[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

THE INTERPRETATION OF THE INFLUENCE OF ACID ON THE OSMOTIC PRESSURE OF PROTEIN SOLUTIONS

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I

The osmotic pressure of protein solutions is influenced by electrolytes in the same way as are the other colloidal properties, such as viscosity, swelling, or membrane potentials, and this suggests that the influence of electrolytes on all these properties may be due to the same cause. In a series of papers[1] and a recent book[2] the writer has tried to show that this common cause is the establishment of Donnan's membrane equilibrium, which is produced when a protein solution containing ionized protein is separated by a collodion membrane from an aqueous solution free from protein; or when a gel containing protein ions is surrounded by an aqueous solution free from protein or containing little protein. When the collodion membrane or the gel is permeable to crystalloidal ions but impermeable to protein ions, an equilibrium condition originates in which the product of the concentrations of each pair of oppositely charged diffusible (crystalloidal) ions is the same on opposite sides of the membrane. This results in an unequal distribution of the different crystalloidal ions on the opposite sides of the membrane, whereby the osmotic pressure of the protein solution is modified. The observed osmotic pressure of a protein solution must therefore be corrected for the Donnan effect before it can be used for theories of the effect of electrolytes on the osmotic pressure of protein solutions. When 1 g. of iso-electric protein, gelatin or crystalline egg albumin or casein or edestin, is dissolved in 100 cc. of water containing varying quantities of 0.1 *N* hydrochloric acid, protein chloride is formed. In this salt formation, the hydrogen ion becomes part of the complex protein cation, while the chlorine ion remains free as before. That the protein chloride formed is highly ionized is shown by the fact that the addition of iso-electric protein to a solution of hydrochloric acid lowers the concentration of hydrogen ion, but not of chloride ion, as measured by the hydrogen and calomel electrodes, respectively,[3] indicating that the acid reacts with protein much as it does with ammonia. There is, however, one essential difference between the two cases, inasmuch as the protein chloride solutions are hydrolyzed to a considerable extent, so that there exists always an equilibrium between

[1] Loeb, *J. Gen. Physiol.*, **3**, (a) 667, (b) 691, (c) 827 (1920–1); (d) **4**, 73 (1921–2).

[2] Loeb, "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Book Company, Inc., New York and London 1922.

[3] Ref. 2, pp. 41–42.

free hydrochloric acid, protein chloride and iso-electric protein not in combination with the acid. The relative part of acid in combination with protein is an unequivocal function of the hydrogen-ion concentration of the protein solution. Since only the ionized part of the protein plays a role in the Donnan equilibrium and since this part is determined by the hydrogen-ion concentration of the solution, a protein solution is only adequately defined if, aside from the concentration of originally iso-electric protein, the hydrogen-ion concentration of the solution is known.

When a solution of a protein chloride is put into a collodion bag, permeable to crystalloidal ions but not to the protein ions, and when this bag is put into water or a weak hydrochloric acid solution, there exist, when osmotic equilibrium is established, inside the collodion bag, free hydrochloric acid, protein chloride and non-combined (iso-electric) protein, while outside there is free hydrochloric acid. At equilibrium let x be the concentration of hydrogen and of chloride ions in the outside aqueous solution (free from protein), y the concentration of the hydrogen and chloride ions of the free acid inside the protein solution, and z the concentration of the chloride ions in combination with the protein ions, then according to Donnan the equilibrium is expressed by the following equation (which was first used by Procter and Wilson in their theory of swelling),[4]

$$x^2 = y(y + z) \qquad (1)$$

Since all the quantities in this equation are positive, x must be greater than y, and $y + z$ must be greater than x. It is also evident from Equation 1 that $2y + z$ is greater than $2x$, showing that the sum of the concentrations of hydrogen and chloride ions must be greater in the inside than in the outside solution at equilibrium. This fact is, as we shall see, of great importance for the understanding of the effect of acids on the osmotic pressure of protein solutions. The values of y and x can be determined directly with the aid of the hydrogen electrode or by titration, and z can be calculated from Equation 1. We can, therefore, calculate with the aid of Donnan's equilibrium equation the correction which the observed osmotic pressure of a protein chloride solution requires, provided that we can prove that the inequality of the concentrations of hydrogen and chloride ions on the opposite sides of the membrane is caused by the membrane equilibrium. This proof has been furnished through the comparison of the membrane potentials of protein chloride solutions with the values of these potentials calculated from differences of the hydrogen-ion or the chlorine-ion concentrations on the opposite sides of the membrane with the aid of Donnan's equilibrium formula. The two sets of values agree perfectly.[5]

[4] Procter, *J. Chem. Soc.*, **105**, 313 (1914). Procter and Wilson, *ibid.*, **109**, 307 (1916).

[5] Ref. 1 a; *J. Gen. Physiol.*, **4**, (a) 351, (b) 617, (c) 621 (1921–22). Ref. 2, p. 120.

When 1 g. of iso-electric crystalline egg albumin or of iso-electric gelatin[6] or of iso-electric edestin[7] is dissolved in 100 cc. of water containing various quantities of 0.1 N hydrochloric acid, and when the osmotic pressure of these solutions is measured after 18 hours it is found that the osmotic pressure rises at first with increasing concentration of the hydrogen ions until a maximum is reached (at about P_H 3.4) and then falls again with a further rise in the hydrogen-ion concentration. The dispersion theory explains this rise on the assumption that the addition of little acid increases the degree of dispersion of the solution thereby raising the osmotic pressure, while the addition of more acid diminishes the degree of dispersion again, thereby diminishing again the osmotic pressure of the solution.[8] This is merely a qualitative speculation which at best does not conflict with the observations on the influence of acid on the osmotic pressure of protein solutions; it conflicts, however, with the observations on the influence of acids on viscosity. The viscosity of solutions of gelatin or casein is also a minimum at the iso-electric point, rising upon the addition of a little acid until a maximum is reached and diminishing upon the addition of more acid. Unfortunately for the dispersion hypothesis, the writer has been able to show by experiments on the viscosity of suspensions of powdered gelatin in water that the viscosity of the suspensions is diminished considerably when the degree of dispersion of the suspension is increased.[9] If the dispersion theory were right in ascribing the increase of the osmotic pressure upon the addition of little acid to an increase in the degree of dispersion of the protein, the same increase in dispersion should diminish the viscosity of the protein solution. In reality, however, the addition of little acid raises the viscosity of the protein solution in the same way as it raises the osmotic pressure.

There exists a second difficulty overlooked by the believers in the dispersion theory, namely, that at the time of osmotic equilibrium the hydrogen-ion concentrations are different on the opposite sides of the membrane. This demands, as already stated, a correction of the observed values for the osmotic pressure of the protein solutions. The writer has already shown in experiments on the influence of acids on the osmotic pressure of solutions of gelatin and crystalline egg albumin that the correction covers practically the whole influence of acids on the osmotic pressure of these protein solutions, so that when the correction is made there is—at least within the limits of the accuracy of the experiments and calculations—little if anything left for the dispersion theory to explain.[6] This has been confirmed by Hitchcock in experiments on the influence of acid on edestin.[7]

[6] Ref. 2, p. 169. Ref. 1b.

[7] Hitchcock, *J. Gen. Physiol.*, **4**, 597 (1921–22).

[8] Zsigmondy, "Kolloidchemie," Otto Spamer, Leipzig, 2nd ed., **1918**, p. 341.

[9] Ref. 1d, p. 97. Ref. 2, p. 232.

What was believed to be the effect of the variation of dispersion of the protein turned out to be the effect of the unequal concentration of crystalloidal ions on the opposite sides of the membrane demanded by Donnan's theory.

It is intended to show in this paper that the same explanation is true also for the influence of acids on the osmotic pressure of casein solutions.

II

Since the method used in these experiments has already been described[6], it may suffice here to state briefly that the protein solutions were put into collodion bags cast in the shape of Erlenmeyer flasks of a volume of about 50 cc. The opening of each collodion bag was closed by a rubber stopper which was perforated by a glass tube serving as a manometer. The bag was dipped into a beaker containing 350 cc. of water which was at the beginning of the experiment usually brought to the same hydrogen-ion concentration as that of the casein solution by adding the necessary quantity of acid. The temperature was kept constant at 24°. The osmotic equilibrium was usually established within 6 hours, but the final reading was taken after about 18 or 24 hours. The osmotic pressure was measured in terms of the height in millimeters of the solution in the tubes.

The material used was casein prepared from skimmed milk after the method of Van Slyke and Baker.[10] According to Michaelis, the iso-electric point of casein is at a Sörensen value of about 4.7 (equivalent to a hydrogen-ion concentration of 2×10^{-5} N). The finely powdered casein used by us was nearly iso-electric. Casein is only sparingly soluble in water at the iso-electric point but it becomes more soluble in hydrochloric or phosphoric acid if enough of the acid is added. Portions of 1 g. of powdered casein were put into 100 cc. of water containing various quantities of 0.1 N hydrochloric acid (see Table I). After 24 hours all the casein was dissolved in those solutions which contained more than 5 and less than 40 cc. of 0.1 N hydrochloric acid in 100 cc. The hydrogen-ion concentration of the casein chloride solutions was determined after the casein was dissolved and each collodion bag containing a casein chloride solution was dipped into a beaker containing 350 cc. of hydrochloric acid of originally the same hydrogen-ion concentration as that of the casein solution.

The first row in Table I gives the volume of 0.1 N hydrochloric acid originally contained in 100 cc. of solution with 1 g. of originally approximately iso-electric casein. The next row states whether or not all the casein went into solution in the next 24 hours, that is, whether or not there was a precipitate at the bottom of the beaker containing the solution. It is evident that no more precipitate was left when the solution contained

[10] Van Slyke and Baker, *J. Biol. Chem.*, **35**, 127 (1918).

TABLE I

DIFFERENCE OF HYDROGEN-ION CONCENTRATION BETWEEN CASEIN SOLUTION AND OUTSIDE SOLUTION AT EQUILIBRIUM

1. Cc. of 0.1 N HCl contained in 100 cc. of solution.....	1	2	3	4	5	6	7	8	10	12.5	15	17.5	20	25	30	40
2. Appearance of solution.....		Precipitate			Slight ppt.					Solution						Precipitate
						P_H at beginning of experiment										
3. P_H of casein (inside) solution.....	3.73	3.43	3.30	3.20	3.10	2.94	2.78	2.64	2.45	2.26	2.13	2.02	1.93	1.81	1.71	1.55
4. P_H of outside solution.....	3.90	3.80	3.50	3.40	3.20	3.00	2.90	2.60	2.50	2.30	2.20	2.10	1.95	1.80	1.70	1.60
						P_H after 18 hours (at equilibrium)										
5. P_H of casein (inside) solution.....	4.04	3.87	3.68	3.61	3.46	3.32	3.22	2.93	2.78	2.62	2.52	2.39	2.22	2.04	1.89	1.73
6. P_H of outside solution.....	3.84	3.69	3.42	3.28	3.13	2.97	2.88	2.67	2.57	2.42	2.35	2.26	2.14	2.00	1.88	1.73

TABLE II

APPROXIMATE AGREEMENT BETWEEN OBSERVED OSMOTIC PRESSURE AND OSMOTIC PRESSURE CALCULATED FROM DONNAN'S EQUATION

1. P_H of casein solution at equilibrium.....	1.73	1.89	2.04	2.22	2.39	2.52	2.62	2.78	2.93	3.22	3.32	3.46	3.61	3.68	3.87	4.04
2. $C_{H^+} \times 10^5$ inside (y)..	1862	1288	912	603	407	302	240	166	118	60.3	47.9	34.7	24.6	20.9	13.5	9.1
3. $C_{H^+} \times 10^5$ outside (x)..	1862	1318	1000	724	550	447	380	269	214	132	107	74.1	52.5	38.0	20.4	14.5
4. $\frac{(x+y)(x-y)}{y} = z$.....	0	61	185	267	337	360	362	270	270	229	191	124	87.5	48.1	17.3	14.0
5. $2y + z - 2x$.....	0	1	9	25	51	70	82	64	78	86	73	45	32	14	3.5	3.2
6. Share of osmotic pressure due to Donnan equilibrium, mm. of water.....	0	2.5	22.5	62.5	128	175	205	160	195	215	183	113	80	35	8.7	8
7. Observed osmotic pressure, mm. of solution..	41	69	85	102	126	145	158	177	187	189	173	135	77	43	23	14

more than 5 and less than 40 cc. of 0.1 N hydrochloric acid in 100 cc. The third row gives the Sörensen value (P_H) of the casein solutions at the beginning of the osmotic pressure experiment. The next row gives the Sörensen value of the outside solution at the beginning. It was approximately identical in each case with that of the inside solution.

The important part of the table is the last two rows (5 and 6) giving the Sörensen values of the inside and outside solutions *after osmotic equilibrium was established*, that is, after 18 hours. The Sörensen value has changed both in the inside and outside solutions, but it is without exception smaller in the outside than in the inside solution; or in other words, the outside solution has at equilibrium a higher hydrogen-ion concentration than the inside solution. This is exactly what should happen according to Donnan's formula for membrane equilibria as stated above.

In Row 7, Table II, are given the observed values of osmotic pressure and the Sörensen value of the casein solutions as found at the end of the experiment after equilibrium was established is given in Row 1 of the same table. It is obvious that the osmotic pressure is a minimum nearest the iso-electric point (P_H 4.04), that it rises with the increase of acid until the maximal osmotic pressure is reached at about P_H 3.0, and that the osmotic pressure falls again when the hydrogen-ion concentration in the solution increases beyond this point. This is typical for the influence of acids on all colloidal properties of proteins and the question arises: What is the cause of this peculiar influence of the hydrogen-ion concentration? It has already been stated that the dispersion hypothesis explains this phenomenon by assuming that the addition of little acid increases the degree of dispersion and hence the osmotic pressure of the solution; while when more acid is added the degree of dispersion and hence the osmotic pressure are diminished again. Even if we were willing to ignore the observations on viscosity, we must consider the fact expressed in Rows 5 and 6 of Table I (and demonstrated for other proteins than casein) that at equilibrium the hydrogen-ion concentration is not the same inside and outside the protein solution. This fact proves that the observed osmotic pressure of a protein chloride solution is the sum of two osmotic pressures, namely, that due to the concentration of the protein molecules, ions, and micells themselves, and that due to the excess of the concentration of crystalloidal ions (in our experiment hydrogen and chloride) inside the protein solution over the concentration of hydrogen and chloride ions in the outside solution. Before entering upon speculations concerning the possible cause of the influence of acid on the osmotic pressure of a casein chloride solution we must, therefore, find out how much of the influence of the acid is due to that part of the osmotic pressure which is the mere consequence of the excess of the concentration of hydrogen and chloride ions inside over that outside. This calculation is made in Table

II, and it turns out that the effect of the hydrogen-ion concentration of the casein solution on its osmotic pressure is practically if not entirely due to the Donnan effect, that is, to the difference of the sum of the concentrations of hydrogen and chloride ions inside over the sum of the concentrations of these two ions outside.

At equilibrium let x be the concentration of free hydrogen and chloride ions in the outside solution and y the concentration of the hydrogen and chloride ions of the free hydrochloric acid in the inside (casein) solution; let z be the concentration of chloride ions in combination with the casein, and let us assume complete electrolytic dissociation of all the electrolytes. In that case the osmotic pressure due to the excess of concentration of hydrogen and chloride ions inside the casein solution over that in the outside solution is determined by the term, $2y + z - 2x$.

We can calculate the value of y from the measurement of the Sörensen value inside, and the value of x from the Sörensen value outside; the Sörensen value being the logarithm of the hydrogen-ion concentration with the minus sign omitted. We can calculate z from y and x with the aid of Equation 1 for the Donnan equilibrium,

$$z = \frac{(x + y)\,(x-y)}{y}$$

if we can furnish the proof that the excess of the concentration of crystalloidal ions inside over that outside is due to the Donnan equilibrium. This proof has been furnished for solutions of gelatin, crystalline egg albumin,[5] and edestin,[7] and we shall furnish it later for casein chloride solutions.

The calculation of $2y + z - 2x$ is carried out in Table II. Here the upper row is a repetition of the Sörensen values of the casein solutions at the end of the experiment from Row 5 of Table I. The second and third rows in Table II give the concentrations of hydrogen and chloride ions of the free acid inside and outside. The values of the concentration of hydrogen ion, C_{H^+}, are multiplied by 10^5. The fifth row gives the values of $2y + z - 2x$. These latter values are the excess of the concentration of hydrogen and chloride ions inside the casein solutions over the concentration of the same ions outside, on the assumption that the difference in P_H inside and outside is due to a Donnan equilibrium.

If we express the theoretical osmotic pressure of a gram molecular solution in terms of millimeters pressure of a column of water we have (with correction for a temperature of 24°)

$$22.4 \times 760 \times 13.6 \times \frac{297}{273} = 2.5 \times 10^5$$

In other words, a theoretical pressure of 2.5 mm. of water corresponds to a concentration of 10^{-5} N. Hence, we need only multiply the values for $2y + z - 2x$ in Row 5 of Table II by 2.5 to obtain that part of the observed

osmotic pressure of our casein solutions which is due to the Donnan effect. These values are given in the sixth row of Table II. When these values are compared with the observed osmotic pressures in the seventh row of Table II it is found that, within the limits of the accuracy of the measurements of Sörensen values and the calculation of z, there is a fair agreement. The observed osmotic pressures should be slightly higher than those due to the excess osmotic pressure of $2y + z - 2x$, namely, by that value which is due to the osmotic pressure of the protein itself. From the experiments with gelatin and crystalline egg albumin, the writer would infer that that share of the osmotic pressure which in this experiment was due to the casein molecules, ions, or aggregates was so small that it was within the limit of error of measurement and calculation.

In comparing the observed and calculated values for osmotic pressure in Table II the discrepancies appear to be rather large. This is simply due to the fact that a slight variation of the measurements of the Sörensen values in the second decimal place (so slight that it is inside the limit of the source of error) causes a considerable variation in the calculated value for $2y + z - 2x$. It is therefore worth while to point out that on the basis of the Donnan equation we can also calculate the Sörensen values inside or outside, and that in this case the calculated and the observed values show excellent agreement. This calculation was made by Dr. D. I. Hitchcock from the values in Table II, and I quote his results in his own words.

"It is possible to show in another way that the osmotic pressure of the casein chloride solutions is due almost entirely to the Donnan equilibrium. The expression for the difference in ion concentrations due to the Donnan equilibrium, $2y + z - 2x$, can be given in terms of x and y alone by substituting the value for z from Equation 1.

$$2y + z - 2x = 2y + \frac{x^2 - y^2}{y} - 2x = \frac{2y^2 + x^2 - y^2 - 2xy}{y} = \frac{x^2 - 2xy + y^2}{y} = \frac{(x-y)^2}{y}$$

(This expression was obtained by Procter and Wilson.[4])

"If it be assumed for the moment that the observed osmotic pressure is the result of the Donnan equilibrium alone, the correctness of this assumption can be tested by using the observed values for osmotic pressure and one hydrogen-ion concentration in the above expression, and solving for the other hydrogen-ion concentration.

"Let p = observed osmotic pressure in mm. of water. Then

$$p = 2.5 \times 10^5 \times \frac{(x-y)^2}{y}$$

if x and y are expressed in moles per liter.

$$(x-y)^2 = \frac{p\,y}{250{,}000}; \quad x - y = \sqrt{\frac{p\,y}{250{,}000}}; \quad x = y + \sqrt{\frac{p\,y}{250{,}000}}; \quad x = y + \frac{\sqrt{p\,y}}{500}$$

Using the values for y obtained from the measurement of the P_H inside, and the observed values for the osmotic pressure (Table II), calculated values for x were obtained. These were translated into terms of P_H (P_H outside = $-\log x$), and are given in Table III, together with the observed values for the P_H outside.

"The results show that in 10 out of 16 cases the agreement is within 0.02 P_H, which is about the experimental error of a single measurement of the P_H. If the other

TABLE III

COMPARISON OF OBSERVED VALUES FOR P_H OUTSIDE WITH THOSE CALCULATED ON THE ASSUMPTION THAT THE OSMOTIC PRESSURE OBSERVED WAS DUE ENTIRELY TO THE DONNAN EQUILIBRIUM

Observed P_H	1.73	1.88	2.00	2.14	2.26	2.35	2.42	2.57
Calculated P_H	1.69	1.83	1.96	2.12	2.26	2.36	2.44	2.56
Observed P_H	2.67	2.88	2.97	3.13	3.28	3.42	3.69	3.84
Calculated P_H	2.67	2.84	2.98	3.11	3.28	3.40	3.61	3.79

measurements of P_H were in error by that much in the opposite direction, the agreement could be considered to be within the experimental error in all except one case. The calculation therefore shows quite conclusively that the observed osmotic pressure is wholly due, at least within the limits of the experimental error, to the differences in concentration of the crystalloidal ions which result from the Donnan equilibrium."

III

We will now furnish the proof that (aside from this agreement between calculated and observed values of osmotic pressures) we had the right to assume that we are dealing here with the Donnan effect. The difference in the hydrogen-ion concentration inside (y) and outside (x) (Rows 2 and 3 in Table II) was not assumed but directly observed. What was assumed was the legitimacy of the calculation of z from Donnan's equilibrium Equation 1. In order to prove that this was justified we must be able to show that the value P_H inside minus P_H outside is exactly that demanded by Donnan's theory. This proof can be furnished by the measurements of the membrane potentials. There exists at osmotic equilibrium a potential difference between a protein chloride solution inside a collodion membrane and the protein-free hydrochloric acid solution outside, and this potential difference can be measured with the aid of indifferent saturated potassium chloride calomel electrodes. Donnan had shown that such a potential difference was to be expected on the basis of his theory.[11]

It follows from Donnan's formula that $E = \frac{RT}{F} \log \frac{x}{y} = \frac{RT}{F} \log \frac{y+z}{x}$.

If the difference between the Sörensen value inside and that outside in Rows 5 and 6 of Table I is the consequence of the Donnan equilibrium, the potential difference across the collodion membrane between the casein chloride solution and the outside solution observed with indifferent calomel electrodes should be equal to the potential difference in millivolts calculated from either of the following two terms (at 24°): 59 (P_H inside minus P_H outside), or 59 (P_{Cl} outside minus P_{Cl} inside), regardless of whether the Sörensen value for hydrogen or chlorine is determined by titration or with a hydrogen electrode or a chloride electrode, respectively. The writer has shown that this is true in the case of gelatin and albumin

[11] Donnan, *Z. Elektrochem.*, **17**, 572 (1911).

chloride solutions,[5] and Hitchcock has shown that it is true for edestin solutions.[7] It is also true for casein chloride solutions as the following experiment shows: 4, 3, 2, 1, 0.5, and 0.25% solutions of iso-electric casein were brought to about P_H 2.5 by adding hydrochloric acid, put into collodion bags as described, and each bag was immersed in 350 cc. of hydrochloric acid solution of initial P_H 2.3. After 18 hours the potential differences between the casein solution (inside solution) and the outside aqueous solution free from casein were determined with the indifferent calomel electrodes and afterwards the Sörensen values of the inside and outside solutions were measured with the hydrogen electrode. Table IV gives the results of the measurements.

TABLE IV

AGREEMENT BETWEEN MEMBRANE POTENTIALS AND THE VALUE $59(P_H$ INSIDE MINUS P_H OUTSIDE)

1. Casein chloride, %	4	3	2	1	0.5	0.25
2. P_H of inside solution at equilibrium	2.595	2.595	2.580	2.53	2.46	2.46
3. P_H of outside solution at equilibrium	2.230	2.270	2.305	2.34	2.36	2.39
4. P_H inside minus P_H outside	0.365	0.325	0.275	0.19	0.10	0.07
5. $59(P_H$ inside minus P_H outside)	21.5	19.2	16.2	11.2	5.9	4.1
6. P. d. observed with indifferent electrodes	20.0	18.0	15.0	10.8	7.2	3.1

The second and third rows give the Sörensen values of the inside and outside solutions as measured with the hydrogen electrode after osmotic equilibrium was established. The fourth row gives the values P_H inside minus P_H outside and the fifth row these values multiplied by 59, since the experiments were made at 24°. These latter values should agree with the values for observed potential differences between inside and outside solutions obtained with the aid of indifferent electrodes, and a comparison of the fifth and sixth rows of Table IV shows that the agreement is good. The agreement between the values in the last two rows of Table IV leaves little doubt that the difference, P_H inside minus P_H outside, is indeed the result of Donnan's membrane equilibrium. This, then, proves that our calculation of z was justified and that in order to obtain the influence of the hydrogen-ion concentration on the osmotic pressure of the protein particles themselves a deduction determined by $2y + z - 2x$ has to be made as we assumed. Of course, we may not have been justified in assuming complete dissociation of all the electrolytes involved, but this would necessitate only a minor correction. The main fact is that if the correction due to the Donnan effect is applied to the osmotic pressure of the casein chloride solution, it is found that the typical influence of the hydrogen-ion concentration upon the osmotic pressure of casein chloride solutions is practically accounted for by the Donnan effect. In this peculiar effect the protein plays only an indirect role, namely, that on

account of the impermeability of the collodion membrane to protein ions the concentration of the diffusible crystalloidal ions becomes higher inside than outside. It is quite possible or probable that the acid also influences the degree of dispersion of the casein solution, but the influence of such an effect on the osmotic pressure of the protein solution is too small to be noticeable in our experiments.

Summary and Conclusions

1. It is shown that the addition of a little acid to a solution of iso-electric casein increases the osmotic pressure until a maximum is reached after which the addition of still more acid depresses the osmotic pressure. This is explained on the basis of the dispersion hypothesis by the assumption that the addition of little acid increases the degree of dispersion and consequently the osmotic pressure of the protein solution, while the addition of more acid diminishes the degree of dispersion and consequently the osmotic pressure.

2. Our observations show that when osmotic equilibrium is established between a solution of casein chloride enclosed in a collodion bag and an outside aqueous solution free from protein, the hydrogen-ion concentration is always greater in the outside solution than in the casein solution.

3. There exists a membrane potential between the casein chloride solution enclosed in a collodion bag and the surrounding aqueous solution free from protein with which the casein solution is in osmotic equilibrium, and this membrane potential can be measured with indifferent calomel electrodes and a Compton electrometer. When this is done at 24°, it is found that the number of millivolts of the observed membrane potential is equal to 59 (P_H inside minus P_H outside), the latter values being measured with the hydrogen electrode. This is the result to be expected if the inequality of the hydrogen-ion concentrations inside and outside the casein chloride solution at equilibrium is determined by Donnan's equation for membrane equilibria.

4. The fact that the hydrogen-ion concentration inside a protein chloride solution is not the same as that of the outside solution free from protein with which it is in osmotic equilibrium, shows that the observed osmotic pressure of the protein chloride solution cannot be entirely due to the protein but must be partly due to the difference in the concentration of the crystalloidal ions (hydrogen and chloride) in the inside and outside solution. It is, therefore, necessary to correct the observed osmotic pressure of a protein chloride solution for this difference in the concentration of hydrogen and chloride ions on the opposite sides of the membrane on the basis of the Donnan equilibrium. It is shown in this paper how this correction can be evaluated with the aid of Donnan's equation.

5. When this evaluation is made, it is found that within the limits

of accuracy of the observations and calculations the entire effect of the hydrogen-ion concentration on the osmotic pressure of the casein chloride solution is covered by the correction required and that there is little if anything left for the dispersion hypothesis to explain.

6. This is in harmony with the conclusion previously reached by the writer that the influence of electrolytes on the osmotic pressure of protein solutions is entirely or almost entirely the consequence of the difference in the concentration of crystalloidal ions inside the protein solutions and the outside aqueous solutions at equilibrium, this difference being caused by the establishment of a membrane equilibrium.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF CLARK UNIVERSITY, I, 12]

THE RESISTANCE-TEMPERATURE COEFFICIENT OF CONCENTRATED SOLUTIONS OF SODIUM IN LIQUID AMMONIA

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Introduction

In a previous article, the writers[1] have shown that the order of magnitude of the electrical conductance of concentrated solutions of sodium and potassium in liquid ammonia approaches that of typical metallic substances. For example, the specific conductance of a saturated solution of sodium in liquid ammonia has a value of 5047.0, which is about one-half that of mercury at ordinary temperatures. The atomic conductance of these solutions, however, is much higher than that of many metallic elements. Thus, the atomic conductance of sodium[2] in liquid ammonia is 1.1×10^6,

[1] Kraus and Lucasse, THIS JOURNAL, **43**, 2529 (1921).

[2] This value of the atomic conductance is based upon the value 0.54 for the density of a saturated solution of sodium in liquid ammonia, which was determined approximately. The great increase in volume accompanying the formation of this saturated solution, which amounts to 53 cc. per atom of sodium, or an increase of 33%, strikingly illustrates the enormous change which the physical properties of these solutions undergo with respect to those of the constituent substances. It is interesting to note that the volume of solutions of sodium and potassium of the same atomic composition is practically identical. Thus the volume of a saturated solution of sodium containing one atom of metal ($V=0.1353$) is 211.5 cc., assuming the density given above. The volume of a solution containing one atom of potassium ($V=0.1295$) is 211.4 cc., assuming as density the value 0.632 (Ref. 1). The difference in the density of sodium and potassium solutions of a given composition is, therefore, due to the difference in the weight of the metallic atoms. This serves to illustrate the striking similarity in the physical properties of solutions of different metals in ammonia.