MEMBRANE POTENTIALS AND CATAPHORETIC POTENTIALS OF PROTEINS.

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(Received for publication, January 15, 1923.)

I.

Hardy observed in 1900 that particles of denatured (boiled) white of egg migrated in an electric field to the cathode in an acid solution, to the anode in alkaline solution, and did not migrate at all at a point between the two; namely, at the so called isoelectric point.[1] It was shown in previous publications[2] that the same influence of the pH on the charge of the protein exists in the case of membrane potentials, inasmuch as a protein solution enclosed in a collodion bag and submerged in water free from protein is positively charged on the acid side of the isoelectric point, negatively on the alkaline side, and not charged at all at the isoelectric point. The question arises, What is the cause of the similarity of the influence of the pH on the two types of potentials? The membrane potentials are due to a difference in the concentration of a diffusible ion, *e.g.* the H ion inside the protein solution and outside; and the membrane potentials can be calculated with a fair degree of accuracy from the ratio of the hydrogen ion concentrations inside and outside with the aid of Donnan's formula. The cataphoretic potentials, however, are determined by the potential difference between the two strata of an electrical double layer situated at the interface between particles and water, but entirely in the water. One stratum or film of this double layer, namely the one adjoining the solid particle, adheres to the solid particle and moves with it, and the charge of this film is the cause of the cataphoretic motion of the particles.

[1] Hardy, W. B., *Proc. Roy. Soc. London*, 1899–1900, lxvi, 110.

[2] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 667. Proteins and the theory of colloidal behavior, New York and London, 1922.

According to the theory of these double layers originally developed by Helmholtz and modified in an essential point by Perrin, the potential difference of this electrical double layer can be calculated from measurements of the velocity of migration of such particles in an electric field with the aid of the following formula

$$v = \frac{\epsilon \cdot E \cdot K}{4 \pi \eta}$$

where v is the velocity of migration of the particle in centimeter per second, ϵ the potential difference between the two strata of the double layer around the solid particle, E the potential gradient in E. S. U. per centimeter of the galvanic field, K the dielectric constant of the water or the solution, and η the viscosity of the water. It may be said that this formula must be nearly correct for the reason that flocculation of suspensions of a given substance always occurs at the same calculated cataphoretic P. D., which would be impossible if the Helmholtz-Perrin formula were not, at least approximately, correct. We shall return to this point later.

We are not so well informed as to the origin of the P.D. of this double layer, but we may assume with a good degree of probability that it is due to the fact that the two oppositely charged ions of an electrolyte are not contained in the same concentration in the two strata of the double layer, and that forces inherent in the water drive an excess of one type of ions—generally the OH or some other negative ion—into the outermost surface of the water, *i.e.* into that film or stratum of the interface which adheres to and moves with the solid particle. Since this film determines the cataphoretic sign of charge of the particle, we notice that, very frequently, suspended particles are negatively charged in water, while the bulk of water, having a corresponding excess of positive ions, is positively charged.

It is obvious, therefore, that, as a rule, the cataphoretic potential has an entirely different origin from the membrane potentials, and this makes it more difficult to account for the fact that the sign of charge of membrane potentials and of cataphoretic potentials of protein particles varies in the same sense with the change in the hydrogen ion concentration.

It was, therefore, important to find out how far the agreement between the two potentials actually goes. For this purpose the measurements of the influence of salts on the cataphoretic potentials of solid protein particles, described in a preceding paper,[3] were used.

In these experiments the following data are of importance for us. At the isoelectric point the charge of the particles is zero, if no salt is present. The addition of NaCl, Na_2SO_4, and $CaCl_2$ had very little effect on the cataphoretic P.D. except that $CaCl_2$ made the particles slightly positive and Na_2SO_4 slightly negative. While these effects were very slight, they seemed to exist in the case of all proteins.

The effects of $LaCl_3$ and of $Na_4Fe(CN)_6$ on the P.D. of the isoelectric casein particles were much greater. $Na_4Fe(CN)_6$ made the particles strongly negative, while $LaCl_3$ made them strongly positive.

At pH 4.0, the protein particles, such as gelatin, casein, and albumin are positively charged without salts, the cataphoretic P.D. being in the neighborhood of 15 millivolts. $LaCl_3$, $CaCl_2$, and NaCl depressed the P.D., and the more the higher the concentration of the salt, and Na_2SO_4 depressed the cataphoretic P.D. still more rapidly. All these effects of salts on the cataphoretic P.D. are similar to the effects of these salts on the membrane potentials at the same pH. What is, however, different is the effect of $Na_4Fe(CN)_6$, which reverses the sign of the cataphoretic charge of the particles in as low a concentration as M/65,000. We shall see later that such a reversal of the sign of charge of proteins by $Na_4Fe(CN)_6$ occurs only in the case of the cataphoretic, but not in the case of membrane potentials of proteins at a pH of 4.0.

At pH 3.0, the above mentioned protein particles are positively charged without salts, the P.D. being about 20 millivolts. At this pH the influence of $Na_4Fe(CN)_6$ can no longer be investigated on account of the chemical changes in the salt. All the four salts, NaCl, Na_2SO_4, $CaCl_2$, and $LaCl_3$, depressed the cataphoretic P.D., and Na_2SO_4 more rapidly than the three chlorides. This effect of these salts on the cataphoretic P.D. is similar to their effect on the membrane potentials at pH 3.0.

[3] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 395.

Without salts the protein particles were negatively charged at pH 5.8, the P.D. being about 12 millivolts. $LaCl_3$ reverses the sign of charge in as low a concentration as M/30,000, while NaCl, $CaCl_2$, and Na_2SO_4 cause no such reversal. $Na_4Fe(CN)_6$ causes an enormous increase in the negative charge of the particles, while Na_2SO_4 causes a slight increase. $CaCl_2$ causes no increase in the cataphoretic P.D. at pH 5.8. Neither the reversal of the sign of charge by $LaCl_3$ nor the increase of the charge by $Na_4Fe(CN)_6$ at pH 5.8 was observed in the case of membrane potentials.

These experiments, then, prove the existence of definite differences between membrane potentials and cataphoretic potentials.

II.

To leave no doubt that these differences are real, experiments were made on the effect of $Na_4Fe(CN)_6$ and $LaCl_3$ on the membrane potentials of 3 per cent and 1 per cent solutions of crystalline egg albumin at pH 4.0 and 5.8 respectively. The membrane potentials were measured after 18 hours, in the way described in a previous publication.

Fig. 1 gives a comparison of the effects of different concentrations of $Na_4Fe(CN)_6$ on the cataphoretic potentials of albumin-coated collodion particles (upper curve) and on the membrane potentials of a 3 per cent and 1 per cent solution of crystalline egg albumin at a pH of 4.0. The ordinates are the P.D. in millivolts, while the abscissæ are the concentrations of $Na_4Fe(CN)_6$ used. When the protein is positively charged, the P.D. is below the zero line; and when the protein is negatively charged, the P.D. is above the zero line.

Without salt the protein at pH 4.0 is positively charged in both membrane and cataphoretic potentials, but the membrane potential is higher than the cataphoretic potential. While a concentration of M/200,000 $Na_4Fe(CN)_6$ suffices to reverse the sign of charge of the protein in the case of cataphoretic potentials, no such reversal occurs in the case of the membrane potentials of the 3 per cent albumin solution. In this latter case the salt depresses the P.D. in accordance with Donnan's theory and at a concentration of M/1,024 the P.D. is zero and stays so even if the concentration of the salt is as high as M/64 or above.

A slight reversal seems to occur in the case of the membrane potentials of a 1 per cent solution of albumin at a concentration of M/2,048; but this reversal is in reality due to a change of the pH in the protein solution caused by the $Na_4Fe(CN)_6$ on standing. Measurements of

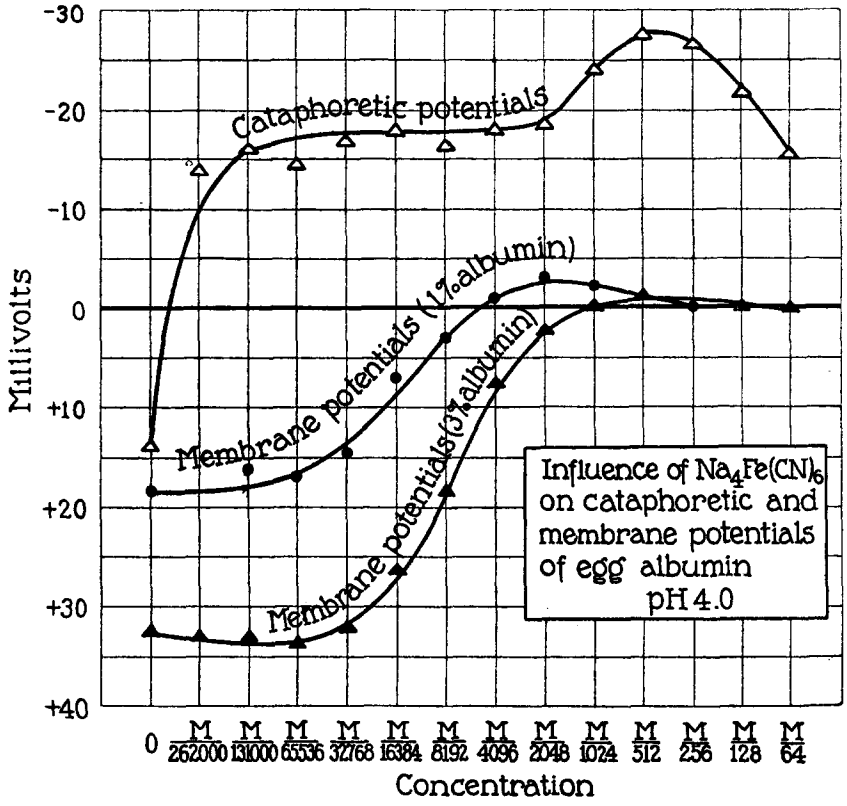

FIG. 1. Comparison of the influence of $Na_4Fe(CN)_6$ on membrane potentials of 1 per cent and 3 per cent solutions of crystalline egg albumin and the cataphoretic potentials of collodion particles coated with crystalline egg albumin at pH 4.0. While low concentrations of the salt reverse the sign of charge of the cataphoretic potentials, no reversal occurs in the case of membrane potentials.

the pH of the protein solution show that it rises in 18 hours beyond that of the isoelectric point and this causes the reversal of the sign of charge at M/4,096 or M/2,048 $Na_4Fe(CN)_6$. When the concentration of the salt becomes higher the depressing effect of the salt brings

the membrane potentials again to zero. This reversal of the membrane potentials, due to a change in the pH, did not occur in the 3

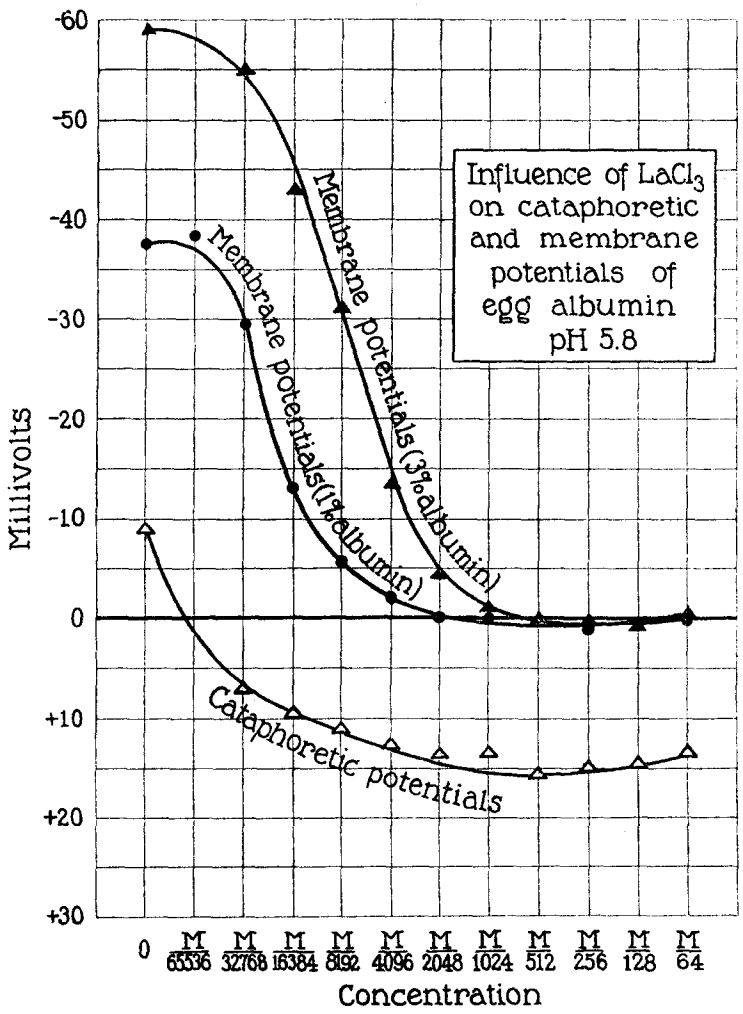

FIG. 2. Comparison of influence of $LaCl_3$ on membrane potentials and cataphoretic potentials of albumin at pH 5.8.

per cent protein solution, possibly because the protein acts as a buffer against the pH changes and this buffer action is the greater the higher the concentration of the protein. In the measurements of the cataph-

oretic potentials no such pH changes occurred, since the measurements of the cataphoretic potentials were made after 20 minutes instead of after 18 hours. In 20 minutes the pH undergoes no material change.

Fig. 2 compares the influence of $LaCl_3$ on membrane and cataphoretic potentials at pH 5.8. Without salt the protein particles as well as the protein solution are negatively charged at pH 5.8. While a low concentration of $LaCl_3$, about M/32,000 or even less, reverses the sign of charge of the cataphoretic potentials, the salt causes no such reversal in the case of the membrane potentials even in high concen-

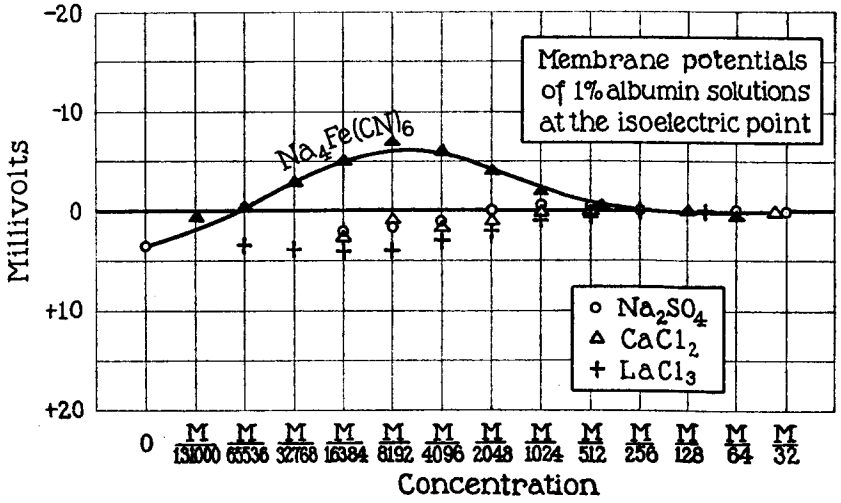

FIG. 3. Influence of various salts on membrane potentials of albumin solutions at the isoelectric point.

trations. $LaCl_3$ can only bring the membrane potentials of a protein solution at pH 5.8 to zero but cannot reverse their sign of charge.

Near the isoelectric point, low concentrations of $Na_4Fe(CN)_6$ produce a considerable negative cataphoretic charge, and low concentrations of $LaCl_3$, produce a considerable positive cataphoretic charge of the albumin particle. Fig. 3 shows that they produce no such charge in the membrane potentials of albumin solution at the isoelectric point except that caused by a change in the hydrogen ion concentration of the protein solution by the salt. $Na_4Fe(CN)_6$ brings the solution of crystalline egg albumin in 18 hours to a pH slightly above 4.7.

Previous experiments on the influence of $Na_4Fe(CN)_6$ or $LaCl_3$ on the membrane potentials of gelatin solutions at the isoelectric point had given results similar to the new experiments on egg albumin, and the writer also noticed a tendency of the solution to change its pH.[4] The writer was not certain at that time that the slight effect of $Na_4Fe(CN)_6$ and $LaCl_3$ on the membrane potentials of isoelectric gelatin was due exclusively to a change of the pH occurring gradually on standing. The new experiments make it more probable that this must have been the case.

Experiments on the membrane potentials between solid gels of gelatin and aqueous solutions free from gelatin showed that the influence of $LaCl_3$ and $Na_4Fe(CN)_6$ is the same as in membrane potentials of solutions of albumin. On the other hand, the action of salts of the type of NaCl, $CaCl_2$, and Na_2SO_4 is alike in the case of membrane potentials and cataphoretic potentials since these salts depress the P.D. of both potentials without causing a definite reversal of either. There exists, however, one effect of these latter salts on the cataphoretic potentials which does not occur in the case of membrane potentials; namely, sulfates make the cataphoretic charge of the protein slightly more negative and $CaCl_2$ slightly more positive than NaCl. The effect is slight and noticeable only at or near the isoelectric point. This slight effect was not observed in the case of membrane potentials.

We come, therefore, to the conclusion that a reversal of the sign of charge of protein by low concentrations of salts with trivalent or tetravalent ions occurs in the case of cataphoretic potentials, but not, or practically not, in the case of membrane potentials. The fact that such a reversal is brought about in the cataphoretic potentials by low concentrations of $LaCl_3$ and $Na_4Fe(CN)_6$ was corroborated by experiments on two types of phenomena which depend on cataphoretic potentials; namely, on the stability of protein particles and on electrical osmosis through protein films.

If we assume the validity of the Helmholtz-Perrin theory of cataphoretic migration, the sign of cataphoretic migration is determined by that film of water which adheres to and moves with the protein particle. This film is usually negatively charged, probably because it has an excess of negative ions which are forced into the film by

[4] Loeb, J., *J. Gen. Physiol.*, 1921–22, iv, 741.

forces inherent in the water—presumably surface tension forces. If the two ions of an electrolyte lower the surface tension of water to a different extent, that ion must be driven in excess into the outermost stratum of the double layer (*i.e.* into the stratum which adheres to and moves with the particle) which has the greater depressing effect on the surface energy. We may conceive that the molecules of water are oriented by such forces at the surface of the water, the oxygen atom forming, as a rule, the outermost, the hydrogen the deeper stratum of the surface.

While thus negative ions are generally forced in excess into the outermost stratum at the surface of the water, the positive ions are in excess in the stratum beneath or in the bulk of the solution. It seems, however, that the force with which cations are driven away from the surface deeper into the water decreases with increasing valency of the cation, so that in the case of salts like $LaCl_3$ the trivalent La ion is driven with greater force into the outermost stratum of the water than the Cl ions, as a consequence of which this stratum becomes positive. That the force determining this ionic stratification may be surface tension seems to be also supported by the fact (observed by Freundlich and Gyemant[5]) that organic cations like basic dyes have also the tendency to reverse the generally negative cataphoretic charge of "oily" particles in water, such basic dyes having a tendency to accumulate in the surface of the water.

These facts and suggestions probably explain the fact that particles of gelatin chloride of pH 4.0, which are positively charged, assume a negative cataphoretic charge in a weak solution of $Na_4Fe(CN)_6$; while particles of Na gelatinate of pH 5.8, which are negatively charged, assume a strong positive charge in a solution of $LaCl_3$. We may also understand on this basis why Na_2SO_4 has a tendency to make the cataphoretic charge of the protein particles near the isoelectric point slightly more negative and why $CaCl_2$ makes the particles a little more positive than does NaCl.

All these facts agree with the idea that the cataphoretic migration is, indeed, determined by the P.D. of an electrical double layer situated entirely in the water and determined at least partly by forces inherent in the water.

[5] Freundlich, H., and Gyemant, A., *Z. physik. Chem.*, 1922, c, 182.

III.

But this leaves the equally striking fact unexplained that changes in the hydrogen ion concentration affect the cataphoretic P.D. of protein particles similarly as they affect the membrane potentials of protein solutions. The influence of acids and alkalies on the cataphoretic P.D. of gelatin-coated particles of collodion had been shown in a preceding article.[3] At the isoelectric point the cataphoretic P.D. of gelatin-coated collodion particles was zero, but both acid and alkali increased the P.D., the particles being negatively charged in alkali and positively charged in acid, just as in the case of the membrane potentials. When the anion of the acid or the cation of the alkali was monovalent, the cataphoretic P.D. was greater than when the respective ions were bivalent. This agrees with the valency effect in the case of membrane potentials.

When the concentration of acid or alkali exceeded a certain limit, the further increase in concentration diminished the cataphoretic P.D. again, and this was also the case with the membrane potentials. It would have been of importance to find out whether the agreement was quantitative, but this was impossible, since we do not know the concentration of protein in the solid particles. The membrane potential of a 1 per cent solution of protein was for the same pH always greater than the cataphoretic P.D. of solid particles of the same protein.

The question arises, What causes this qualitative agreement between the two potentials in regard to the pH effect? We have a mathematical theory of the effect of acid and alkali on the membrane potentials but, unfortunately, not on the cataphoretic potentials, so that we cannot offer more than a provisional suggestion.

On the basis of Helmholtz's theory of double layers the motion of protein particles to the cathode must be due to an excess of H (or other positive) ions in that film of water which adheres to and moves with the protein particles; and the motion of the solid protein particles to the anode must be due to an excess of OH or other anions in the same film of water. This film of water must, therefore, have an excess of cations when the pH of the protein particle is on the acid side of the isoelectric point, and an excess of anions when the pH is on the

alkaline side of the isoelectric point of the protein; and this excess of cations and anions respectively in the layer of water adhering to the protein particle (and not the charges of the protein particle itself) must, on the basis of Helmholtz's theory of double electrical layers, be responsible for the sign of the cataphoretic migration of the particles.

The difficulty which confronts us is the uncertainty concerning the exact location of the P.D. determining the membrane potentials of protein particles. J. A. Wilson[6] assumes that the membrane potential between a solid protein particle and the solution is located at the boundary between a stratum of liquid adhering to the surface of the particle and the bulk of the solution. This assumption is based on the idea that the concentration of diffusible ions, *e.g.* H and Cl, at the surface of a particle of gelatin chloride will be the same as in the interior of the gel. On that assumption the P.D. of the membrane potential must be located between this surface stratum of liquid and the bulk of solution.

Now if this surface stratum of liquid is identical with the stratum of liquid which adheres to the particle in its cataphoretic motion, it is obvious why a solid particle of protein chloride must move to the cathode and a solid particle of Na proteinate must move to the anode. The ultimate cataphoretic P.D. would then be the resultant between the membrane potentials and the P.D. determined by the (surface tension?) forces inherent in the water.

On this assumption it is difficult to understand why there should ever be a difference between the membrane potentials and the cataphoretic potentials, and yet our experiments have shown such a difference to exist. We can only state at present that the cataphoretic P.D. of the protein particles is influenced both by the (surface tension) forces inherent in the water and the membrane potentials.

IV.

Throughout this paper we have assumed the correctness of the Helmholtz-Perrin formula for the calculation of the cataphoretic P.D. which had been used in our experiments. The question might be raised whether the discrepancies between the influence of salts on

[6] Wilson, J. A., *J. Am. Chem. Soc.*, 1916, xxxviii, 1982.

the cataphoretic potentials and the membrane potentials might not be due to the fact that the Helmholtz-Perrin formula for the calculation of the cataphoretic P.D. is not correct, and that if this formula is corrected the discrepancies might disappear. It should be pointed out that there exists a group of facts which offers a strong support for the assumption that the Helmholtz-Perrin formula for the calculation of the cataphoretic P.D. cannot be far from correct; namely, the existence of a critical P.D. for the flocculation of suspensions. Regardless of the nature of the salt used, flocculation of suspensions of collodion particles occurs always when the P.D. falls below a certain critical value.[7] Powis[8] had observed the same fact in his experiments on emulsions, and Northrop and De Kruif[9] in suspensions of bacteria. Moreover, the numerous observations of Burton, at least, do not contradict the fact. Furthermore, in the next paper we shall see that a critical potential for flocculation exists also in the case of suspended particles of proteins. This seems, therefore, to justify our assumption that the figures for the cataphoretic potentials given in this paper are essentially correct. The critical P. D. is apparently different for different substances.

V.

Freundlich and Rona[10] have noticed a difference between Haber's phase boundary potentials at the boundary of glass and water and the cataphoretic potentials of water against glass. The phase boundary potential depends in this case only on the hydrogen ion concentration of the solution, while other ions, except H and OH, have no direct influence on this potential, as had already been shown by Haber and Klemensiewicz.[11] The "electrokinetic potential" at the boundary of glass and water measured cataphoretically by Freundlich and Rona showed, however, a striking influence of other ions besides hydrogen and hydroxyl ions, and showed especially the

[7] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 109.

[8] Powis, F., *Z. physik. Chem.*, 1914, lxxxix, 186.

[9] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, iv, 639, 655.

[10] Freundlich, H., and Rona, P., *Sitzungsber. Preuss. Akad. Wissensch.*, 1920, xx, 397.

[11] Haber, F., and Klemensiewicz, Z., *Z. physik. Chem.*, 1909, lxvii, 385.

valency effect so characteristic of all cataphoretic potentials. Freundlich and Rona assume that the difference between the two kinds of potential is as follows. The phase boundary potential is the potential difference between the interior of the solid phase and the interior of the liquid and is therefore influenced only by such ions of the liquid which can go into the solid phase, and which seem to be in the case of glass only H and OH ions. The electrokinetic potential, however, is in accordance with Helmholtz's theory, the potential difference between a film of water adhering to the solid particle and the interior of the water. This P.D. of the double electrical layer is influenced by all the ions of the liquid and the authors assume that adsorption plays the chief rôle in the electrokinetic potentials.

Freundlich and Gyemant[5] compared the thermodynamic and electrokinetic potentials between water-immiscible liquids (phenol, guaiacol, benzonitrile, and aniline) and aqueous solutions, and confirmed the conclusions arrived at by Freundlich and Rona. The thermodynamic potentials between these "oily" liquids and water had been investigated by Beutner[12] in a series of excellent experiments, and his results and conclusions in regard to the origin of these potentials were confirmed by Freundlich and Gyemant. Beutner found that the non-aqueous phase was the more positively or negatively charged the more soluble the cation or anion of a salt was respectively in the non-aqueous phase. Freundlich and Gyemant found that in the cataphoretic potentials between these four non-aqueous liquids and water the non-aqueous droplets were always negatively charged, even the basic aniline, and the sign of the cataphoretic charge of these water-immiscible droplets could be reversed by polyvalent inorganic cations and by organic cations (*e.g.* basic dyes). This influence of the cations on the cataphoretic potentials they ascribe to adsorption.

These experiments bring out the difference between thermodynamic potentials and cataphoretic potentials in the cases which Freundlich and his collaborators investigated. The ideas on adsorption can, however, not be used to explain why the membrane potentials of proteins are modified in the same way by H and OH ions as are the

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

cataphoretic potentials, since adsorption, according to Freundlich and Rona, influences only the cataphoretic potentials.

We may state, as a result of our experiments, that the cataphoretic migration and the cataphoretic P.D. of protein particles or of suspended particles coated with a protein are the result of two groups of forces; namely, first, forces inherent in the protein particles (these forces being linked with the membrane equilibrium between protein particles and the outside aqueous solution); and second, forces inherent entirely in the aqueous solution surrounding the protein particles.

The forces inherent in the protein particles and linked with the membrane equilibrium prevail to such an extent over the forces inherent in the water, that the sense of the cataphoretic migration of protein particles is determined by the forces resulting from the membrane equilibrium.

VI.

SUMMARY AND CONCLUSIONS.

1. It has been shown in preceding publications that the membrane potentials of protein solutions or gels are determined by differences in the concentration of a common ion (*e.g.* hydrogen ion) inside a protein solution or protein gel and an outside aqueous solution free from protein, and that the membrane potentials can be calculated with a good degree of accuracy from Donnan's equation for membrane equilibria.

2. On the basis of the theory of electrical double layers developed by Helmholtz, we are forced to assume that the cataphoretic potentials of protein particles are determined by a difference in the concentration of the two oppositely charged ions of the same electrolyte in the two strata of an electrical double layer surrounding the protein particle but situated entirely in the aqueous solution.

3. The membrane potentials of proteins agree with the cataphoretic potentials in that the sign of charge of the protein is negative on the alkaline side and positive on the acid side of the isoelectric point of the protein in both membrane potentials and cataphoretic potentials. The two types of potential of proteins disagree, especially in regard to the action of salts with trivalent and tetravalent ions on the sign of charge of the protein. While low concentrations of these salts

bring about a reversal of the sign of the cataphoretic potentials of protein particles (at least in the neighborhood of the isoelectric point), the same salts can bring the membrane potentials of proteins only to zero, but can bring about no or practically no reversal of the sign of charge of the protein. Where salts seem to bring about a reversal in the membrane potential of protein solutions, the reversal is probably in reality always due to a change in the pH.

4. We may state, as a result of our experiments, that the cataphoretic migration and the cataphoretic P.D. of protein particles or of suspended particles coated with a protein are the result of two groups of forces; namely, first, forces inherent in the protein particles (these forces being linked with the membrane equilibrium between protein particles and the outside aqueous solution); and second, forces inherent entirely in the aqueous solution surrounding the protein particles.

The forces inherent in the protein particles and linked with the membrane equilibrium prevail to such an extent over the forces inherent in the water, that the sense of the cataphoretic migration of protein particles is determined by the forces resulting from the membrane equilibrium.