DONNAN EQUILIBRIUM AND THE PHYSICAL PROPERTIES OF PROTEINS.

II. Osmotic Pressure.

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I. INTRODUCTION.

It was pointed out in two preceding papers[1] that the curves representing the influence of electrolytes on the membrane potentials between gelatin solutions and water at the point of equilibrium show a marked similarity to the curves representing the influence of the same electrolytes on osmotic pressure, swelling, and viscosity of gelatin. There was no doubt left that the Donnan equilibrium accounted quantitatively for these variations in the case of membrane potentials[2] and the question therefore arose whether the same equilibrium condition can account also for the corresponding variations of the osmotic pressure of protein solutions. An attempt was, therefore, made to calculate the osmotic pressures on the basis of the Donnan equilibrium in order to find out whether the curves for the calculated values would show the characteristics of the curves representing the observed values for osmotic pressures. The depressing effect of the addition of a neutral salt on the osmotic pressure of colloidal solutions was predicted by Donnan and offered no theoretical difficulty.[3] The real test was whether the Donnan equilibrium would be able to account for the peculiar curves obtained when the observed osmotic pressures of 1 per cent solutions of gelatin-acid salts are plotted as a function of the pH.

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[1] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 557, 667.

[2] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 667.

[3] 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.

Fig. 1 shows a set of such curves where the abscissæ are the pH of the gelatin solution at equilibrium, and the ordinates the observed osmotic pressures. There are two outstanding peculiarities in these curves, namely, first, that they all rise from a minimum at pH 4.7 (the isoelectric point of gelatin) until they reach a maximum at pH

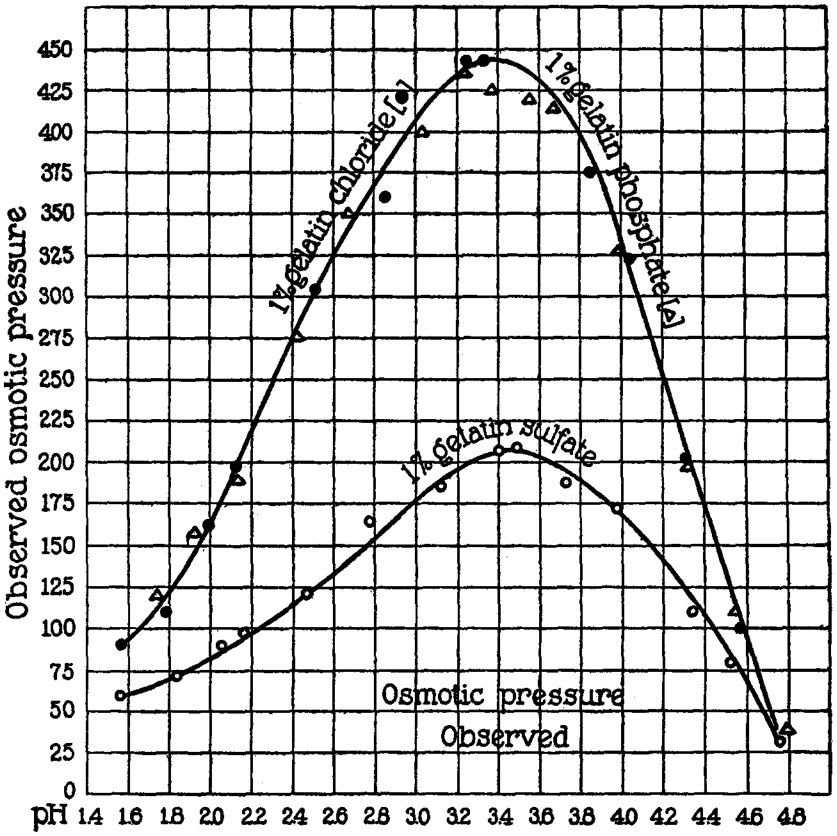

FIG. 1. Curves representing the influence of pH and valency of anion on osmotic pressure of solutions of gelatin-acid salts containing 1 gm. of originally isoelectric gelatin in 100 cc. solution. The curves for gelatin chloride and gelatin phosphate are identical since the anions, Cl and H_2PO_4, of these two gelatin salts are monovalent. The curve for gelatin sulfate is less than half as high as the curve for the two other salts because the anion of gelatin sulfate is bivalent. Both curves rise from the isoelectric point at 4.7 to a maximum at pH about 3.4 or 3.5, and then drop rapidly again.

about 3.5, and then drop again; and second, that only the valency of the ion in combination with a protein influences its osmotic pressure (or degree of swelling, etc.), while the specific nature of the ion aside from its valency has no influence. The latter fact is really the crucial point which decides between colloid chemistry and classical physical chemistry. If only the valency of the ion in combination with a protein is of importance and if gelatin-acid salts of the same pH and the same concentration of originally isoelectric gelatin have the same osmotic pressure, provided the anion of the gelatin-acid salt has the same valency, a suspicion must arise that we are dealing with some equilibrium condition for which classical physical chemistry is able to account. The writer has shown in preceding papers that gelatin chloride, nitrate, acetate, succinate, tartrate, citrate, and phosphate have at the same pH and the same concentration of originally isoelectric gelatin the same osmotic pressure; and it was shown by titration curves that the anion in all these salts is monovalent. The titration curves show also that the anion in gelatin sulfate is bivalent and we have found that the osmotic pressure of gelatin sulfate is less than half that of gelatin chloride or phosphate at the same pH and for the same concentration of originally isoelectric gelatin.[4]

Fig. 1 illustrates this valency effect in the observed osmotic pressure. The curves for the observed osmotic pressure of gelatin chloride and gelatin phosphate are identical while the curve for gelatin sulfate is considerably lower.

It is the purpose of this paper to show that we can calculate with a fair degree of accuracy the osmotic pressure of gelatin solutions on the assumption of the validity of Donnan's equilibrium equation and the validity of van't Hoff's theory of osmotic pressure.

II. Theoretical Data.

A gelatin chloride solution contains free hydrochloric acid, gelatin chloride (which dissociates electrolytically like any other salt in watery solution), and non-ionogenic protein molecules. A 1 per cent gelatin chloride solution of about pH 3.5 is in equilibrium with a

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[4] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 85, 247, 391; *Science*, 1920, lii, 449.

HCl solution (free from protein) of a pH of about 3.0, both solutions being separated by a collodion membrane.

Let y be the concentration of the H and Cl ions of the free HCl inside a gelatin chloride solution (containing 1 gm. of originally isoëlectric gelatin in 100 cc.), z the concentration of the Cl ions held by the gelatin ions, and a the sum of the gelatin ions and non-ionized molecules of gelatin. For the sake of simplification we assume complete electrolytic dissociation of the gelatin chloride and of the HCl. In this case the real osmotic pressure of the inside solution is determined by

$$2y + z + a$$

Since, however, the outside solution is at equilibrium not H_2O but a HCl solution—in the example selected of about pH 3.0—the *observed* osmotic pressure is the difference between the osmotic pressure of the inside solution against H_2O and the osmotic pressure of the outside solution.

Let x be the concentration of the H ions in the outside solution, then the osmotic pressure of the outside solution is determined by $2x$.

Hence the observed osmotic pressure P_o of the gelatin chloride solution is determined by

$$P_o = 2y + z + a - 2x$$

P_o is observed experimentally, y can be calculated from the pH inside, and x from the pH outside.

z can be calculated from Donnan's equilibrium equation in the form given it by Procter[5]

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

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

where x, y, and z have the significance stated above. We have seen in the preceding paper that this equation leads to correct results in regard to the P.D.

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

a is unknown, and we therefore can only calculate for the present the values of

$$2y + z - 2x$$

If we express the theoretical osmotic pressure of a grammolecular solution in terms of mm. pressure of a column of H_2O we get (with correction for a temperature of 24°C.)

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

In other words, a theoretical pressure of 2.5 mm. H_2O corresponds to a concentration of 10^{-5} N. In the following tables all concentrations are expressed in terms of 10^{-5} N and hence we only need to multiply the values for $2y + z - 2x$ given in our tables by 2.5 to obtain the calculated osmotic pressure of the gelatin solution (neglecting the osmotic pressure of the gelatin ions and molecules).

Equation (1) holds in the case of solutions of all gelatin-acid salts with monovalent anion; *i.e.* gelatin chloride, acetate, phosphate, tartrate, citrate, etc. When, however, the anion of a gelatin-acid salt is divalent, as in the case of gelatin sulfate, the equilibrium equation becomes one of the third degree. If x be the hydrogen ion concentration of the outside solution, the concentration of the SO_4 ion in the outside solution becomes $\frac{x}{2}$. If y be the concentration of the H ions of the free sulfuric acid in the inside solution, $\frac{y}{2}$ is the concentration of the SO_4 ions of the free acid inside the gelatin sulfate solution. In the case of gelatin chloride z represented the concentration of chlorine ions in combination with the gelatin; hence $\frac{z}{2}$ will represent the concentration of SO_4 ions in combination with the same number of gelatin ions.

The equilibrium equation, therefore, assumes in the case of gelatin sulfate the following form

$$x^2 \cdot \frac{x}{2} = y^2 \frac{(y + z)}{2} \qquad (2)$$

From equation (2) follows

$$z = \frac{x^3 - y^3}{y^2}$$

The osmotic pressure of the gelatin solution should therefore be calculated from the following values (omitting the share of the osmotic pressure due to the gelatin)

$$\frac{3}{2}y + \frac{z}{2} - \frac{3}{2}x$$

III. The Calculated Curves.

Solutions containing 1 gm. of originally isoelectric gelatin in 100 cc. and containing different quantities of acid were prepared. Collodion bags cast in the form of Erlenmeyer flasks of 50 cc. volume were filled with the 1 per cent solution of a gelatin-acid salt and put into a beaker containing 350 cc. of H_2O. In order to accelerate the establishment of the equilibrium between inside and outside solutions a certain amount of acid was added to the outside water (*e.g.*, HCl in the experiments with gelatin chloride, H_3PO_4 in the experiments with gelatin phosphate, etc.). Each Erlenmeyer flask was closed with a rubber stopper perforated by a glass tube serving as a manometer. All this was described in more detail in previous publications.

In Fig. 2 are plotted the values of the calculated osmotic pressures for 1 per cent solutions of gelatin chloride, gelatin phosphate, and gelatin sulfate, and Tables I, II, and III give the data on the basis of which the curves in Fig. 2 are calculated. The experiments from which these calculations were made are identical with the experiments from which the curves for the observed osmotic pressures in Fig. 1 were plotted. The abscissæ in Fig. 2 are the pH in the inside solution at the point of equilibrium, the ordinates are the values for osmotic pressure calculated on the basis of the Donnan equilibrium as discussed before. The reader will notice that the three curves plotted in Fig. 2 show not only the same qualitative characteristics as the curves for the observed osmotic pressures in Fig. 1, but show them almost quantitatively; except that a correction for the value of osmotic pressure due to the gelatin particles itself may have to be

TABLE I.

Gelatin Chloride.

Observed and Calculated Osmotic Pressures of Gelatin Chloride Containing 1 gm. of Originally Isoelectric Gelatin in 100 cc. Solution at Equilibrium.

pH inside	4.56	4.31	4.03	3.85	3.33	3.25	2.85	2.52	2.13	1.99	1.79	1.57
pH outside	4.14	3.78	3.44	3.26	2.87	2.81	2.53	2.28	2.00	1.89	1.72	1.53
$y = C_H$ inside $\times 10^5$	2.7	4.9	9.3	14.1	46.8	56.2	141	302	741	1023	1622	2692
$x = C_H$ outside $\times 10^5$	7.2	16.6	36.3	54.9	135.0	155.0	295	524	1000	1288	1905	2951
$z = \frac{(x+y)(x-y)}{y}$	16.5	51.4	132.5	200.0	343.0	372.0	477	608	609	600	612	544
$2y + z - 2x$	7.5	28.0	78.5	118.4	166.6	174.4	169	164	91	70	46	26
Observed osmotic pressure	100	202	322	375	443	442	360	303	198	162	110	90
Calculated osmotic pressure, neglecting osmotic pressure of protein	19	70	196	296	416	436	422	410	227	175	115	65

TABLE II.

1 Per Cent Gelatin Phosphate.

Observed and Calculated Osmotic Pressures at Equilibrium.

pH inside	4.79	4.54	4.31	3.98	3.68	3.56	3.38	3.24	3.02	2.67	2.42	2.12	1.92	1.74
pH outside	4.70	4.10	3.77	3.40	3.14	3.04	2.90	2.80	2.66	2.39	2.22	1.98	1.83	1.67
$y = C_H$ inside $\times 10^5$	1.6	2.9	4.9	10.5	20.9	27.5	41.7	57.5	95.5	213.8	380.2	758.6	1202	1820
$x = C_H$ outside $\times 10^5$	2.0	7.9	16.9	39.8	72.4	91.2	125.9	158.5	218.8	407.4	602.6	1047.0	1479	2138
$z = \frac{(x+y)(x-y)}{y}$	0.9	18.6	53.3	140.0	228.0	231.0	338.0	380.0	405.0	556.0	575.0	686.0	617	690
$2y + z - 2x$	0.1	8.6	31.3	81.4	125.0	103.6	169.6	178.0	158.0	169.0	130.0	109.0	63	54
Observed osmotic pressure	34	111	199	328	416	420	426	436	401	350	275	190	158	121
Calculated osmotic pressure, neglecting osmotic pressure of protein		22	77	203	310	258	423	445	395	420	324	273	157	135

TABLE III.

1 Per Cent Gelatin Sulfate.

Observed and Calculated Osmotic Pressure at Equilibrium.

pH inside	4.76	4.52	4.34	3.98	3.73	3.49	3.41	3.12	2.78	2.47	2.16	2.06	1.84	1.57
pH outside	4.61	4.20	3.99	3.60	3.38	3.18	3.14	2.88	2.61	2.35	2.09	2.00	1.80	1.54
$y = C_H$ inside $\times 10^5$	1.7	3.0	4.6	10.4	18.6	32.3	38.9	75.9	166	339	692	871	1445	2692
$x = C_H$ outside $\times 10^5$	3.1	6.3	10.2	25.1	41.7	66.0	72.4	131.8	245.5	447	813	1000	1585	2884
$z = \frac{x^3 - y^3}{y^2}$	8.3	24.7	45.8	136	191.5	243	212	322	390	435	433	449	466	620
$\frac{3}{2}y + \frac{z}{2} - \frac{3}{2}x$	2	7.35	14.5	46	64	71	55.8	77	77	55	37	31	23	20
Observed osmotic pressure	33	79	110	172	188	208	208	185	164	122	98	89	72	61
Calculated osmotic pressure, neglecting osmotic pressure of protein	5	18.5	36	115	160	178		192	192	138	92.5	77.5	57.5	50

added, a point which will be discussed in the next chapter. What is of importance here is the following. The curves for osmotic pressure calculated on the basis of the Donnan equilibrium and plotted in Fig. 2 resemble the curves for the osmotic pressure observed in the same experiments represented in Fig. 1 in the following essential points.

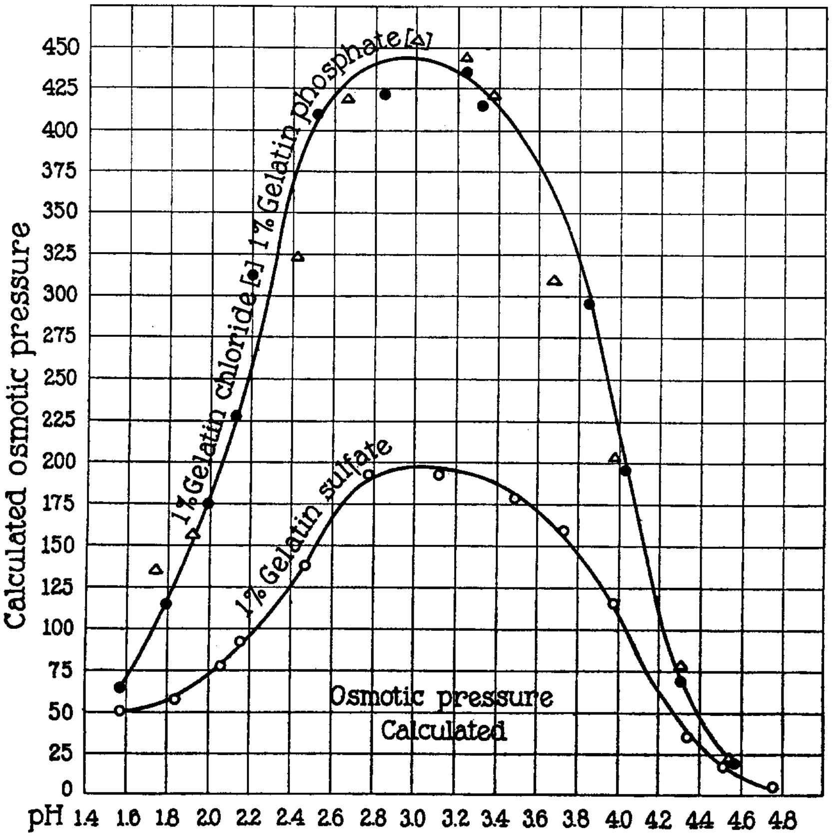

FIG. 2. Calculated curves of osmotic pressure taken from the data of the experiments represented in Fig. 1. The calculation is made on the basis of the validity of Donnan's theory of membrane equilibrium. The calculations lead to curves resembling the curves in Fig. 1 in all essential points, in regard to valency effect of the anion, as well as in regard to influence of pH. (See legend in Fig. 1.)

1. The curve for the calculated osmotic pressure of gelatin chloride is identical with the curve for the calculated osmotic pressure of gelatin phosphate, and the same is true for the two corresponding curves representing the observed osmotic pressures (Figs. 1 and 2).

2. The curve for the calculated osmotic pressure of gelatin sulfate is a little less than half as high as the curves for the calculated osmotic pressures of gelatin chloride and gelatin phosphate; and the same is true for the curves representing the observed osmotic pressures of gelatin sulfate and gelatin chloride.

3. All the curves in Figs. 1 and 2 rise from a minimum at pH 4.7, reach a maximum (which lies at pH 3.4 or 3.5 for the observed, and at 3.0 for the calculated curves), and then drop again as steeply as they rose on the other side. Moreover, the absolute values of observed and calculated osmotic pressures agree almost quantitatively, a fact which will be discussed more fully in Chapter V.

It may be added that the curve for the calculated values of the osmotic pressure of gelatin oxalate solutions agrees also with the curve for the observed values of the osmotic pressure of solutions of the same gelatin salt, both being slightly lower than the curves for gelatin chloride.

We can therefore say that (with the exception of two minor discrepancies to be discussed further on) the Donnan equilibrium accounts not only qualitatively but almost quantitatively for (*a*) *the valency effect of the anion with which the gelatin is in combination;* (*b*) *for the effect of the pH.*

Thus two of the most puzzling problems of the colloid chemistry of proteins seem to find their solution on the basis of classical physical chemistry.

IV. The Presumable Osmotic Pressure of Gelatin Particles.

The question now arises what the possible share of the protein particles in the osmotic pressure may be. Different concentrations of gelatin phosphate from 2 per cent to $\frac{1}{2}$ per cent were prepared, all having a pH of 3.5. The gelatin phosphate solutions were put into Erlenmeyer flasks of 50 cc. volume, connected with a glass tube serving as a manometer as described, and these flasks were put into beakers containing 350 cc. of H_2O, the pH of which was brought at the beginning of the experiment to 3.5 through the addition of H_3PO_4. When the bags containing gelatin phosphate solutions are put into water the latter diffuses rapidly into the gelatin solution thereby lowering the concentration of the gelatin solution. To avoid this error so much gelatin phosphate solution was poured into each bag and glass tube that at the beginning of the experiment the liquid reached already to about that level which from preceding experiments we knew the gelatin solution would reach in the manometer at the point of osmotic equilibrium. All experiments were made in duplicate. In addition to the osmotic pressure we measured the pH inside and outside after equilibrium was reached. From these latter data the osmotic pressure due to the H and PO_4 ions could be calculated, being equal to

$$(2y + z - 2x) \times 2.5 \text{ mm } H_2O$$

By deducting this value from the observed osmotic pressure in each case it was hoped to obtain a rational value for the share of the protein particles in the observed osmotic pressure. Table IV gives the results.

The reader's attention is called to the last two rows of figures (Table IV) giving the difference between the observed and the calculated osmotic pressures, since if this difference actually represents the osmotic pressure due to the gelatin particles, the figures should be in direct proportion to the concentration of the gelatin. The experiments were all made in duplicate to give some idea of the magnitude of error and it is obvious that the error may be considerable, 25 per cent or more, because the errors in the observed and the calculated values are additive. Thus the "difference" is for $\frac{3}{4}$ per cent solution in one case 92, in the other 61, a variation of 50 per cent! If we take this into consideration we may conclude that the differences between the observed and the calculated osmotic pressures are compatible with the idea that the difference is the value for the osmotic pressure due to the gelatin particles in solution.

This would lead us to the conclusion that the osmotic pressure due to the gelatin particles in a 1 per cent solution (of originally isoelectric gelatin) of gelatin phosphate of pH 3.60 is about 100 mm. H_2O. Since the osmotic pressure of 1 grammolecule is about 250,000 mm.

TABLE IV.

Influence of Concentration of Gelatin Phosphate of pH of About 3.6 on the Osmotic Pressure. (All Experiments Were Made in Two Sets.)

	Concentration of gelatin in per cent.									
	2	2	1½	1½	1	1	¾	¾	½	½
pH inside at equilibrium	3.64	3.66	3.60	3.60	3.65	3.66	3.60	3.60	3.61	3.62
pH outside at equilibrium	3.02	3.02	3.02	3.01	3.12	3.11	3.14	3.12	3.21	3.19
$y = C_H$ inside $\times 10^5$	22.9	21.9	25.1	25.1	22.4	21.9	25.1	25.1	24.6	24.0
$x = C_H$ outside $\times 10^5$	95.5	95.5	95.5	97.7	75.9	77.6	72.4	75.9	61.7	64.6
$z = \frac{(x + y)(x - y)}{y}$	375	395	338	355	235	253	184	204	130	150
$2y + z - 2x$	230	248	197	210	128	142	89	102	56	69
Observed osmotic pressure	860	860	715	680	420	445	314	316	186	186
Calculated osmotic pressure (ignoring gelatin)	576	620	493	523	320	355	222	255	140	172
Difference (osmotic pressure due to gelatin)	284	240	222	157	100	90	92	61	46	14
Mean	262		190		95		73		26	

H_2O and since 1 liter of a 1 per cent solution of gelatin contains 10 gms. of gelatin, the molecular weight of gelatin should be expected to be in the neighborhood of 25,000. The experiment just described for gelatin phosphate was repeated for gelatin chloride, with similar results.

According to Dakin's[6] recent analyses gelatin contains 1.4 per cent phenylalanine. Since 1 molecule of gelatin cannot contain less than 1 molecule of phenylalanine and since the molecular weight of this amino-acid is 165 the lowest possible molecular weight of gelatin is 11,800. If a molecule of gelatin contains two molecules of phenylalanine, the molecular weight should be about 23,600. This would be approximately the figure we might expect from the data of Table IV on the assumption that the differences in the last two rows may be considered to be the values of the osmotic pressure of the protein particles.

TABLE V.

Influence of Concentration of Albumin Chloride of pH of About 3.4 on the Osmotic Pressure.

	Concentration of Egg Albumin in per cent.					
	4	3	2	1	½	¼
pH inside at equilibrium.....	3.34	3.32	3.38	3.40	3.40	3.40
pH outside at equilibrium....	2.98	2.97	3.07	3.14	3.19	3.24
$y = C_H$ inside $\times 10^5$.........	45.7	47.9	41.7	39.8	39.8	39.8
$x = C_H$ outside $\times 10^5$.......	104.7	107.2	85.1	72.4	64.5	57.5
$z = \frac{(x+y)(x-y)}{y}$.........	194.0	192.0	132.0	92.0	64.6	43.3
$2y + z - 2x$...............	76	74	45	27	15	8
Observed osmotic pressure....	776	555 + x	375	163	75	36
Calculated osmotic pressure (ignoring albumin)........	190	185	113	67	39	20
Difference (osmotic pressure due to albumin)..........	586	370 + x	262	96	36	16

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[6] Dakin, H. D., *J. Biol. Chem.*, 1920, xliv, 499.

A similar experiment was made with different concentrations of solutions of the chloride of crystalline egg albumin. The original pH of the albumin chloride solution was 3.5 and that of the outside solution 3.0. After equilibrium was established the pH both inside and outside was slightly changed as is shown in Table V. The osmotic pressures for $\frac{1}{4}$ to 4 per cent solutions of albumin chloride were measured and calculated for $2y + z - 2x$. The difference, which should be the osmotic pressure of the albumin particles in solution, is found in the last row. It is almost identical with the difference found for gelatin chloride for the same concentration of gelatin.

V. Difference between the Curves for Calculated and Observed Values.

The curves representing the values for calculated osmotic pressures differ in one or two respects from the curves representing the values for the observed osmotic pressures. These differences are not great but they are constant and can therefore not be due to an accidental error. Fig. 3 shows the difference between the curves for the observed and the calculated osmotic pressures in the case of a gelatin chloride solution containing 1 gm. of originally isoelectric gelatin in 100 cc. If we start with the ascending branch of the two curves of Fig. 3, we notice that the observed osmotic pressures for pH 4.4 to pH 3.8 are about 100 mm. higher in each case than the calculated pressures. It may be a mere accident but 100 mm. happens to be the approximate value for the osmotic pressure of 1 gm. of gelatin in 100 cc. and if the gelatin particles participate in the osmotic pressure of solutions of gelatin salts our calculated values should be about 100 mm. lower than the observed values for the same pH in the case of a 1 per cent gelatin solution. Near the summit the difference becomes a little less but we have seen that we may expect such irregularities on account of experimental error. Besides at pH 3.4 the concentration of the gelatin solution was diminished by about 20 per cent on account of diffusion of water into the gelatin bag. The difference in the ascending branch of the observed and calculated values occurs in every experiment.

The second constant difference between the curves for observed and calculated osmotic pressures lies in the fact that the drop in the calculated curves begins at a lower pH than the drop for the curves of observed values. This or both discrepancies may be due to a constant experimental error or they may find their explanation in the influence of one or more factors not taken into consideration in our calculations.

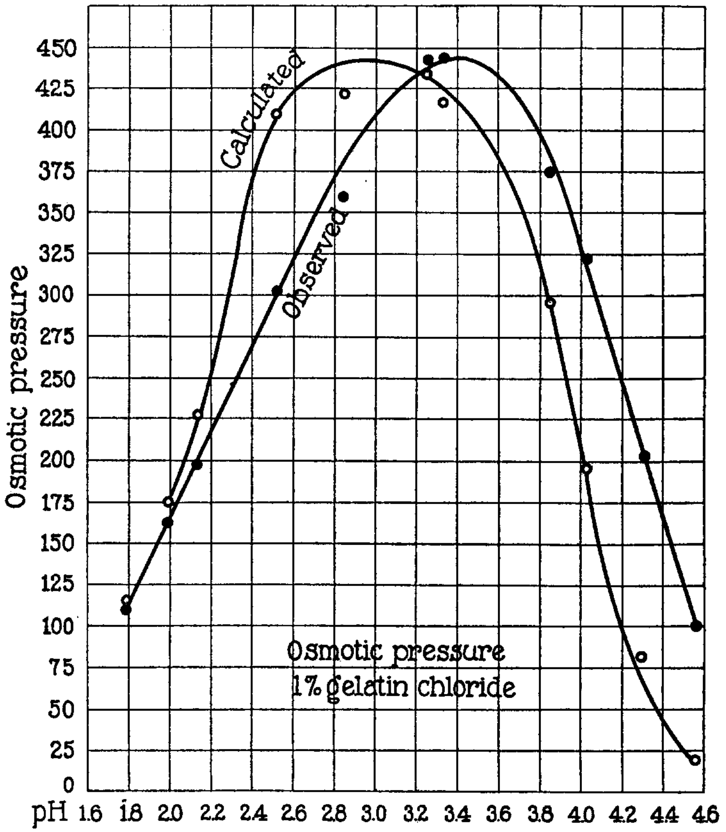

Fig. 3. Showing agreement and minor discrepancies between the curves of observed and calculated osmotic pressures of 1 per cent gelatin chloride solutions.

The calculated and observed curves for gelatin phosphate are identical with those for gelatin chloride given in Fig. 3, and we therefore omit these curves.

Fig. 4 gives the curves for observed and calculated values for gelatin sulfate. It seemed of interest to calculate the osmotic pressures from some of our older experiments on the osmotic pressure of crystalline egg albumin (containing 1 gm. of isoelectric albumin in 100 cc. solution). Fig. 5 gives a comparison of the curves for the

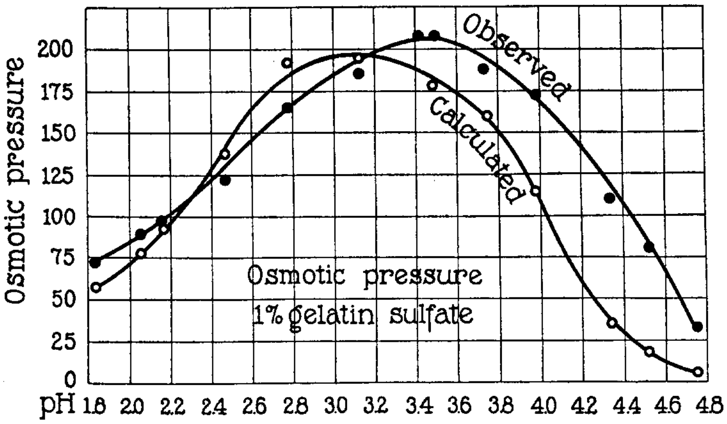

FIG. 4. Comparison of curves for observed and calculated values of osmotic pressure of solutions of 1 per cent gelatin sulfate.

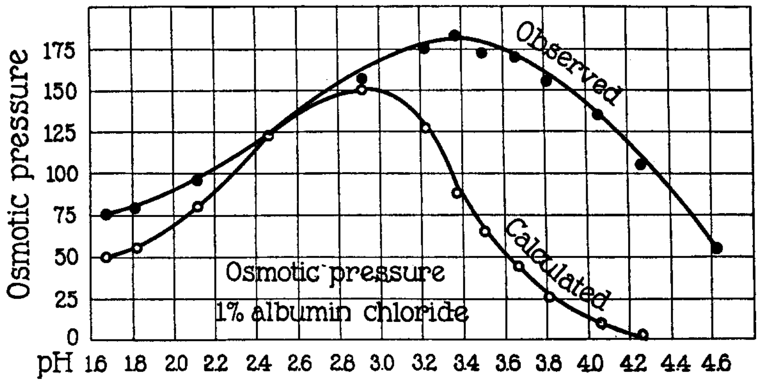

FIG. 5. Comparison of curves for observed and calculated values of osmotic pressure of solutions of 1 per cent crystalline egg albumin chloride.

observed and calculated osmotic pressures of 1 per cent albumin chloride and Fig. 6 the curves for observed and calculated osmotic pressures of 1 per cent albumin sulfate. In Fig. 6 appears the constant difference between the curves for observed and calculated values which the neglect of the protein value in the calculated curves postulates.

Procter assumes in his theory of swelling that the protein particles do not participate in the osmotic pressure inside the gel, and the writer is willing to admit that the same assumption may be necessary for the osmotic pressure of the protein solutions. The fact that the maximal observed osmotic pressure of gelatin solutions agrees with the maximal pressure calculated, without regard to the possible osmotic pressure caused by the protein ions, seems to agree with Procter's view. In this case, we should have to say that the curves for the observed values coincide with the curves for the calculated values if the latter curves are moved to the right, parallel with themselves.

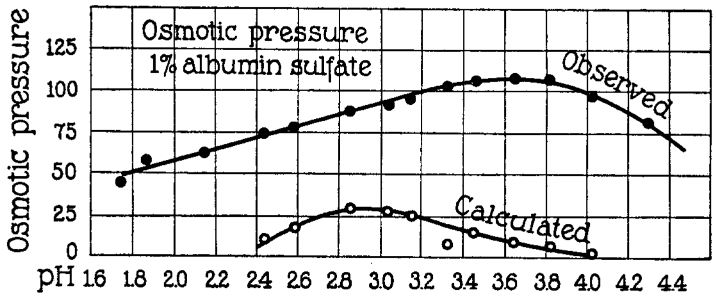

FIG. 6. Comparison of curves for observed and calculated values of osmotic pressure of solutions of 1 per cent crystalline egg albumin sulfate.

VI. The Depressing Influence of Neutral Salts on the Osmotic Pressure of a Gelatin Chloride Solution.

The question arises whether the membrane equilibrium could also be responsible for the depressing influence of salts on the osmotic pressure of protein solutions of a given pH and concentration of originally isoelectric protein. Although Donnan has shown that such a result is to be expected from his theory, the theory does not include all the facts in the case of gelatin chloride solutions. If on one side of the membrane there be a solution of NaCl, on the other side a colloidal salt NaR, where R is the colloidal ion, the real osmotic pressure (P_o) of the colloidal solution NaR can, according to Donnan, be calculated from the observed osmotic pressure (P_1) by the following equation,

$$\frac{P_1}{P_o} = \frac{C_1 + C_2}{C_1 + 2C_2}$$

assuming that gelatin is monovalent, which is improbable, where C_1 is the concentration of NaCl and C_2 that of NaR.` If C_1 is small compared with C_2, $P_1 = \frac{1}{2} P_o$; if, however, C_2 is small in comparison with C_1, $P_1 = P_o$. It follows from this that the greater the concentration of NaCl (or of any neutral salt) added to a colloidal solution, the smaller the observed osmotic pressure of the colloidal solution becomes, and this is what actually happens. It follows also from this theory that the observed osmotic pressure cannot be depressed below a certain minimum. This seems to agree with the observation that when the solution of the salt reaches the value M/8 a further increase in the concentration of the salt can no longer increase the depressing effect of the salt (see Figs. 1 and 2 in the preceding paper of this series).[2] To this extent Donnan's theory accounts for the depressing action of the salt on the osmotic pressure. A discrepancy between theory and observed value of osmotic pressure arises, however, in the fact that according to the theory the maximal depression caused by a salt should be ½ the real osmotic pressure of the colloidal solution. The observed osmotic pressure of a 1 per cent solution of gelatin chloride of pH 3.5 is about 425 mm. H_2O while the osmotic pressure in the presence of M/8 NaCl is only about 30 mm. According to the theory the latter value should be at least ½ of 425; *i.e.*, 212.

Donnan's theory can only give approximate results in this case since in his theory complicating factors were intentionally ignored for the sake of simplification. Thus the depressing effect of the addition of a neutral salt on the electrolytic dissociation of the electrolyte is not taken into consideration.

Northrop[7] has shown by conductivity measurements that the degree of ionization of gelatin chloride is noticeably depressed when the pH is 2.0 or below and is considerable when the pH is 1.0. There may be other complicating factors besides this depression of ionization.

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[7] Northrop, J. H., *J. Gen. Physiol.*, 1920–21, iii, 211.

VII. Theoretical Remarks.

It is of interest to compare the colloidal speculations with the almost quantitative results at which we arrived. All colloidal theories would agree in ascribing the effect of the hydrogen ion concentration or of the valency of the ions with which the protein is in combination to a modification in the state of the protein particles, such as hydratation or degree of dispersion. The hydratation theory is advocated by Pauli[8] who assumes that the ionized protein particle is strongly hydrated while the non-ionized protein particle is not hydrated. If this were the correct explanation of the peculiar type of curves, such as those reported in Fig. 1 of this paper, we should expect the curves for the specific conductivity of the protein in solution to show a close similarity to the curves in Fig. 1. But such is

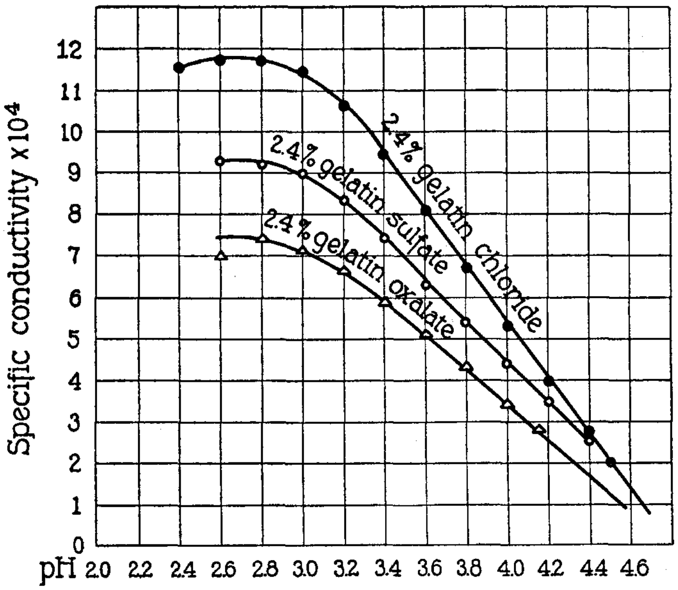

FIG. 7. Curves for the specific conductivity of 2.4 per cent solutions of gelatin chloride, sulfate, and oxalate, showing the entirely different character of these curves from that of the osmotic pressure curves in Figs. 1 and 5 in the preceding paper.

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[8] Pauli, W., Kolloidchemie der Eiweisskörper, Dresden and Leipsic, 1920.

not the case. In Fig. 7 are given the curves for the specific conductivity of 2.4 per cent solutions of gelatin chloride, sulfate, and oxalate after deduction of the specific conductivity of the free acid in the gelatin solution, as described in a previous paper. A comparison between Fig. 7 and Fig. 1 fails to show any close similarity. In the conductivity curves there is no maximum followed by a drop at pH 3.5, as there is in the osmotic pressure curves. Fig. 8 shows that the difference between the conductivity curve and the osmotic pressure curve is still more pronounced in the case of albumin chloride.

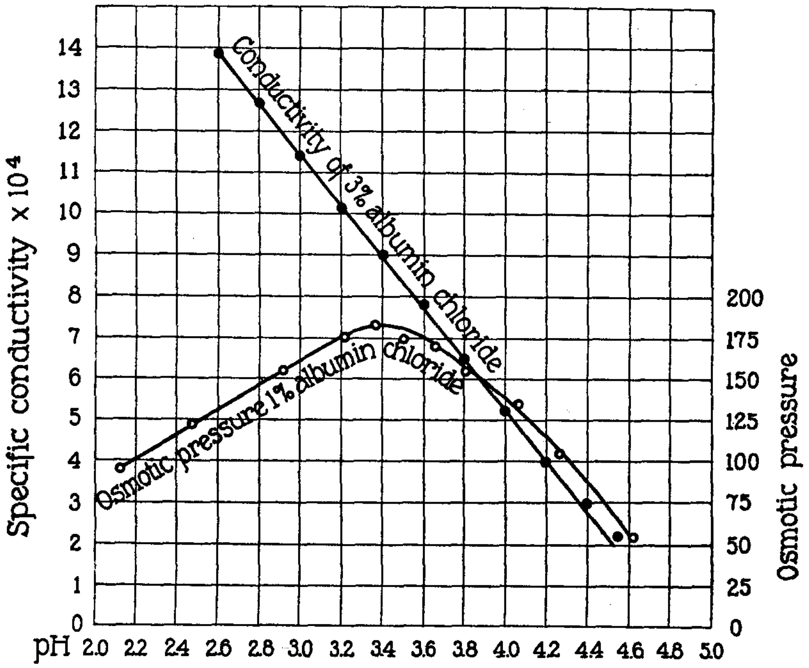

FIG. 8. Comparison of conductivity curve and osmotic pressure curve for albumin chloride, showing the entirely different character of the two curves.

Manabe and Matula,[9] who claim to have proved Pauli's theory, speak of a maximum of the physical properties of protein solutions at pH 2.0 or 2.1. There seems to be some misunderstanding, since the maximum for osmotic pressure of solutions of protein-acid salts;

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[9] Manabe, K., and Matula, J., *Biochem. Z.*, 1913, lii, 369.

e.g., gelatin or crystalline egg albumin, lies at a pH of 3.5 or 3.4, and for casein at a pH above 3.0. The maximal swelling lies at a pH of gelatin of about 3.2 or 3.3. Those who state that it lies at a lower pH (*e.g.* Michaelis[10]) must have mistaken the pH of the supernatant liquid for the pH of the solid gelatin, thus ignoring the effects of the Donnan equilibrium. The maximum for the viscosity of gelatin-acid salts lies at pH of 3.0 and for the viscosity of casein chloride or phosphate at pH 3.0 or above.

Northrop[7] has observed that a drop in the conductivity of gelatin solutions occurs when the pH falls below 2.0, but this cannot explain the drop in the osmotic pressure curves observed at pH above 3.0, and Northrop's results agree entirely with my own in not having noticed a drop in the conductivity curves at pH 3.0 or 3.5.

Moreover, Fig. 7 shows that there is only a slight difference between the conductivity curves for gelatin sulfate and gelatin chloride, while there is a greater difference between the conductivity of gelatin chloride and gelatin oxalate. All this disagrees entirely with the osmotic pressure curves in Fig. 5 in the preceding paper. Furthermore, the idea of a noticeable hydratation of the protein ion seems to be no longer tenable on the basis of Lorenz[11] and Born's[12] experiments and conclusions.

A second colloidal hypothesis would lead us to assume that variations in the degree of dispersion of the protein particles are responsible for the osmotic pressure curves represented in Fig. 1. We need not dwell on this hypothesis since we have no way of putting it to a quantitative test.

The results of this paper show that if we assume the correctness of Donnan's theory of membrane equilibrium the characteristic influences of pH and valency on the osmotic pressure appear as a necessary consequence of the theory; with the exception of the two minor differences discussed in Chapter IV. Donnan's theory leads to a view radically different from all colloidal speculations since on the basis of this theory the variations in osmotic pressure depend on the

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[10] Michaelis, L., Praktikum der physikalischen Chemie insbesondere der Kolloidchemie, Berlin, 1921.

[11] Lorenz, R., *Z. Elektrochem.*, 1920, xxvi, 424.

[12] Born, M., *Z. Elektrochem.*, 1920, xxvi, 401.

unequal distribution of the crystalloidal ions on the opposite sides of the membrane and not on variations in so called colloidal properties of proteins. The quantity which changes with the pH and the valency of the anion of a protein-acid salt is on this assumption not the degree of hydratation or dispersion of the protein particles but the value pH inside minus pH outside, as stated more fully in the two preceding papers.[1]

SUMMARY.

1. It had been shown in previous publications that the osmotic pressure of a 1 per cent solution of a protein-acid salt varies in a characteristic way with the hydrogen ion concentration of the solution, the osmotic pressure having a minimum at the isoelectric point, rising steeply with a decrease in pH until a maximum is reached at pH of 3.4 or 3.5 (in the case of gelatin and crystalline egg albumin), this maximum being followed by a steep drop in the osmotic pressure with a further decrease in the pH of the gelatin or albumin solution. In this paper it is shown that (aside from two minor discrepancies) we can calculate this effect of the pH on the osmotic pressure of a protein-acid salt by assuming that the pH effect is due to that unequal distribution of crystalloidal ions (in particular free acid) on both sides of the membrane which Donnan's theory of membrane equilibrium demands.

2. It had been shown in preceding papers that only the valency but not the nature of the ion (aside from its valency) with which a protein is in combination has any effect upon the osmotic pressure of the solution of the protein; and that the osmotic pressure of a gelatin-acid salt with a monovalent anion (*e.g.* Cl, NO_3, acetate, H_2PO_4, HC_2O_4, etc.) is about twice or perhaps a trifle more than twice as high as the osmotic pressure of gelatin sulfate where the anion is bivalent; assuming that the pH and gelatin concentrations of all the solutions are the same.

It is shown in this paper that we can calculate with a fair degree of accuracy this valency effect on the assumption that it is due to the influence of the valency of the anion of a gelatin-acid salt on that relative distribution of the free acid on both sides of the membrane which Donnan's theory of membrane equilibrium demands.

3. The curves of the observed values of the osmotic pressure show two constant minor deviations from the curves of the calculated osmotic pressure. One of these deviations consists in the fact that the values of the ascending branch of the calculated curves are lower than the corresponding values in the curves for the observed osmotic pressure, and the other deviation consists in the fact that the drop in the curves of calculated values occurs at a lower pH than the drop in the curves of the observed values.