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THE COLLOIDAL BEHAVIOR OF PROTEINS.

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I.

Colloids show a number of peculiarities which at first appearance seem to be lacking in crystalloids, and these properties are generally accounted for by differences in the degree of dispersion. We have repeatedly discussed these peculiarities in our analysis of the chemical behavior of proteins, *e.g.* the depressing effect of neutral salts on the osmotic pressure, swelling, and viscosity of certain proteins; the peculiar influence of the hydrogen ion concentration of the solution on these properties; and finally the peculiar influence of the valency of the ion with which the protein is in combination and the apparent lack of influence of the other chemical properties of the ion except valency and sign of charge.¹ The dispersion theory accounts for these difficulties by the assumption of differences in the degree of aggregation of the protein particles. If, for example, the addition of some salt to a protein solution of a definite pH lowers its osmotic pressure, the assumption is made that the salt diminishes the degree of dispersion of the colloidal particles in solution. It is not possible to submit the dispersion theory to a quantitative test since we cannot measure the degree of dispersion of a protein.

A second theory to account for the influence of salts and hydrogen ion concentration is Pauli's ionization theory which ascribes the osmotic pressure, the swelling, and viscosity chiefly to the hydration of ionized protein, while the non-ionized protein molecule is assumed not to be hydrated. The idea of such a hydration of protein ions has become doubtful in view of recent experimental and theoretical investigations by Lorenz² and by Born,³ and, moreover, the writer

¹ Loeb, J., *J. Gen. Physiol.*, 1920-21, iii, 85, 247, 391; *Science*, 1920, lii, 449.

² Lorenz, R., *Z. Elektrochem.*, 1920, xxvi, 424.

³ Born, M., *Z. Elektrochem.*, 1920, xxvi, 401.

has been able to show that the conductivity measurements of protein solutions contradict Pauli's ionization theory.

The solution of the problem seems to lie in a field altogether foreign to the speculations current in colloid chemistry, namely in the Donnan equilibrium, which exists when a membrane separates two solutions, one of an electrolyte for the ions of which the membrane is permeable, and one of an electrolyte for one ion of which the membrane is not permeable.⁴ It is immaterial whether the latter ion is a colloid or a crystalloid; it is only necessary that it cannot diffuse through the membrane. When a collodion membrane separates a gelatin or albumin chloride solution of pH 3.3 from an aqueous solution of HCl of originally the same pH (but without gelatin), the pH is no longer the same on both sides of the membrane at the time of equilibrium but is lower on the outside than in the gelatin solution. The Donnan equilibrium demands in this case that acid be given off from the gelatin chloride solution to the outside aqueous solution (containing no gelatin). The writer has found that, *e.g.* a 1 per cent gelatin chloride solution of pH 3.5 is in equilibrium with an aqueous HCl solution of about pH 3.0.⁵

II.

Gelatin chloride solutions containing 1 gm. of originally isoelectric gelatin in 100 cc. solution and having a pH of 3.5 were made up in H₂O and in different concentrations of NaNO₃ varying from M/4,096 to M/32 NaNO₃, all of pH of 3.5. These solutions were put into collodion bags connected with a manometer to measure the final osmotic pressure of the solution. The collodion bags were put into HCl solution of pH 3.0 made up in water and different concentrations of NaNO₃, the pH of the NaNO₃ solutions being also 3.0. These outside solutions contained no gelatin. The collodion bags were put into these aqueous solutions free from gelatin in such a way that the concentration of the NaNO₃ solution inside the collodion bag was always the

⁴ Donnan, F. G., *Z. Elektrochem.*, 1911, xvii, 572. Donnan, F. G., and Harris, A. B., *J. Chem. Soc.*, 1911, xcix, 1554. Donnan, F. G., and Garner, W. E., *J. Chem. Soc.*, 1919, cxv, 1313.

⁵ Loeb, J., *J. Gen. Physiol.*, 1920-21, iii, 247.

same as outside. The osmotic pressure of the 1 per cent gelatin chloride solution, which was after 18 hours 435 mm. when no salt was present, was only 63 mm. when the inside and outside solutions were made up in $m/32$ NaNO_3 .

Table I gives the influence of the concentration of NaNO_3 on the osmotic pressure. It is obvious that the osmotic pressure diminishes with the concentration of the salt. This phenomenon had already been described by Lillie⁶ and by the writer.⁷ Donnan has shown that this depressing effect of a salt on the osmotic pressure of a colloidal solution is a necessary consequence of his theory of membrane equilibrium, and this conclusion is supported by the following experiments.

TABLE I.

Original inside solution, 1 per cent gelatin chloride of pH 3.5 made up in various concentrations of NaNO_3 of the same pH.

Outside solution, HCl of pH 3.0 made up in the same concentrations of NaNO_3 of pH 3.0 as the inside solution.

	Concentration of NaNO_3 .								
	0	$m/4,096$	$m/2,048$	$m/1,024$	$m/512$	$m/256$	$m/128$	$m/64$	$m/32$
Osmotic pressure in mm. of H_2O ..	435	405	371	335	280	215	134	85	63
P.D. inside solution in millivolts.....	+31	+26	+24	+22	+16	+12	+7	+4	0

The writer undertook measurements of the potential difference between the gelatin chloride solutions inside the collodion bag and the aqueous solutions outside the collodion bag with the aid of a Compton electrometer. It was found that the gelatin chloride solution was always positively charged while the outside aqueous solution was negatively charged, as was to be expected. The second important fact is that the P.D. diminishes with the increase in concentration of the neutral salt added and (if the necessary corrections are made) in approximately the same ratio as the osmotic pressure diminishes (lower row in Table I).

⁶ Lillie, R. S., *Am. J. Physiol.*, 1907-08, xx, 127.

⁷ Loeb, J., *J. Gen. Physiol.*, 1920-21, iii, 391.

III.

What is the origin of these potential differences? Beutner⁸ has shown that the potential differences at the boundary of water and water-immiscible substances obey Nernst's well-known logarithmic formula

$$E = \frac{RT}{nF} \ln \frac{C_1}{C_2}$$

or at room temperature and for $n = 1$

$$0.058 \log \frac{C_1}{C_2}$$

Loeb and Beutner have found the same formula to hold for the potential differences at the boundary of water and the skin of an apple, a tomato, or the leaf of a rubber plant.

It can be shown that the potential differences mentioned in Table I follow Nernst's formula, if we assume that only the hydrogen ion concentration need be considered for the potential difference. If C_1 is the concentration of free HCl in the gelatin solution, and C_2 the concentration of HCl in the outside aqueous solution (without gelatin) the value $\log \frac{C_1}{C_2}$ becomes equal to (pH inside - pH outside).

We measured the pH of the gelatin chloride solution (inside solution) and of the outside HCl solution (without gelatin) after the osmotic and the Donnan equilibria were established. The surprising result was noticed that the difference of pH inside the gelatin solution minus the pH of the outside solution (without gelatin) becomes the smaller the greater the concentration of the NaNO_3 , as shown in Table II.

We can calculate from this difference of pH inside minus pH outside the p.d. between inside and outside solution in millivolts by multiplying the differences by 58 or 59 (correcting for temperature of 24°C.). If the Nernst formula holds the values for p.d. thus calculated should be identical with the observed values for p.d. Table III shows that this is true to a remarkable degree.

⁸ Beutner, R., *Die Entstehung elektrischer Ströme in lebenden Geweben*, Stuttgart, 1920.

TABLE II.

pH Inside Minus pH Outside after 18 Hours.

Original inside solution, 1 per cent originally isoelectric gelatin dissolved in various concentrations of NaNO_3 made up with HCl to pH 3.5.

Outside solution, same concentrations of NaNO_3 all made up with HCl to pH 3.0.

	Concentration of NaNO_3 .								
	0	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32
pH of inside solution.....	3.58	3.56	3.51	3.46	3.41	3.36	3.32	3.29	3.25
“ “ outside “	3.05	3.08	3.10	3.11	3.14	3.17	3.20	3.22	3.24
Difference pH inside minus pH outside.....	0.53	0.48	0.41	0.35	0.27	0.19	0.12	0.07	0.01

TABLE III.

Potential Difference between Gelatin Solution and Outside Solution.

Concentration of NaNO_3 .	Calculated by Nernst's formula from pH.	Observed.
	<i>millivolts</i>	<i>millivolts</i>
0	31.2	31
M/4,096	28.3	28
M/2,048	24.0	24
M/1,024	20.7	22
M/512	16.0	16
M/256	11.2	12
M/128	7.0	7
M/64	4.1	4
M/32	0	0

IV.

The greatest puzzle in the physical chemistry of the proteins is the fact that at the isoelectric point of gelatin and crystalline egg albumin the osmotic pressure is a minimum, that it rises when acid is added, at first with the increase of acid added, reaching a maximum when the pH is about 3.5, and that on further addition of acid a rapid fall of osmotic pressure occurs.

It was also reported in the preceding papers that the osmotic pressure of gelatin chloride and gelatin phosphate solutions of the same pH and the same concentration of originally isoelectric gelatin was about the same, that the osmotic pressure of gelatin oxalate was slightly

lower, while that of gelatin sulfate was only half as high or not quite half as high as that of gelatin chloride.

Measurements of the potential differences between the gelatin solution and the outside solution revealed the fact that the curves presenting the P.D. as a function of the hydrogen ion concentration resemble the curves for osmotic pressure. The P.D. curves have a minimum at the isoelectric point, rise steeply with increasing hydrogen ion concentration until a pH of 3.9 is reached, then drop equally steeply again when the pH falls further. Moreover, the maximum of the P.D. curve for gelatin sulfate is about one-half of that of the maximum of the P.D. curve for gelatin chloride and the P.D. curve for gelatin chloride is about equal to the P.D. curve for gelatin phosphate, while that of gelatin oxalate is only slightly lower than that of gelatin chloride.

The next question was whether or not the Nernst formula can account for these differences. The pH of the gelatin solutions and of the outside solutions were measured at the point of equilibrium and it was found that the difference of pH inside minus pH outside multiplied by 58 gave approximately the number of millivolts actually measured. The agreement between the calculated P.D. and the observed P.D. was not as perfect as in the case of the salt effect (Table III) but sufficient to leave no doubt that Nernst's theory accounts for these P.D.

v.

Procter⁹ has applied the Donnan equilibrium to the theory of swelling of gelatin, reaching the conclusion that swelling is an osmotic phenomenon and that the amount of swelling of a gelatin chloride solution is determined by the concentration of the free ions inside the gel minus the concentration of the free ions in the outside solution. He did not measure the pH. By filling this gap the writer was able to satisfy himself that the depressing influence of salts upon the swelling of gelatin is due to a diminution of the difference of pH inside

⁹ Procter, H. R., *J. Chem. Soc.*, 1914, cv, 313. Procter, H. R., and Wilson, J. A., *J. Chem. Soc.*, 1916, cix, 307.

and outside the gel, and that the curves expressing the influence of neutral salts on the value of pH inside minus pH outside the gel, and on the swelling run approximately parallel.

SUMMARY AND CONCLUSION.

1. It is well known that neutral salts depress the osmotic pressure, swelling, and viscosity of protein-acid salts. Measurements of the P.D. between gelatin chloride solutions contained in a collodion bag and an outside aqueous solution show that the salt depresses the P.D. in the same proportion as it depresses the osmotic pressure of the gelatin chloride solution.

2. Measurements of the hydrogen ion concentration inside the gelatin chloride solution and in the outside aqueous solution show that the difference in pH of the two solutions allows us to calculate the P.D. quantitatively on the basis of the Nernst formula $E = \frac{RT}{nF} \ln \frac{C_1}{C_2}$ if we assume that the P.D. is due to a difference in the hydrogen ion concentration on the two sides of the membrane.

3. This difference in pH inside minus pH outside solution seems to be the consequence of the Donnan membrane equilibrium, which only supposes that one of the ions in solution cannot diffuse through the membrane. It is immaterial for this equilibrium whether the non-diffusible ion is a crystalloid or a colloid.

4. When acid is added to isoelectric gelatin the osmotic pressure rises at first with increasing hydrogen ion concentration, reaches a maximum at pH 3.5, and then falls again with further fall of the pH. It is shown that the P.D. of the gelatin chloride solution shows the same variation with the pH (except that it reaches its maximum at pH of about 3.9) and that the P.D. can be calculated from the difference of pH inside minus pH outside on the basis of Nernst's formula.

5. It was found in preceding papers that the osmotic pressure of gelatin sulfate solutions is only about one-half of that of gelatin chloride or gelatin phosphate solutions of the same pH and the same concentration of originally isoelectric gelatin; and that the osmotic pressure of gelatin oxalate solutions is almost but not quite the same as that of the gelatin chloride solutions of the same pH and concentra-

tion of originally isoelectric gelatin. It was found that the curves for the values for P.D. of these four gelatin salts are parallel to the curves of their osmotic pressure and that the values for pH inside minus pH outside multiplied by 58 give approximately the millivolts of these P.D.

In this preliminary note only the influence of the concentration of the hydrogen ions on the P.D. has been taken into consideration. In the fuller paper, which is to follow, the possible influence of the concentration of the anions on this quantity will have to be discussed.

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