THE ORIGIN OF THE ELECTRICAL CHARGES OF COLLOIDAL PARTICLES AND OF LIVING TISSUES.

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(Received for publication, November 19, 1921.)

I. Stability of Suspensions, Electrical Charges of Micellæ, and Donnan Equilibrium.

The stability of suspensions is, perhaps, the chief problem of a theory of colloidal behavior. Hardy has shown that this problem is linked with the problem of the origin of the electrical charges of the particles in suspension (which we will term micellæ, when they consist of aggregates of ions or molecules) inasmuch as the micellæ carrying a sufficiently large electrical charge will be forced by mutual electrostatic repulsion to stay in suspension. By his experiments on the migration of suspended particles of coagulated white of egg in an electrical field he proved that they have a positive charge in the presence of acid, a negative charge in the presence of alkali, and no charge at an intermediate point which he termed the isoelectric point of the particles. He was able to demonstrate that the stability of colloidal suspensions is a minimum at the isoelectric point.[1]

He and others found, moreover, that low concentrations of neutral salts diminish the stability of colloidal suspensions in the presence of acids or alkalies and that the efficient ion of the salt has the opposite sign of charge from the colloidal particle; since the precipitating efficiency of a salt increases rapidly with the valency of that ion of the salt which has the opposite sign of charge from the colloidal particle. It seemed natural to infer that the precipitation of colloidal

[1] Hardy, W. B., *Proc. Roy. Soc. London, Series B*, 1899–1900, lxvi, 110; *J. Physiol.*, 1903, xxix, p. xxvi. Wood, T. B., and Hardy, W. B., *Proc. Roy. Soc. London*, 1909, lxxxi, 38.

suspensions by low concentrations of a salt was caused by an annihilation of the charge of the colloidal particle. The problem of the stability of the colloidal suspension then developed into the problem of accounting for this peculiar behavior of the electrical charges of colloidal particles.

Hardy's original idea was that the H ions of the acid or OH ions of the alkali were adsorbed by the colloidal particle in preference to the other ions on account of their greater rapidity of migration; and this idea was also accepted by Perrin in his experiments on electrical endosmose, where it was necessary to account for the fact that certain membranes become positively charged in the presence of acid and negatively in the presence of alkali.[2] Those who accept this adsorption hypothesis explain the fact that the electrical charges of the particles are apparently diminished or destroyed by the addition of a salt on the assumption of a preferential adsorption of one of the ions of the salt; yet such an assumption is incompatible with the purely stoichiometrical behavior of proteins. It is also difficult to account for the fact that the addition of little acid increases while the addition of more acid depresses the electrical charge of micellæ on the basis of the adsorption hypothesis.

A second possibility was pointed out by the writer in 1904; namely, that Hardy's migration experiments might be explained in the case of proteins by the fact that proteins are amphoteric electrolytes which, in the presence of alkali, dissociate electrolytically by giving rise to a protein anion and, in the presence of acid, by giving rise to a protein cation while at the isoelectric point no protein ion would be formed.[3] While this idea is correct if applied to the migration of isolated protein ions in the electrical field, it cannot explain why the addition of a salt in low concentration should diminish the charge of aggregates of molecules and ions, the micellæ, except by assuming that in this case the electrolytic dissociation of the protein salts should be repressed. The concentration of salts required for the precipitation of colloidal suspensions is, however, much too small to make such a suggestion acceptable.

[2] Perrin, J., *J. chim. physique*, 1904, ii, 601; 1905, iii, 50.
[3] Loeb, J., *Univ. California Pub., Physiol.*, 1903–04, i, 149.

In 1916 J. A. Wilson suggested that the electrical charges of micellæ were caused by the establishment of a Donnan equilibrium between the colloidal particle and the surrounding solution.[4] There were, however, no measurements of membrane potentials available at that time and this was probably the reason that his suggestion was not accepted.

We may consider a solution of a protein inside a collodion bag surrounded by a watery solution (free from protein) as a model of a micella. The measurements of the P. D. with the aid of a Compton electrometer with saturated calomel electrodes show that the electrical charges of this model vary in the same way as the charges possessed by colloidal particles in general (*e.g.* coagulated egg albumin) in suspension; namely, (1) The electrical charge of the micella model is zero at the isoelectric point of the protein. (2) The charge of the model is positive on the acid side and negative on the alkali side of the isoelectric point, and increases with the addition of little acid and diminishes with the addition of more acid to isoelectric particles. (3) The charge of the model is depressed by the addition of low concentrations of neutral salts and the depressing action of the salt increases rapidly with the valency of that ion of the neutral salt which has the opposite sign of charge to that of the micella.[5]

If these charges are due to the Donnan equilibrium it must be possible to prove that the concentration of the crystalloidal ions inside the micella (or its model) is different from their concentration in the surrounding liquid and that this difference in the concentration of crystalloidal ions on the opposite sides of the membrane is able to account quantitatively for the observed P. D. The difference in the concentration of crystalloidal ions in the two phases (micella and surrounding water) is due to the fact that the protein ion cannot diffuse into the watery solution. When a solution of a protein-acid salt is inside a collodion membrane, the diffusion of the protein ion is prevented by the membrane and when the protein ion forms part of a gel the diffusion of the protein ions is prevented by the forces of

[4] Wilson, J. A., *J. Am. Chem. Soc.*, 1916, xxxviii, 1982.
[5] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 667.

cohesion between the protein ions constituting the gel, while both the collodion membrane and the solid gel are permeable for crystalloidal ions. We have already shown that when we separate a solution of gelatin chloride from water by a collodion membrane, at equilibrium the concentration of chlorine ions inside the gelatin solution is greater than the concentration of chlorine ions in the outside solution, and the concentration of the hydrogen ions inside the gelatin solution is smaller than outside, as Donnan's theory demands.[5] The concentration of the Cl ions was determined by titration and it was shown that the P.D. calculated with the aid of Nernst's formula from the difference of concentration of chlorine ions inside and outside, was within the limits of accuracy of the measurements, identical with the P.D. directly observed between the gelatin chloride solution and the outside solution with the aid of a Compton electrometer with saturated calomel electrodes. In other words, it was found that

$$\text{P.D. observed} = 58 \log \frac{C_{\text{Cl outside}}}{C_{\text{Cl inside}}} \text{ millivolts}$$

or, since $\log C_{\text{Cl outside}} = \text{pCl outside}$, and $\log C_{\text{Cl inside}} = \text{pCl inside}$,

$$\text{P.D. observed} = 58 \text{ . (pCl outside minus pCl inside) millivolts}$$

If Donnan's theory accounts quantitatively for the observed P. D., the value 58 (pH inside minus pH outside) should also agree quantitatively with the observed P.D.

The values of pH inside and pH outside were determined in our experiments with the potentiometer (*i.e.* the hydrogen electrode), and the value 58 (pH inside minus pH outside) was therefore also an observed value. In order to avoid confusion of terms we call the P.D. observed with the Compton electrometer the *observed* P.D., since this gives us the empirical charge found in the micella or its model, free from any theory. On the other hand, we will call the value 58 (pH inside minus pH outside) the *calculated* P.D. since we could find this value by calculation from Nernst's formula if we determined the hydrogen ion concentration of the inside and outside solutions by titration instead of by the potentiometer.

The determinations of the value pH inside minus pH outside give the following results already published in a preceding paper:[5] (1)

At the isoelectric point of gelatin the value of pH inside minus pH outside is zero. (2) When the collodion bag contains a solution of gelatin-acid salt, the value pH inside minus pH outside is positive. (3) The value pH inside minus pH outside increases at first when acid is added to isoelectric gelatin with the increase in acid, but soon reaches a maximum and diminishes again upon the addition of further acid. It is shown that the P.D. observed with the Compton electrometer between the solution and the water varies in exactly the same way. (4) The addition of a neutral salt to a solution of gelatin chloride at the pH where the observed P.D. is about a maximum diminishes the value of pH inside minus pH outside in the same way as it diminishes the observed P.D. (5) The main fact was that the value 58 (pH inside minus pH outside) agreed quantitatively with the observed P.D.

These facts show that the P.D. between a gelatin chloride solution and a watery solution (separated by a collodion membrane) is caused exclusively by a difference in the concentration of diffusible ions inside and outside the gelatin solution. If there were a second source for the P.D., the P.D. obtained from the value 58 (pH inside minus pH outside) would be always smaller than the P.D. observed with the aid of a Compton electrometer. The reader will therefore see that the quantitative agreement between the values of 58 (pH inside minus pH outside) with the observed P.D. between the gelatin chloride solution and the outside solution is the essential proof that only the Donnan equilibrium is responsible for the difference of potential between the gelatin chloride solution and an outside watery solution.

This paper intends to fill out several gaps left in the preceding publication. Thus it was not proven that on the basis of the Donnan equilibrium the gelatin must have a negative charge on the alkali side of the isoelectric point. When we put a solution of Na gelatinate into a collodion bag and dip the bag into water, the Donnan equilibrium demands that NaOH be expelled from the solution of Na gelatinate through the collodion membrane into the outside solution, and that when equilibrium is established between the solutions of Na gelatinate and water the concentration of NaOH must be greater in the outside watery solution than in the solution of Na

gelatinate (inside solution). As a consequence the pH in the outside solution should be higher than in the inside solution, and the value pH inside minus pH outside should become negative when the inside solution is Na gelatinate. This is the reason why powdered particles of Na gelatinate must assume a negative charge. In the case of a gelatin chloride solution the value pH inside minus pH outside is positive and this explains why powdered particles of gelatin chloride are positively charged.

We will now show that when we separate a solution of Na gelatinate from a watery solution by a collodion membrane and allow both solutions to reach or approach equilibrium, the value pH inside minus pH outside actually becomes negative.

II. The Electrical Charge of Na Gelatinate.

It is necessary to prevent the CO_2 of the air from diffusing into the solutions of Na gelatinate, and therefore the outside solution was put into stoppered bottles connected with the outside air by glass tubes filled with soda lime. The pH measurements were less certain than in the experiments with acid on account of the CO_2 error. There may be other experimental shortcomings, *e.g.*, the slowness of the establishment of the Donnan equilibrium between inside and outside solutions near the isoelectric point.

Collodion bags of a volume of about 50 cc. were filled with solutions of Na gelatinate containing 1 gm. of originally isoelectric gelatin and varying amounts of 0.1 N NaOH in 100 cc. solution. The collodion bags were dipped into flasks containing 500 cc. of aqueous solutions of NaOH of various concentrations and free from gelatin. The flasks were sealed, communicating with the air only through tubes filled with soda lime, as stated. The collodion bags containing the gelatin were closed by a rubber stopper perforated by a glass tube which served as a manometer. The experiment lasted 6 hours at a temperature of 24°C. The results of the experiments are given in Table I. The upper horizontal row gives the number of cc. of 0.1 N NaOH originally in 100 cc. of the gelatin solution; the second row gives the original concentration of NaOH in the outside aqueous solution free from gelatin; the third row gives the osmotic pressure in millimeters H_2O after 6 hours. The next row gives the pH inside

TABLE I.

1 Per Cent Na Gelatinate.

Cc. 0.1 N NaOH added to 1 gm. gelatin in 100 cc.	0	1	2	3	4	5	6	8	10	12.5	15	20
Concentration of NaOH of outside solution	0	0	0	N/25,600	N/12,800	N/6,400	N/3,200	N/1,600	N/800	N/400	N/200	N/100
Osmotic pressure, millimeters	26	164	265	353	375	385	366	335	340	265	192	150
pH inside	5.02	5.40	5.76	6.64	7.15	9.02	9.68	10.16	10.45	—	11.30	11.58
pH outside	5.60	5.82	5.92	6.37	7.70	9.50	9.96	10.60	10.85	—	11.46	11.70
pH inside minus pH outside	−0.58	−0.42	−0.16	+0.27	−0.55	−0.48	−0.28	−0.44	−0.40	—	−0.16	−0.12
P.D. calculated, millivolts	−34.0	−24.5	−9.4	+15.8	−32.0	−28.0	−16.5	−25.7	−23.4	—	−9.4	−7.0
P.D. observed, millivolts	−3.5	−19.5	−18.0	−37.5	−37.5	−36.0	−30.0	−22.0	−19.5	—	−10.0	−7.0

and the following row the pH outside after the experiment was finished (*i.e.* after 20 hours), and the sixth row gives the difference pH inside minus pH outside. The reader will notice that this difference is always negative with one exception which is obviously an error. The last two rows give the calculated P.D. (*i.e.* the value 58 (pH inside minus pH outside)), and the observed P.D. Observed P.D. means the millivolts between the solution of Na gelatinate and watery solution measured directly in the Compton electrometer, as described in preceding papers.

It is obvious that there is no quantitative agreement between the observed and calculated P.D. near the isoelectric point, probably on account of the CO_2 error. As soon as the pH is above 7.0 the agreement between observed and calculated P.D. becomes better so that we are entitled to say that the difference of potential between a Na gelatinate solution and an outside solution is due to the Donnan equilibrium which forces the expulsion of NaOH from the inside into the outside solution. As a consequence the pH inside becomes lower than the pH outside.

III. Valency Effect on the P.D.

It had been shown in a preceding paper that the charge of a solution of gelatin sulfate is lower than a charge of a solution of gelatin chloride or gelatin phosphate or any other gelatin-acid salt with a monovalent anion of the same pH and the same concentration of originally isoelectric gelatin.[5] It should, however, be pointed out that on the basis of the theory the ratio of the charges in the two cases should be exactly as 3:2. It is needless to say that if we can prove that this postulate is fulfilled the probability that the charges of micellæ are due to the Donnan equilibrium becomes very strong.

The equilibrium equation which is of the second degree when the anion is monovalent, *e.g.* Cl, in the case of gelatin chloride, becomes of the third degree when the anion is bivalent, *e.g.* SO_4, in the case of gelatin sulfate. Let x be the concentration of hydrogen ions and Cl ions of the outside, y that of the H and Cl ions of the free HCl inside the gelatin chloride solution, and z the concentration of the Cl in combination with gelatin. Then the equilibrium equation is,

$$x^2 = y\,(y+z)$$
$$x = \sqrt{y\,(y+z)}$$

Substituting this term $\sqrt{y\,(y+z)}$ for x in $\frac{x}{y}$ we get

$$\frac{x}{y} = \frac{\sqrt{y\,(y+z)}}{y} = \sqrt{1+\frac{z}{y}}$$

The P.D. is $58 \log \sqrt{1+\frac{z}{y}} = \frac{58}{2} \log \left(1+\frac{z}{y}\right)$.

In the same way we can arrive at the term for $\frac{x}{y}$ in the case of gelatin sulfate solution. If we call x the concentration of hydrogen ions in the outside solution, y that of the hydrogen ion concentration in the inside solution; then $\frac{x}{2}$ is the concentration of the SO_4 ions in the outside and $\frac{y}{2}$ the concentration of the SO_4 ions of the free H_2SO_4 in the inside (gelatin) solution. The concentration of SO_4 ions in combination with gelatin becomes $\frac{z}{2}$. Then the equilibrium equation is as follows:

$$\frac{x^3}{2} = \frac{y^2\,(y+z)}{2};\quad x = [y^2\,(y+z)]^{\frac{1}{3}}$$

The value which interests us is $\frac{x}{y}$, *i.e.* the ratio of the hydrogen ion concentration outside over that inside.

Substituting $[y^2\,(y+z)]^{\frac{1}{3}}$ for x in $\frac{x}{y}$ we get

$$\frac{x}{y} = \sqrt[3]{\frac{y^2\,(y+z)}{y^3}} = \sqrt[3]{\frac{y+z}{y}}$$

The P. D. is therefore in the case of gelatin sulfate

$$\text{P.D.} = \frac{58}{3} \log \left(1+\frac{z}{y}\right) \text{millivolts}$$

while in the case of gelatin chloride it is

$$\text{P.D.} = \frac{58}{2} \log \left(1+\frac{x}{y}\right) \text{millivolts}$$

Hence the P.D. of gelatin sulfate solution should be two-thirds of the P. D. of a gelatin chloride solution of the same pH and the same concentration of originally isoelectric gelatin.

1 gm. of isoelectric gelatin was dissolved in 100 cc. of water containing in one case 5 cc. of 0.1 N HCl, in the other, 5 cc. of 0.1 N H_2SO_4. One collodion bag with a volume of 50 cc. was filled with the gelatin chloride solution and this bag was dipped into a beaker containing 350 cc. N/1000 HCl. A second collodion bag was filled with the gelatin sulfate solution and this bag was dipped into 350 cc. N/1000 H_2SO_4. The solutions were kept for 24 hours at 24°C. and the pH inside, and pH outside were measured. The pH of the two gelatin solutions was identical, namely 3.64, but the value pH inside minus pH outside was for the gelatin chloride solution 0.49 and for the gelatin sulfate solution 0.31, which is as near 3:2 as the accuracy of the measurements permits. A confirmation of this result can be found in the experiments published in a preceding paper where this relation had not yet been recognized.[5] Thus it was found that for gelatin phosphate of pH 3.98 the value of pH inside minus pH outside was 0.58, while for gelatin sulfate of pH 3.98 the value of pH inside minus pH outside was 0.38, which is again the ratio of 3:2. For pH 4.31 the value pH inside minus pH outside was 0.53 for gelatin chloride while it was for pH 4.34, 0.35 for gelatin sulfate, which is again as 3:2. The P.D. observed directly with the Compton electrometer agreed quantitatively with the value 58 (pH inside minus pH outside).

Quantitative results, such as these, leave little doubt that the P. D. between solutions of gelatin-acid salts and outside watery solutions when separated by a collodion membrane are determined entirely by the Donnan equilibrium; and that there can be no other source of the charge of this micella model.

IV. The P. D. *of Solutions of Crystalline Egg Albumin.*

The experiments published thus far had all been done on gelatin. It was of importance to make sure whether or not these results can be confirmed with crystalline egg albumin. This was found to be the case, and the experiments on the membrane potentials of the

solutions of the chloride of crystalline egg albumin showed a perfect quantitative agreement with the theory.

Collodion bags of about 50 cc. volume were filled with a solution of 1 per cent crystalline egg albumin containing varying amounts of 0.1 N HCl, and the bags were put, as usual, into beakers containing 350 cc. of HCl solutions of different concentration but free from albumin. The first two horizontal rows of Table II give the amount of 0.1 N HCl in each solution. The experiments were carried out at a temperature of 24°C. and after 22 hours the osmotic pressure, P. D., and pH of inside (albumin) solution and pH of the outside solution were measured, the P. D. with the Compton electrometer and the pH with the hydrogen electrode. The albumin used was not isoelectric, but, since it had been prepared after Sörensen's method, it was probably partly ammonium albuminate, with a pH of near 6.0. The table shows that the observed P. D. agree with the value 58 (pH inside minus pH outside), *i.e.* the calculated P. D. (especially on the acid side of the isoelectric point); that the P. D. is a minimum near pH 4.7 of the albumin (*i.e.* near its isoelectric point which is at pH 4.8), and that the albumin is positively charged on the acid and negatively charged on the alkaline side of the isoelectric point. This is again in harmony with what we should expect on the basis of the Donnan equilibrium.

The next problem was to determine the influence of the addition of a neutral salt to a solution of the chloride of crystalline egg albumin. A 1 per cent solution of crystalline egg albumin containing 7 cc. of 0.1 N HCl in 100 cc. was made up in various concentrations of NaCl. The collodion bags containing these albumin chloride-NaCl mixtures were dipped into beakers containing 350 cc. of the same concentration of NaCl as that of the albumin solution, and all made up in N/1000 HCl. The experiment was carried out at 24°C. and the measurements were made after 22 hours.

Table III gives the results which show again a good agreement between the observed P. D. and the value 58 (pH inside minus pH outside), our so called calculated P.D.

We may, therefore, conclude that the P.D. of both gelatin solutions and solutions of crystalline egg albumin separated by a collodion membrane from a watery solution free from protein is accounted for

TABLE II.

1 Per Cent Albumin Chloride.

Cc. 0.1 N HCl added to 1 gm. albumin in 100 cc.	0	1	2	3	4	5	6	7	8	10	15	20	30	40
Cc. 0.1 N HCl in 350 cc. outside solution	0	0.1	0.3	0.5	1	1.5	2.1	2.8	4	7.1	16.4	32	60	80
Osmotic pressure, millimeters	155	100	52	114	178	205	214	219	218	180	136	100	81	74
pH inside	5.80	5.40	4.70	4.30	4.00	3.75	3.64	3.42	3.24	3.00	2.53	2.20	1.89	1.73
pH outside	6.14	5.64	4.67	4.06	3.65	3.38	3.22	3.07	2.91	2.71	2.37	2.10	1.82	1.70
pH inside minus pH outside	−0.34	−0.24	+0.03	+0.24	+0.35	+0.37	+0.42	+0.35	+0.33	+0.29	+0.16	+0.10	+0.07	+0.03
P.D. calculated, millivolts	−20.0	−14.0	+2.0	+14.0	+20.6	+22.4	+25.5	+21.0	+20.0	+17.5	+9.4	+6.0	+4.0	+2.0
P.D. observed, millivolts	−24.0	−16.0	+3.0	+11.5	+19.0	+19.5	+20.5	+19.5	+18.5	+16.0	+11.0	+10.0	+4.0	+3.5

TABLE III.

Influence of Salt on P.D. *of Albumin Chloride Solution.*

	Concentration of NaCl.									
	0	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8
Osmotic pressure, millimeters.....	210	181	156	131	107	87	73	61	54	45
pH inside.....	3.35	3.32	3.32	3.27	3.25	3.20	3.19	3.22	3.21	3.22
pH outside.....	3.04	3.04	3.07	3.10	3.11	3.13	3.14	3.18	3.21	3.23
pH inside minus pH outside,.....	0.31	0.28	0.25	0.17	0.14	0.07	0.05	0.04	0.00	−0.01
P.D. calculated, millivolts.....	+18.0	+16.2	+14.5	+10.0	+8.0	+4.1	+2.9	+2.3	0	−0.5
P.D. observed, millivolts.....	+18.5	+15.5	+13.5	+10.0	+7.5	+5.0	+3.0	+1.5	+1.0	+0.5

completely by the Donnan equilibrium. There can be no other source for the electrical charge of this model of a protein micella except that due to the membrane equilibrium.

V. The Electrical Charges of Suspended Particles of Powdered Gelatin.

It is possible to show that the electrical charges of the powdered particles of gelatin suspended in a watery solution are determined by the fact that acid is forced from the suspended particles into the watery solution when the particles consist of gelatin chloride, and that alkali is forced from the particles into the solution when they consist of Na gelatinate.

Measurements of the P.D. between solid gels of gelatin and the surrounding solution suffer from inaccuracies (especially near the isoelectric point) which we have not been able to eliminate, so that we must be satisfied with only an approximate confirmation of the theory. In order to prove or to make it probable that the P.D. is due to the Donnan equilibrium we must be able to show that there exists a difference of the value of pH inside and pH outside the gel when the suspended particles of gelatin chloride or Na gelatinate are in equilibrium with the watery solution.

1 gm. of powdered gelatin of grain size between mesh 30 and 60, and rendered isoelectric was put into each of a series of closed flasks containing 350 cc. of distilled water with varying quantities of 0.1 N HCl or NaOH per 100 cc. (see Table IV). The temperature was 20°C. After 4 hours the powdered gelatin was separated from its liquid by filtration, the gelatin was melted and the pH of the melted gelatin and of the outside solution (filtrate) were measured. The gelatin was then solidified and the P. D. between the solid gelatin and the filtrate (outside solution) determined, as will be described a little later. The results of the experiments are given in Table IV. The first row gives the number of cc. of 0.1 N HCl or NaOH contained originally in 100 cc. outside solution. The next row gives the relative volume of the solid mass of gelatin, *i.e.* the degree of swelling. The rest of the table needs no explanation. It is obvious that pH inside minus pH outside is positive as long as the pH of the gelatin is on the acid side of the isoelectric point, while it is negative when the gelatin is on the alkaline side of the isoelectric point. The turning point is

TABLE IV.

Suspensions of Powdered Gelatin.

	HCl.				NaOH.						
Cc. 0.1 N HCl or NaOH in 100 cc. solution.	1.0	0.5	0.2	0.1	0	0.1	0.2	0.5	1.0	2.0	4.0
Volume of gelatin, millimeters	28	20	18	16	17	18	28	37	40	47	48
pH of melted gelatin (inside)	4.44	4.56	4.79	4.85	4.89	4.98	5.06	5.50	6.74	9.54	10.15
pH of supernatant liquid (outside)	3.35	3.55	3.92	4.24	4.97	5.96	6.24	6.46	7.30	10.56	11.08
pH inside minus pH outside	+1.09	+1.01	+0.87	+0.61	−0.08	−0.98	−1.18	−0.96	−0.56	−1.02	−0.93
P.D. calculated, millivolts	+63.0	+58.6	+51.0	+36.0	−4.5	−57.0	−68.0	−56.0	−33.0	−59.0	−48.0
P.D. observed, millivolts	+56.0	+55.5	+36.5	+15.0	−17.5	−59.0	−61.0	−70.0	−66.0	−46.0	−36.0

approximately at the isoelectric point, but the measurements near the isoelectric point are obviously vitiated by experimental errors or by some other factor so that we cannot demonstrate more by the experiment than that the suspended particles of solid metal gelatinate have the opposite sign of charge from the gelatin chloride and that this difference is accompanied by a reversal of the sign of the value of pH inside minus pH outside, which is positive in the case of gelatin chloride and negative in the case of Na gelatinate. It may also be pointed out that the minimum of swelling (volume) coincides with the minimum of P.D.

While the experimental errors are rather great in the neighborhood of the isoelectric point and on the alkaline side, they are fortunately less annoying on the acid side when the hydrogen ion concentration is sufficiently large. In this case the agreement between the value pH inside minus pH outside and the P.D. observed is at least sufficient to show that the P.D. is caused by the Donnan equilibrium.

1 gm. of powdered isoelectric gelatin going through mesh 30 but not through mesh 60 was put into 350 cc. of water containing various quantities of HCl (see first horizontal row of Table V), and left in this solution for 24 hours at 20°C. The flasks were occasionally stirred. After 24 hours the volume of the particles was measured and they were put on a filter to allow the outside solution to drain off. The gelatin was then melted by heating to 45°C. and poured into glass cylinders which at their lower end had two glass side tubes attached. The mass was then allowed to solidify and the P. D. between gelatin and watery solution was ascertained. One of the two glass tubes dipped into a beaker containing the outside HCl solution (the filtrate) with which the gelatin had been in equilibrium, and the other dipped into a beaker containing a saturated solution of KCl. Each beaker was connected with one of the calomel electrodes (filled with saturated KCl) of a Compton electrometer. The last row in Table V gives the observed P.D. in millivolts.

The gelatin was then melted and its pH was determined potentiometrically. This is called pH inside in Table V. The pH of the outside solutions (filtrate) was also determined at 24°C.

While the agreement between the observed P.D. and the values of 58 (pH inside minus pH outside) is not as complete as in the experi-

TABLE V.

Suspensions of Powdered Gelatin.

Cc. 0.1 N HCl in 100 cc. H_2O....	0.5	1.0	2.0	4.0	6.0	8.0	10.0	12.0	15.0	20.0	30.0	40.0
Volume of gelatin, millimeters..	30	40	62	73	75	73	66	64	54	50	41	37
pH of melted gelatin (inside)	4.58	4.27	3.76	3.26	2.92	2.57	2.41	2.29	2.11	1.96	1.78	1.59
pH of supernatant liquid (outside)	3.89	3.45	3.04	2.65	2.44	2.27	2.16	2.07	1.95	1.82	1.65	1.49
pH inside minus pH outside.....	0.69	0.82	0.72	0.61	0.48	0.30	0.25	0.22	0.16	0.14	0.13	0.10
P.D. calculated, millivolts.....	+40.7	+48.4	+42.5	+36.0	+28.4	+17.7	+14.7	+13.0	+9.5	+8.3	+7.7	+5.9
P.D. observed, millivolts.....	+37.5	+39.0	+38.0	+29.5	+22.0	+17.7	+17.7	+18.2	+17.0	+10.7	+8.6	+5.4

ments with solutions inside collodion bags, it is at least sufficient to leave no doubt that this difference in pH inside and outside causes the P.D. In other words, there is no doubt that the P.D. between the powdered particles and the surrounding liquid with which they are in equilibrium is due to the Donnan equilibrium.

We have already shown in a preceding paper that the addition of a salt to a solution containing suspended particles of powdered gel of gelatin chloride diminishes the P.D. between the particles and surrounding liquid and that this diminution is due to a diminution of the value pH inside minus pH outside; *i.e.*, to the Donnan equilibrium.[5]

These facts then leave no doubt that the difference in the hydrogen ion concentration between micellæ of protein and the surrounding solution which the Donnan equilibrium demands is the only cause of the electrical charges of micellæ of proteins or of their models.

The experiments on the solution of casein chloride published by Robert F. Loeb and the writer in the preceding number of this Journal indicate that aside from the electrical charges osmotic forces may play a rôle in maintaining the stability of colloidal suspensions.[6] These forces are also a consequence of the Donnan equilibrium and hence vary in a similar way as the P.D. No other theory except the Donnan theory can account for this similarity.

VI. The Origin of the Electrical Charges of Living Cells and Tissues.

In his first paper on the theory of membrane equilibria Donnan suggested that the membrane potentials postulated by his theory might contribute towards an explanation of the action of nerves and even of electrical fish. In 1911 the writer suggested to Dr. Beutner that he investigate the P.D. between such organs as apples or leaves of the rubber plant and water, instead of the P.D. of muscles or nerves which had usually been used by physiologists for this purpose. In these experiments Dr. Beutner made the important observation that the P.D. between the surface of an apple or a leaf was a maximum when the bounding liquid was pure water, while the P. D. was depressed when a salt was added to the water the depressing effect on

[6] Loeb, J., and Loeb, R. F., *J. Gen. Physiol.*, 1921–22, iv, 187.

the P. D. increasing with the concentration of the salt.[7] MacDonald[8] had observed a similar phenomenon; namely, the increase in P.D. between nerve and surrounding salt solution with increasing dilution. Donnan's theory was not known to us and we were not able to give an explanation of the depressing effect of salt on the P.D.

We next searched for those substances in the cortex of an apple or leaf which might be responsible for these peculiar concentration effects on the P. D. When the P. D. between solid gels of gelatin and of coagulated egg albumin and water was investigated no potential differences were observed,[9] to the great surprise and disappointment of the writer who had hoped that the investigations of the P. D. might lead to an explanation of the antagonistic ion effects in which he was then interested. It is possible that the negative results with protein were due to the fact that the measurements were accidentally made near the isoelectric point. On the other hand, it was found that there exists a P.D. at the boundary of lipoids (lecithin dissolved in guaiacol) which is depressed by the addition of salts, and the more the higher the concentration of the salt.[9]

This analogy between lipoids and living cells gave us the impression that the proteins had no share in the potential differences observed between living tissues or living cells and watery solutions. The experiments recorded in this paper leave no doubt that this conclusion was wrong; any ion in a cell or on its surface which cannot diffuse into the surrounding watery solution (no matter whether the ion is a protein or a fatty acid or some complicated lipoid or a complicated carbohydrate or even a crystalloid) can or must give rise to a P.D. which is depressed when a diffusible salt is added to the surrounding watery solution.

The idea that lipoids are the substances responsible for the P.D. of tissues led Beutner to an extensive and most interesting investigation of the P.D. at the boundary of water-immiscible substances and water.[10] He found always a depressing effect of the addition of salt. Beutner

[7] Loeb, J., and Beutner, R., *Biochem. Z.*, 1912, xli, 1.

[8] MacDonald, J. S., *Proc. Roy. Soc.*, 1900, lxvii, 310.

[9] Loeb, J., and Beutner, R., *Biochem. Z.*, 1913, li, 288; 1914, lix, 195.

[10] Beutner, R., Die Entstehung elektrischer Ströme in lebenden Geweben, Stuttgart, 1920.

tried to explain this on the basis of differences in the electrolytic dissociation in the watery and the water-immiscible (oily) phase. Such an explanation cannot be applied to the experiments with protein solutions and yet these latter solutions also show the depressing effect of the addition of salt on the P.D. in a most striking way. In this latter case the depressing effect of the salt on the P.D. is due to the Donnan equilibrium and there is no reason why the theory of membrane equilibria should not apply to the P.D. between oily and watery phases since this theory only demands that one ion of the oily phase should be prevented from migrating into the watery phase. Any lipoid ion would fulfill this postulate of the theory. The peculiarities of electrolytic dissociation found by Beutner in non-aqueous solutions must, however, influence the Donnan equilibrium in a secondary way since this equilibrium depends on ionization.

SUMMARY AND CONCLUSIONS.

1. When a solution of a salt of gelatin or crystalline egg albumin is separated by a collodion membrane from a watery solution (free from protein) a potential difference is set up across the membrane in which the protein is positively charged in the case of protein-acid salts and in which the protein is negatively charged in the case of metal proteinates. The turning point is the isoelectric point of the protein.

2. Measurements of the pH of the (inside) protein solution and of the outside watery solution show that when equilibrium is established the value pH inside minus pH outside is positive in the case of protein-acid salts and negative in the case of metal proteinates. This is to be expected when the P.D. is caused by the establishment of a Donnan equilibrium, since in that case the pH should be lower outside than inside in the case of a protein-acid salt and should be higher outside than inside in the case of a metal proteinate.

3. At the isoelectric point where the electrical charge is zero the value of pH inside minus pH outside becomes also zero.

4. It is shown that a P. D. is established between suspended particles of powdered gelatin and the surrounding watery solution and that the sign of charge of the particles is positive when they contain gelatin-acid salts, while it is negative when the powdered particles contain metal gelatinate. At the isoelectric point the charge is zero.

5. Measurements of the pH inside the powdered particles and of the pH in the outside watery solution show that when equilibrium is established the value pH inside minus pH outside is positive when the powdered particles contain a gelatin-acid salt, while the value pH inside minus pH outside is negative when the powdered particles contain Na gelatinate. At the isoelectric point the value pH inside minus pH outside is zero.

6. The addition of neutral salts depresses the electrical charge of the powdered particles of protein-acid salts. It is shown that the addition of salts to a suspension of powdered particles of gelatin chloride also diminishes the value of pH inside minus pH outside.

7. The agreement between the values 58 (pH inside minus pH outside) and the P. D. observed by the Compton electrometer is not only qualitative but quantitative. This proves that the difference in the concentration of acid (or alkali, as the case may be) in the two phases is the only cause for the observed P.D.

8. The Donnan theory demands that the P.D. of a gelatin chloride solution should be $1\frac{1}{2}$ times as great as the P.D. of a gelatin sulfate solution of the same pH and the same concentration (1 per cent) of originally isoelectric gelatin. This is found to be correct and it is also shown that the values of pH inside minus pH outside for the two solutions possess the ratio of 3:2.

9. All these measurements prove that the electrical charges of suspended particles of protein are determined exclusively by the Donnan equilibrium.