CLIII. THE EFFECT OF HYDROGEN ION CONCENTRATION AND PROTEIN CONCENTRATION ON THE OSMOTIC PRESSURE OF SERUM-PROTEINS.

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SEVERAL workers have shown that the osmotic pressure of serum- or plasma-proteins, in solutions of increasing protein concentration, increases more rapidly than the protein concentration.

Verney [1926] has shown that this relation is such as would be found if the protein in solution occupied many times its dry volume. There are, however, other factors which will cause a deviation from a linear relation between observed osmotic pressure and protein concentration. Particularly, there is an excess of diffusible ions in the protein solution over that in the outside solution, due to the Donnan effect; this excess of diffusible ions would, if the solutions were ideal, have an osmotic pressure, which we will call the Donnan pressure, over and above that due to the protein itself; and this Donnan pressure would be proportional to the square of the protein concentration, when the salt concentration of the outside fluid and the reaction were fixed. As a consequence the observed osmotic pressure (y) and protein concentration (x), at any particular p_H , would be connected by an equation of the form

$$y = ax + bx^2 \quad \dots\dots(1),$$

a parabola convex to the axis of x , very similar to the curves found by Mayrs [1926] and Verney [1926].

The term b in equation (1) would vary with the p_H , so that the observed osmotic pressure should also vary with the p_H . Mayrs however was unable to detect such variation.

We have therefore studied the effect of varying protein concentration and p_H on the observed osmotic pressure, with the idea that although the actual effect of the Donnan equilibrium would not agree with that calculated for ideal solutions, it would still be demonstrable, and with the hope of showing deviations from ideality which will affect the relation between the observed osmotic pressure and the protein concentration.

The excess of diffusible ions in the protein solution, and the osmotic pressure resulting therefrom.

Symbols.

[Pr] = concentration of protein in the protein solution in g. per 1000 g. H₂O.

[Pr⁻] = concentration of protein ions in the protein solution in millequivalents per 1000 g. H₂O.

[BPr] = concentration of base bound by protein in the protein solution in millequivalents per 1000 g. H₂O.

[A_P⁻] = concentration of diffusible anions in the protein solution in millequivalents per 1000 g. H₂O.

[B_P⁺] = concentration of diffusible kations in the protein solution in millequivalents per 1000 g. H₂O.

[B_P] = concentration of total base in the protein solution in millequivalents per 1000 g. H₂O.

[Cl_P] = concentration of chlorine in the protein solution in millequivalents per 1000 g. H₂O.

[Cl_P⁻] = concentration of chlorine ions in the protein solution in millequivalents per 1000 g. H₂O.

[HCO_{3P}⁻] = concentration of bicarbonate ions in the protein solution in millequivalents per 1000 g. H₂O.

[A_E⁻] = concentration of diffusible anions in the external solution in millequivalents per 1000 g. H₂O.

[B_E⁺] = concentration of diffusible kations in the external solution in millequivalents per 1000 g. H₂O.

α_{A_P} , α_{B_P} , α_{Cl_P} , etc. = the activities of the ions in the protein solution.

α_{A_E} , α_{B_E} , α_{Cl_E} , etc. = the activities of the ions in the external solution.

f_{A_P} , f_{B_P} , f_{Cl_P} , etc. and f_{A_E} , f_{B_E} , f_{Cl_E} , etc. = the activity coefficients of the ions in the two solutions.

Then $\alpha_{A_P} = f_{A_P} [A_P^-]$; $\alpha_{A_E} = f_{A_E} [A_E^-]$, etc.(2).

Let $[Pr^-] = k [BPr]$.

Now $[B_P^+] = [A_P^-] + [Pr^-] = [A_P^-] + k [BPr]$ (3),

and

$$f_{A_P} [A_P^-] \cdot f_{B_P} [B_P^+] = \alpha_{A_P} \cdot \alpha_{B_P} = \alpha_{A_E} \cdot \alpha_{B_E} = f_{A_E} [A_E^-] \cdot f_{B_E} [B_E^+] = f_{A_E} \cdot f_{B_E} \cdot [A_E^-]^2 \quad \text{.....(4)}.$$

$$\therefore f_{A_P} \cdot f_{B_P} \cdot [A_P^-] \{ [A_P^-] + k [BPr] \} = f_{A_E} \cdot f_{B_E} \cdot [A_E^-]^2.$$

$$\begin{aligned} \therefore [A_P^-] &= \left[\frac{f_{A_E} \cdot f_{B_E}}{f_{A_P} \cdot f_{B_P}} [A_E^-]^2 + \frac{k^2}{4} [BPr]^2 \right]^{\frac{1}{2}} - \frac{k}{2} [BPr] \\ &= \left(\frac{f_{A_E} \cdot f_{B_E}}{f_{A_P} \cdot f_{B_P}} \right)^{\frac{1}{2}} [A_E^-] + \frac{k^2}{8} \left(\frac{f_{A_P} \cdot f_{B_P}}{f_{A_E} \cdot f_{B_E}} \right)^{\frac{1}{2}} \cdot \frac{[BPr]^2}{[A_E^-]} - \frac{k}{2} [BPr] \quad \text{.....(5)}. \end{aligned}$$

If $k [\text{BPr}]$ is small compared with $[\text{A}_E^-]$;

$$\text{similarly } [\text{B}_P^-] = \left(\frac{f_{A_E} \cdot f_{B_E}}{f_{A_P} \cdot f_{B_P}} \right)^{\frac{1}{2}} [\text{A}_E^-] + \frac{k^2}{8} \left(\frac{f_{A_P} \cdot f_{B_P}}{f_{A_E} \cdot f_{B_E}} \right)^{\frac{1}{2}} \frac{[\text{BPr}]^2}{[\text{A}_E^-]} + \frac{k}{2} [\text{BPr}] \quad \dots(6).$$

$$\therefore [\text{A}_P^-] + [\text{B}_P^-] = 2 \left(\frac{f_{A_E} \cdot f_{B_E}}{f_{A_P} \cdot f_{B_P}} \right)^{\frac{1}{2}} [\text{A}_E^-] + \frac{k^2}{4} \left(\frac{f_{A_P} \cdot f_{B_P}}{f_{A_E} \cdot f_{B_E}} \right)^{\frac{1}{2}} \frac{[\text{BPr}]^2}{[\text{A}_E^-]} \quad \dots\dots(7).$$

If the effect of the protein on the activity coefficients of the diffusible ions is negligible, this becomes

$$[\text{A}_P^-] + [\text{B}_P^-] = 2 [\text{A}_E^-] + \frac{k^2 [\text{BPr}]^2}{4 [\text{A}_E^-]},$$

that is, the excess of diffusible ions in the protein solution equals

$$\frac{k^2}{4} \cdot \frac{[\text{BPr}]^2}{[\text{A}_E^-]}.$$

The osmotic pressure at 37° due to this excess, when $[\text{A}_E^-] = 160$, will be

$$26 \times 0.938 \times \frac{k^2 [\text{BPr}]^2}{4 [\text{A}_E^-]} \text{ cm. H}_2\text{O}.$$

The coefficient 0.938 is derived from Bronsted's [1921] formula

$$1 - \phi = az_1z_2\sqrt{\mu} + \beta c,$$

and the values for the activity coefficients at this ionic strength from Scatchard [1925].

If we assume that the activity coefficient of the protein does not change with varying protein concentration, the observed osmotic pressure (π) at constant p_{H} will be given by the equation

$$\pi = 26,000 \frac{[\text{Pr}]}{M} + 26 \times 0.938 \times \frac{k^2}{4} \times \frac{[\text{BPr}]^2}{160} \quad \dots\dots(9),$$

(where M is the molecular weight of the protein), which is a parabola convex to the $[\text{Pr}]$ axis. Also, if the activity coefficient of the protein is not affected by change of hydrogen ion concentration, the observed osmotic pressure of a solution of constant protein concentration will rise with rising p_{H} by the amount,

$$\Delta\pi = \frac{26 \times 0.938}{4 \times 160} \times k^2 \times 0.097^2 [\text{Pr}]^2 [(p_{\text{H}_2} - 5.26)^2 - (p_{\text{H}_1} - 5.26)^2] \quad \dots\dots(10)$$

using equation (11) (see p. 1135) for $[\text{BPr}]$.

If $k = 1$, the second term in equation (9) is equal to 13.1 cm. when $[\text{Pr}] = 70$ and the p_{H} is 8.0; and $\Delta\pi$ in equation (10) is equal to 7.6 cm. when $[\text{Pr}] = 70$ and $p_{\text{H}_1} = 7.0$, $p_{\text{H}_2} = 8.0$; thus the effect of this unequal distribution of diffusible ions will be by no means negligible if the assumptions with regard to activity coefficients are correct and the protein salt is fully dissociated.

METHODS.

Horse serum was separated from blood obtained from the slaughter house and allowed to clot. Human serum was obtained by shaking blood from two normal males (J. M. and L. H.) with glass beads to remove fibrin and centrifuging. The human sera were obtained under sterile conditions and both horse and human sera were filtered through a Seitz filter before use. The lower concentrations of protein were obtained by dilution with 0.9 % sodium chloride solution.

Fig. 1.

The osmotic pressures were measured with the apparatus shown in Fig. 1. The large bottle contained about 1 l. of a bicarbonate-carbon dioxide buffer mixture, containing phenolsulphonephthalein. The bicarbonate solution was first sterilised by boiling in a litre flask; CO_2 was then passed into it through

a sterile cotton-wool filter, until the p_H was approximately that required. When necessary the p_H was adjusted more accurately after the solution had been placed in the bottle by running a CO_2 -air mixture of known composition in through the tap *A* and out through *B*.

When the apparatus was in a water-bath the pressure inside the bottle was adjusted to atmospheric pressure by opening tap *B*.

Collodion sacs were made inside test-tubes using an 8 % solution of collodion (British Drug Houses) in a mixture of alcohol (50 volumes), ether (50 volumes) and glacial acetic acid (10 volumes). We are indebted to Mrs Dover who prepared many of the sacs used.

These sacs were attached to pressure tubing with rubber bands; they were made to hold about 8 cc. To check their impermeability to protein they were filled with serum, the rubber tubing was closed with a glass rod, and the sacs were placed for a night in normal saline, which was then tested with sulphosalicylic acid; if the faintest cloud appeared the sacs were rejected.

The sacs were found to be readily permeable to phenolsulphonephthalein; if they were filled with water and placed in a phenolsulphonephthalein solution over night, the concentrations of dye in the external and internal fluids were found to be the same next morning. The permeability was not altered by the process of sterilisation.

The capillary tubes had a bore of about 1 mm.; it was found that narrower tubes were unsatisfactory as the protein solution was liable to stick. Dilution of the protein solutions could be avoided by forcing the capillary tubes well into the collodion sacs so that the fluid rose in the capillary to about the height anticipated.

Those parts of the apparatus which could safely be heated were sterilised by autoclaving or by dry heat. The remainder, including the collodion membranes, were sterilised by soaking for 15 minutes with 70 % alcohol, which was washed off with sterile saline, or with alcohol and ether if the parts were required to be dry. The whole apparatus was assembled with aseptic precautions by an operator wearing sterile gloves.

These methods of sterilisation proved entirely satisfactory; even in subsequent experiments with egg-albumin we have had no bacterial contamination. The Seitz filters have proved most valuable when dealing with protein solutions which were not sterile to start with.

The apparatus was kept at 0° in a bath of ice and water for one day in order to let substances such as urea and electrolytes other than those present in the buffer solution dialyse out. The external fluid was then changed and the apparatus placed in a water-bath at 37° for 2 or more days. It was gently rocked and finally allowed to stand with the capillary tubes vertical; this final period without rocking was necessary, as during the short periods during which the bottle was tilted the vertical heights of the columns in the capillary tubes were reduced.

In the later experiments the gas pressure above the column of fluid was

equal to that above the external fluid; in order to read the osmotic pressures it was only necessary to mark the heights of the columns of fluid in the capillary tubes, remove the apparatus from the water-bath and read the levels of these marks and of the surface of the external fluid with a vertical mirror scale.

In the earlier experiments the apparatus was slightly different. Taps were carried on the ends of the capillary tubes to which the collodion sacs were attached. These taps were kept closed during the dialysis. When an osmotic pressure was to be read the height of the column of fluid in a capillary was marked, and without removing the apparatus from the water-bath the tap was connected with a water manometer and opened. The manometer pressure was then adjusted until the surface of the protein solution in the capillary was the same as before the tap was opened; this gave the gas pressure above the column in the capillary tube. The height of the mark on the capillary tube was then measured in the same way as in the later experiments; the osmotic pressure was equal to the pressure of this column of fluid plus the manometer pressure. This method was much less satisfactory than the simpler method of the later experiments and the results are given only to show that even with less accurate methods fairly consistent results can be obtained.

In calculating the osmotic pressure, allowance was made for the specific gravity of the protein solutions, and for the rise due to surface tension.

After the osmotic pressures had been read, the apparatus was replaced in the water-bath to reheat (little cooling took place during the short time required for reading the heights of the columns) and a sample of gas was taken into a gas sampler full of mercury attached to tap *B*; clean mercury was run in by tap *A* at the same time to drive the gas out. Before this process dead spaces were filled with mercury. This gas sample was analysed in a Haldane apparatus.

A sample of the external fluid was then taken and the protein solutions were run into test-tubes with precautions to avoid letting the fluids in the capillaries run out, as these fluids might differ from those in the sacs.

The *total nitrogen* in the internal fluids was estimated by Kjeldahl's method; since other nitrogenous substances would have diffused away into the large volume of external fluid this nitrogen was assumed to be that of protein and the protein present was calculated, using 6.45 as the factor.

Chlorides were estimated by heating 1 cc. of fluid in a water-bath with excess of silver nitrate and nitric acid, and titrating the excess of silver nitrate with potassium thiosulphate, using iron alum as indicator. The error in this estimation was not more than 0.5 %.

Bicarbonates were estimated in the external fluid by boiling 20 cc. with a slight excess of *N*/10 HCl and titrating the excess with *N*/10 NaOH with methyl red as indicator. The bicarbonate concentrations in the protein solutions were calculated on the assumption that the ratio

$$\frac{\text{HCO}_3 \text{ in protein solution}}{\text{HCO}_3 \text{ in external solution}} \text{ was the same as } \frac{\text{Cl in protein solution}}{\text{Cl in external solution}}.$$

The p_H of the external fluid was calculated from the CO_2 tension and bicarbonate concentration by the formula

$$p_H = 6.1 + \log [\text{BHCO}_3^-] - \log p\text{CO}_2 - \log 0.0326.$$

The *total base* was estimated by the method of Stadie and Ross [1925]. The proteins of the protein solutions were first precipitated with trichloroacetic acid. In the external fluids the total base was found to be equivalent to the sum of the chloride and bicarbonate found, within 1 % in almost all cases.

In the estimations of chlorides and total base all quantities under 5 cc. were weighed, in order to avoid errors in the discharge of pipettes.

Specific gravities were estimated by weighing in a pycnometer; dry weights by drying a known volume to constant weight in an oven at 100° – 110° and cooling in a desiccator.

In preliminary experiments it was found that appreciable loss of CO_2 by diffusion through the rubber stopper, and changes in alkalinity of the buffer mixture from solution of glass, did not occur.

Base bound by protein.

The values for $[\text{BPr}]$ given in Table I are obtained by subtracting $[\text{Cl}^-] + [\text{HCO}_3^-]$ from the total $[\text{B}_P]$. The estimation by this means must be inaccurate as it is given by the small difference between two large quantities; nevertheless the results are fairly consistent. For human serum-proteins the results and their relation to p_H (Fig. 2) agree fairly well with the formula given by Hastings, Salvesen, Sendroy and Van Slyke [1927]

$$[\text{BPr}] = 0.097 [\text{Pr}] (p_H - 5.26) \quad (\text{curve A, Fig. 2}) \quad \dots\dots(11),$$

though they are slightly higher¹, but our results for horse serum-protein are consistently higher than those given by the formula of Van Slyke, Wu and MacLean [1923]

$$[\text{BPr}] = 0.068 [\text{Pr}] (p_H - 4.80) \quad (\text{curve B, Fig. 2}) \quad \dots\dots(12).$$

Fig. 2. Relation of base bound by protein to p_H .

¹ The p_H in our calculations is that of the external fluid, not of the protein solution; the effect of this however is very small and in the opposite direction to the difference found.

Table I. *Distribution of Cl, HCO₃ and base.*

Expt. No.	Serum	External fluid					Protein solution						
		<i>p_H</i>	[Cl]	[HCO ₃]	$\frac{[\text{Cl}]}{[\text{HCO}_3]} +$	[Pr]	[Cl]	[HCO ₃]	[B]	[BPr]	[Pr]	$\frac{[\text{Cl}_P]}{[\text{Cl}_E]}$	$\frac{[\text{B}_E]}{[\text{B}_P]} \frac{[\text{Cl}_P][\text{B}_E]}{[\text{Cl}_E][\text{B}_P]}$
11 b	J. M.	6.4	142.0	—	—	57.2	142.3	—	—	—	—	1.002	—
5 a	"	6.7	139.9	14.5	154.4	63.9	138.7	14.4	161.8	8.7	0.136	0.991	0.9545 0.963
5 b	"	6.7	139.9	14.5	154.4	26.3	140.4	14.5	159.5	4.6	0.175	1.003	0.969 0.966
3 a	"	6.9	144.9	15.0	159.9	50.4	143.7	14.9	168.0	9.4	0.187	0.9915	0.952 0.961
3 b	"	6.9	144.9	15.0	159.9	34.5	145.0	15.0	—	—	—	1.001	—
12 a	"	7.8	142.9	14.8	157.7	50.3	140.1	14.5	170.3	15.7	0.314	0.980	0.9265 0.943
13 a	"	8.0	141.6	14.9	156.5	62.3	136.2	14.4	171.1	20.5	0.330	0.962	0.915 0.951
4 a	"	8.3	139.9	14.5	154.4	67.8	135.1	14.0	167.9	18.8	0.282	0.965	0.9195 0.948
4 b	"	8.3	139.9	14.5	154.4	37.1	137.1	14.2	163.2	11.9	0.315	0.987	0.946 0.965
6 a	"	6.4	288.9	14.6	303.5	66.9	292.2	14.8	316.1	9.1	0.136	1.011	0.960 0.949
6 b	"	6.4	288.9	14.6	303.5	36.7	292.3	14.8	—	—	—	1.021	—
7 a	"	8.2	288.2	14.6	302.8	64.8	283.1	14.3	318.6	21.2	0.328	0.982	0.950 0.969
7 b	"	8.2	288.2	14.6	302.8	32.2	287.0	14.5	—	—	—	0.996	—
11 a	L. H.	6.4	142.0	15.0	157.0	74.0	140.8	14.9	167.5	11.8	0.160	0.9915	0.9375 0.944
9 a	"	6.7	142.6	15.0	157.6	67.6	139.7	14.7	166.6	12.2	0.180	0.9795	0.946 0.965
9 b	"	6.7	142.6	15.0	157.6	40.9	141.5	14.9	—	—	—	0.992	—
10 a	"	7.8	142.4	14.9	157.3	63.6	137.1	14.4	—	—	—	0.963	—
12 b	"	7.8	142.9	14.8	157.7	68.6	138.2	14.3	171.5	19.0	0.277	0.967	0.920 0.949
1 a	Horse	6.9	125.4	30.6	156.0	70.5	122.0	29.7	166.4	14.7	0.208	0.971	0.9375 0.963
1 b	"	6.9	125.4	30.6	156.0	42.5	124.0	30.2	163.0	8.8	0.207	0.989	0.957 0.966
2 a	"	8.2	124.4	29.4	153.8	68.9	118.3	27.9	167.4	21.2	0.308	0.951	0.919 0.966
2 b	"	8.2	124.4	29.4	153.8	42.6	121.6	28.7	162.5	12.2	0.286	0.9775	0.9465 0.969

Distribution of ions.

We find in all our experiments (Table II) that

$$\frac{[\text{Cl}_P]}{[\text{Cl}_E]} > \frac{[\text{B}_E^+]}{[\text{B}_P]} \quad \text{.....(13),}$$

or
$$\frac{[\text{Cl}_P]}{[\text{Cl}_E]} \frac{[\text{B}_P]}{[\text{B}_E^+]} > 1 \quad \text{.....(13 a)}^1.$$

Now
$$\frac{f_{\text{Cl}_P} [\text{Cl}_P]}{f_{\text{Cl}_E} [\text{Cl}_E]} = \frac{\alpha_{\text{Cl}_P}}{\alpha_{\text{Cl}_E}} = \frac{\alpha_{\text{B}_E}}{\alpha_{\text{B}_P}} = \frac{f_{\text{B}_E} [\text{B}_E^+]}{f_{\text{B}_P} [\text{B}_P^+]} \quad \text{.....(14).}$$

It follows that either $\frac{f_{\text{Cl}_P}}{f_{\text{Cl}_E}} < \frac{f_{\text{B}_E}}{f_{\text{B}_P}}$ or the ions form undissociated compounds with protein (making $[\text{Cl}_P^-] < [\text{Cl}_P]$ or $[\text{B}_P^+] < [\text{B}_P]$).

Our experiments give us no means of estimating the actual values of $\frac{\alpha_{\text{Cl}_P}}{\alpha_{\text{Cl}_E}}$ or $\frac{\alpha_{\text{B}_E}}{\alpha_{\text{B}_P}}$; but in somewhat similar experiments Hastings, Salvesen, Sendroy,

and Van Slyke found $\frac{[\text{B}_E]}{[\text{B}_P]}$ about equal to $\frac{\alpha_{\text{H}_E}}{\alpha_{\text{H}_P}}$.

Now
$$\frac{\alpha_{\text{H}_E}}{\alpha_{\text{H}_P}} = \frac{\alpha_{\text{B}_E}}{\alpha_{\text{B}_P}} = \frac{f_{\text{B}_E} [\text{B}_E^+]}{f_{\text{B}_P} [\text{B}_P^+]}$$

so that
$$\frac{[\text{B}_E^+]}{[\text{B}_P]} = \frac{f_{\text{B}_E} [\text{B}_E^+]}{f_{\text{B}_P} [\text{B}_P^+]} \quad \text{.....(15),}$$

from which it appears probable that

$$f_{\text{B}_E} = f_{\text{B}_P} \quad \text{.....(16),}$$

¹ The sum of the errors in these estimations should not exceed 2 %; the actual difference found between $[\text{Cl}_P][\text{B}_P]$ and $[\text{Cl}_E][\text{B}_E^-]$ always exceeds 3 %.

and that $[B_P] = [B_P^+]$, in other words that all the kation is ionised (if un-ionised kation were present f_{B_P} would have to be actually greater than f_{B_E} , which is improbable).

From (13), (14) and (15) we infer that $f_{Cl_P} < f_{Cl_E}$, or that $[Cl_P] > [Cl_P^-]$ (that is, that some of the Cl present in the protein solution is in an undissociated condition even on the alkaline side of the iso-electric point). Since $\frac{[Cl_P][B_P]}{[Cl_E^-][B_P^+]}$ decreases, if anything, with increasing p_H , it is probable that the first is the correct explanation, that is, that $f_{Cl_P} < f_{Cl_E}$.

The lowest value of $\frac{f_{Cl_P}}{f_{Cl_E}}$ so reckoned is 0.935, so that the effect of protein on the activity coefficient is not great; it appears to increase only slightly when the p_H increases from about 6.7 to about 8.0, that is when the charge on the protein is about doubled. Although the protein ion is polyvalent, the charges are probably so far apart that they have little more effect on the activity coefficients of other ions than would equivalent charges on separate ions; it is quite possible that weak electric fields round numerous polar groups at the links between individual amino-acids have more effect than do the few charges on the molecule.

The excess of diffusible ions found in the protein solution is much greater than calculated by equation (9) on the assumption that the activity coefficients of the diffusible ions were unaffected by the protein. It is not possible to reckon the osmotic pressure due to this difference simply with the aid of the calculated activity coefficients, for the osmotic coefficient involves an integration and requires a knowledge of the equation connecting activity coefficient and protein concentration; but the actual osmotic pressure due to this difference is not necessarily less than that calculated for ideal solutions.

Observed osmotic pressures.

It can be seen from Fig. 3 and Table II that the osmotic pressures found for the same serum, at the same hydrogen ion concentration and salt concentration in the external fluid, agree very well; the differences between the three sera used (two human and one horse) are what would be expected from the difference of the albumin to globulin ratios. The only result which is out of agreement is that of Exp. 4 *a*, which was obtained by the first method and the discrepancy in the case is not too great to be accounted for by the disturbances that occurred in this experiment.

Our results agree well with those of Verney [1926] and Mayrs [1926]; the osmotic pressures are uniformly higher owing to the higher temperature at which we have worked.

An increase of osmotic pressure with a rise of the p_H was detected in our experiments in which the more accurate method was used, even with low protein concentrations (Exps. 13 *c* and *d*), and with such small changes of p_H

Table II. *Osmotic pressures of serum-proteins.*

Experiment Later method	Serum	p_H ext. fluid	[Pr]	Albn. $\frac{\text{Gtobn.}}{\text{Gtobn.}}$	Osmotic pressure cm. of water		Remarks
					0°	37°	
9 a	L. H.	6.7	67.6	$\frac{2.9}{1}$	29.9	32.8	
9 b	"	6.7	40.9	$\frac{2.9}{1}$	15.4	17.6	
11 a	"	6.4	74.0	$\frac{2.8}{1}$	33.6	36.0	
12 a	"	7.5	68.6	$\frac{2.8}{1}$	33.2	36.8	
12 b	"	7.8	68.6	$\frac{2.8}{1}$	—	37.5	
10 a	"	7.8	63.6	$\frac{2.9}{1}$	30.1	33.65	
10 b	"	7.8	36.4	$\frac{2.9}{1}$	13.5	15.9	
8 a	J. M.	6.4	64.8	$\frac{2.3}{1}$	27.7	29.9	
8 b	"	6.4	21.2	$\frac{2.3}{1}$	—	7.4	
11 b	"	6.4	57.2	$\frac{2.2}{1}$	—	24.9	
12 c	"	7.5	50.3	$\frac{2.2}{1}$	—	22.9	
12 d	"	7.8	50.3	$\frac{2.2}{1}$	—	23.3	
13 a	"	6.8	62.3	$\frac{2.2}{1}$	27.8	29.9	
13 b	"	8.0	62.3	$\frac{2.2}{1}$	—	32.2	
13 c	"	6.8	38.6	$\frac{2.2}{1}$	15.0	15.6	
13 d	"	8.0	38.6	$\frac{2.2}{1}$	—	16.4	
Earlier method							
3 a	"	6.9	50.4	—	—	22.3	
5 a	"	6.7	63.9	$\frac{2.37}{1.00}$	—	30.3	
5 b	"	6.7	26.3	$\frac{2.37}{1.00}$	—	11.1	
4 a	"	8.3	67.8	$\frac{2.40}{1.00}$	—	33.3	Temperature regulation disturbed
4 b	"	8.3	37.1	$\frac{2.40}{1.00}$	—	15.8	Temperature regulation disturbed
6 a	"	6.4	66.9	$\frac{2.31}{1.00}$	—	33.3	Ext. fluid about 0.3 M
6 b	"	6.4	36.7	$\frac{2.31}{1.00}$	—	17.7	" " "
7 a	"	8.3	64.4	$\frac{2.31}{1.00}$	—	32.0	" " "
7 b	"	8.3	32.3	$\frac{2.31}{1.00}$	—	13.5	" " "
1 a	Horse	6.9	70.5	$\frac{0.88}{1.00}$	—	29.6	
1 b	"	6.9	42.5	$\frac{0.88}{1.00}$	—	15.9	
2 a	"	8.2	68.9	$\frac{0.88}{1.00}$	—	29.7	
2 b	"	8.2	42.6	$\frac{0.88}{1.00}$	—	15.7	

as that from 7.5 to 7.8 (Exps. 12 *a*, *b*, *c*, *d*). The changes of osmotic pressure found were, however, much less than those calculated for ideal solutions by equation (9). It is probable that the increase of total osmotic pressure due to the greater excess of diffusible ions in the protein solution at the higher p_H is to some extent balanced by a reduction of the activity coefficient of the protein molecules when they are more highly ionised; such a reduction of activity coefficient with increased ionisation has been found by Cohn and Prentiss [1927] in the case of haemoglobin.

Fig. 3.

It is not therefore necessary to assume from the slight effect of change of p_H that the Donnan osmotic effect is much less than that calculated for ideal solutions and inadequate to account for a large part of the deviation of the protein concentration-osmotic pressure curve from a straight line.

With increase of the salt concentration of the external fluid the Donnan effect should be proportionately reduced, and consequently the relation between protein concentration and osmotic pressure should be more nearly linear. This we have found in Exps. 6 *a*, *b* and 7 *a*, *b*.

The changes of osmotic pressure with temperature are on the whole rather less than would be expected. The irregularity in the results is probably due to inaccuracies in the estimation at 0°, as the time allowed at this temperature was probably insufficient for the full pressure to be registered in all cases; if this is the case the lower values for $\frac{\text{O.P. at } 37^\circ}{\text{O.P. at } 0^\circ}$ are probably the more correct.

The results at any rate suggest that there is no great change in the physical condition of the proteins in passing from 0° to 37°.

Hydration of protein.

The relation of osmotic pressure to protein concentration can be explained as shown by Verney by supposing that the protein in solution occupies a much greater volume than it occupies when dry; this could only be the case if the protein was surrounded by a large water sheath ("hydration") or enclosed by a large volume of water (as probably occurs in gelatin solution). The latter supposition can be shown (from consideration of the osmotic pressures found and the possible area of the protein molecule) to be impossible in the case of the proteins we are considering. The solubility of non-polar substances, such as nitrogen [Stoddart, 1927], in protein solutions shows that hydration of proteins, in the sense that lithium ions, for example, are hydrated, does not occur to any great extent. It may be supposed that proteins, in some unknown way, hold a sheath of water which behaves as a solvent just as does the main bulk of the water, the whole mass forming an "omnibus" of protein, water and other solutes. It must however be remembered that the chief evidence for this so-called "hydration" is based on the high viscosities of protein solutions which can be explained in other ways.

We are now further studying osmotic pressures and the effect of proteins on activity coefficients, using individual proteins.

SUMMARY.

1. The observed osmotic pressures of serum-proteins rise with increase of p_H .
2. This increase is less than that calculated for ideal solutions if the compound of protein and base is fully dissociated.
3. The activity coefficients of anions are slightly reduced by the presence of proteins in solution.
4. Both the excess of diffusible ions in the protein solution (due to the Donnan effect) and deviations from ideality will cause the observed osmotic pressures of proteins not to bear a linear relation to the protein concentration. It is not necessary to suppose that proteins in solution occupy a greater bulk than they do when dry.

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