .68	Water	5.6	.7462	1.4
Ovalbumin, lot B4902	2.695	.13– .68	Water	1.6–1.8	.7584	2.3

[a] 1.5 hr. after mixing.

TABLE V

PARTIAL SPECIFIC VOLUME DIFFERENCES

Protein sample	$\bar{v}$ (Isoelectric point) Calcd. (electrostriction neglected)	Obsd.	Electrostriction (calcd.)	Excluded vol. (deduced)	$\bar{v}$ (*p*H2) − $\bar{v}$ (*p*H5) Calcd.	Obsd.
Edestin	0.719	0.724[a]	0.024	0.029		
[b]Lysozyme—(lot 003L1)	.717	.714	.022	.019		
Gelatin (calfskin)	.707	.694	.016	.003	0.010	0.009
Gelatin (pigskin)	.707	.696	.016	.005	.010	.009
Bovine serum albumin—(lot N67210)	.734	.735[c]	.026	.027	.016	.009
(lot P67403)	.734	.733[c]	.026	.025	.016	.007
Human serum albumin	.738	.736	.026	.024	.016	.007
Horse serum albumin		.734			.017	.006
Ovalbumin—(lot 7030)	.738	.746	.016	.024	.010	.008
(lot B4902)	.738	.746	.016	.024	.010	.012

[a] Mean value for all three batches near *p*H 7. [b] Figures relate to *p*H 6.8, not to the isoelectric point. [c] Mean values near *p*H 5, in water and in 0.1 *M* potassium chloride.

ing a volume decrease of 18 ml. per charge pair per mole.[28]

The following points emerge from Table V. Edestin, ovalbumin, human serum albumin and bovine serum albumin, which are generally believed to have compact molecules (in water, near the isoelectric point), show an excluded volume of 3–4%, consistent with their probable structure. (No estimate is possible for horse serum albumin, as insufficient details of its composition are available.) On the other hand, gelatin, which behaves in many respects rather like a random coil,[32,33] has little, if any, excluded volume, as might be anticipated.

Acid or Alkaline Conditions.—Titration curves of the proteins used here[34–38] indicate that, at the isoelectric point, all cationic groups are charged. The carboxyl groups, also mostly charged at this point, react with hydrogen ions at low *p*H. This elimination of part of the electrostriction corresponds to a volume increase of about 11 ml./g. equivalent.[39] This increase has been calculated for the gelatins and albumins for comparison with experimental values (Table V). Ovalbumin and gelatin show satisfactory agreement with expectation, but the three serum albumins give a much smaller effect than the calculations suggest. Chloride binding could not account for this, since it would operate in the opposite direction. Hence, it seems necessary to assume that the excluded volume of the serum albumins decreases at low *p*H.

The above observations accord with what is known of the behavior of these proteins. It has been shown that the bovine serum albumin molecule undergoes pronounced changes at low *p*H.[40,41] The large expansion or unfolding which occurs[42,43] would be expected to facilitate penetration by the solvent. The ovalbumin molecule is known to be much less affected by low *p*H.[36] Recent sedimentation studies[44] afford confirmatory evidence for these opinions. With gelatin the possible change of excluded volume is only of the order of experimental error. If real, it is in the direction expected, as a random coil molecule, at low *p*H, would be in a more expanded, readily permeable form.

McMeekin, *et al.*,[45] working with casein, and at much higher concentrations, found a variation of apparent specific volume with concentration at *p*H 3. The result they obtained by extrapolation to zero protein concentration did not differ signifi-

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cantly from the normal $\bar{v}$. If their extrapolation is reliable, this would indicate that, in casein, the volume increase due to diminished electrostriction, and the decrease resulting from a smaller excluded volume effect, are almost of the same magnitude. If this is so, the alteration in excluded volume should be reflected in variations in other properties of the molecule at low *p*H.

The interpretation of results obtained under alkaline conditions is more complicated, as several different types of groups are titrated above the isoelectric point. An estimate was made, using the data of Weber[39] on the volume changes accompanying the reactions of these groups, and the titration figures of Tanford, *et al.*,[38] of the change in $\bar{v}$ to be expected between *p*H 5 and *p*H 10.0–10.5. For bovine serum albumin this was 0.0168 ml./g., compared with the observed change of 0.0141–0.0156 ml./g. (Table III). This is quite good agreement, in view of the uncertainties. Moreover, this finding is also in accord with recent sedimentation measurements.[44]

Urea Solutions.—Neurath and Saum[46] and McMeekin, *et al.*,[45] could find no difference between the partial specific volumes in water and urea solutions for horse serum albumin and casein, respectively, and McKenzie, *et al.*,[47] reported normal values for ovalbumin and bovine serum albumin in urea. All these workers used the pycnometric method. On the other hand, Christensen[48] and Simpson and Kauzmann,[49] using dilatometers, observed a decrease in volume for β-lactoglobulin and ovalbumin, respectively, when solutions of these proteins were exposed to urea. The figure given for ovalbumin[49] is about 300 ml./mole in 6 to 8 *M* urea at 30°. The corresponding figure calculated from the results in Table IV is about 380 ml./mole. Since the accuracy of this estimate is not likely to be better than 15%, and it refers to slightly different conditions, the agreement may be considered quite satisfactory. The direction of the change is consistent with the idea of an opening out of the molecule under the action of urea, the excluded volume thereby being partly eliminated.

The effect of urea on the partial specific volume of bovine serum albumin (Table III) was quantitatively almost the same as with ovalbumin. This is consistent with other observations[50] which were interpreted as indicating some unfolding of the protein molecule in urea solution.

The effect of urea on gelatin is less easy to assess, because of the larger experimental errors. The apparent slight increase in $\bar{v}$ is possibly not significant. Since the gelatin molecule possesses little organized structure, it would not be anticipated that urea would profoundly influence its properties.

The results obtained in this work are consistent with our knowledge of the composition and structure of the proteins used, and to that extent confirm current views. It would be desirable to examine other proteins listed by McMeekin and Marshall[5] to see whether there are any exceptions to the general pattern established for those proteins studied recently in the magnetic float apparatus. It would also be interesting to study the behavior of suitable synthetic polyampholytes.

Acknowledgments.—The author is greatly indebted to Mr. J. Giroux for technical assistance. He also wishes to thank Mr. D. R. Muirhead for doing the sedimentation experiments. Helpful discussions were held with Dr. W. H. Cook, Dr. D. B. Smith and Mr. W. G. Martin, during both the work and the preparation of the final manuscript. Thanks are also due to Dr. L. R. Wetter for a sample of lysozyme and to Dr. C. A. Mitchell of the Animal Diseases Research Institute, Hull, Que., for supplying the horse serum.

OTTAWA, CANADA

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