ELECTROPHORESIS OF BACTERIA AS INFLUENCED BY HYDROGEN ION CONCENTRATION AND THE PRESENCE OF SODIUM AND CALCIUM SALTS.*

By C.-E. A. WINSLOW, I. S. FALK, and M. F. CAULFIELD.

(*From the Department of Public Health, Yale School of Medicine, New Haven.*)

(Received for publication, September 21, 1923.)

I.

INTRODUCTION.

Since the fundamental work of Hardy (1899, 1900) (18, 19) a number of investigators have reported experimental results directly or indirectly bearing upon the electrophoretic charge of the bacterial cell. These studies indicate clearly that in solutions at or near the neutral point bacteria commonly migrate to the anode in an electrical field. (2, 26, 39, 6, 7, 32, 3, 34, 1, *a*, 20, 33, 16, 35, 36, 37, 31, 29.) Only Thornton (40) and Abbott (1), report conflicting results. Yeasts also carry a negative charge (20, 37) while one author (7) describes a mold, viable in high concentrations of acid, carrying a positive charge. Results with spirochetes, trypanosomes and other protozoa are diversified (37, 31, 41, 21). Heat-killed bacterial cells exhibit in general the same electrophoretic phenomena as living cells (7, 32, 1, *a*, 33, 16, 31). An increase in hydrogen ion concentration tends to diminish the electrophoretic charge and an isoelectric point is found near pH = 3.0 (39, 32, 1, *a*, 33, 16, 31, 29). A positive charge may under certain circumstances, be developed beyond this point (39, 1, *a*, 29). The presence of other electrolytes also exerts a marked influence upon electrophoresis, depending upon concentration and upon the free valency and nature of the ions concerned (39, 32, 20, 16, 35, 36, 37, 29)—particularly in the case of ions bearing a charge opposite to that of the bacterial cells.

* Studies here reported were aided by a grant from the Loomis Research Fund of the Yale School of Medicine.

II.

Technique.

The experiments here reported[1] were undertaken to obtain comparable data in regard to the influence of various hydrogen ion concentrations in water and in sodium and calcium chloride solutions (over a wider range than had hitherto been studied) upon the electrophoresis of a large bacillus (*Bacillus cereus*). The bacteria were grown in Kolle flasks at 37°C. (except when otherwise stated in the specific protocol of an experiment). Growth was removed with a small volume of water or other test fluid by a gently rotary motion of the culture flask. The suspension was then shaken vigorously in a mechanical shaker or by hand to break up clumps of bacteria and to give a uniform distribution of the organisms through the menstruum. The organisms were then washed by repeated precipitations in a centrifuge at some 3,000 revolutions per minute and taken up each time in the fluid in which the electrophoresis was to be measured.

The test fluids were prepared in carefully distilled water using pure salts and pure acid and alkali. To prepare a suspension of bacteria in water or in a salt solution at a particular hydrogen ion concentration our practice was to suspend the bacteria in the water or salt solution and then to adjust to the required pH by adding the necessary amount of acid or alkali as determined by the titration of an aliquot portion of the suspension. Such a titration was accomplished by adding to a 5 or 10 cc. fraction the appropriate indicator and adding acid or alkali to the required pH as measured colorimetrically against solution color standards or—more commonly—against standard color plates (Clark, 8). When adjusting solutions to the extremes of the pH range we obtained more satisfactory results by conducting the titrations electrometrically with the hydrogen electrode, using the titration chambers briefly described by Falk and Shaughnessy (13). Adjustments of pH which were made colorimetrically were occasionally checked electrometrically and found to be satisfactorily precise for the purposes of the experiment. No buffer substances were added to our test fluids because of the desire to avoid the single or

[1] Certain of these experiments have already been presented in a preliminary form (45).

mutual effects of salts other than those specifically studied. That the phenomena studied may be seriously modified by the presence of buffer salts is well illustrated by the experiments of Beniasch (3). He failed to obtain acid agglutination of *Bacterium coli* in the presence of buffer salts. Eggerth and Bellows (9) avoided this salt interference by conducting their experiments without the buffer salts. This procedure necessarily introduces an undesirable instability of the pH (except where the salt content of the fluid was sufficiently high to serve as an effective buffer or where the pH was so high or so low as to be unaffected by the buffering powers of the bacteria) which necessitates a check reading of the pH *after* the electrophoretic manipulation. (The tendency of bacteria to adjust the pH of their menstrua has been discussed at length by Winslow and Falk (43, 44).) Our measurements were conducted at room temperature.

The measurement of the velocity of migration of *Bacillus cereus* in any particular fluid under the influence of an impressed voltage was made by the direct microscopic method described by Northrop (27) with certain modifications courteously demonstrated to us by Dr. Jacques Loeb. These modifications consist in the addition of a pair of funnels connected through stop-cocks to the vessels containing the zinc-zinc sulfate electrodes. The design of this cell permits of the application of a direct current potential through non-polarizable electrodes and of the observation under a magnification of 525 diameters of the velocity with which any individual bacterial cell moves towards the positive or negative pole. The heating effect of the lamp illuminating the field of the microscope was controlled by the interposition of a flat walled flask containing cold water. The basic theoretical and technical aspects of such an apparatus are thoroughly treated by Ellis (10, 11), Powis (30), and Northrop (27) and need not be repeated here. Inasmuch as there is a potential difference between the surfaces of the liquid and the glass,[2] upon application of an external potential there is an electrical-endosmose movement of the liquid. The cell as a whole, however, is a closed system. Therefore, the total movement of the water must be zero. That is, the water which moves towards the cathode at the glass surfaces returns towards the anode in the midregions of the fluid in

[2] Water is electropositive to glass.

the cell. Obviously, then, the electrophoretic movement of the particles will be impeded at certain levels in the cell and accelerated at others by the independent movement of the water. In addition, of course, there is the electrical-endosmose due to the difference in potential between the water and the particles. The true mobility of the particles is the average mobility at all levels in the cell. We have followed Northrop's modification of the technique of Ellis in making measurements at the levels corresponding to the middle of each sixth of the distance between the under surface of the cover-glass and the upper surface of the base of the cell and taking the average of these values as the true mean velocity of the bacteria.[3] Calculation of the potential fall per cm. in the cell (X in the Helmholtz-Lamb equation) was made by the usual method (*vide* Northrop, 27).

The results of our experiments are expressed throughout in terms of actually observed velocity of migration (in micra per second). Observations were made by the use of an ocular micrometer and stopwatch, each figure representing the mean of at least fifteen observations, one-third at each level. Where movement in opposite directions was apparent at different levels an average velocity was obtained for each level and the three level values were then algebraically averaged. It would be eminently desirable to convert the velocity figures into terms of electrical potential difference at the interface between the bacterium and the menstruum. If we adopt the Helmholtz-Lamb equation (Burton, 4, 5) this value can be found from the following formula.

$$V \cdot \frac{l}{d} = \frac{4\pi}{K} \cdot \frac{\eta v}{X}$$

in which

V = P.D., the difference of potential between the solid particle and the liquid.
l = a linear magnitude measuring the "slip."
d = the distance between the plates of an air condenser equivalent to that virtually formed by the opposed surfaces of solid and fluid.
K = the specific inductive capacity (dielectric constant) of the liquid.
η = the coefficient of viscosity of the liquid.
v = the mobility (velocity of migration) of the particle.
X = the strength of the electrical field,
all measurements being made in electrostatic units.

[3] Inasmuch as the upper and lower halves of the cell are symmetrical we dispensed with three readings at the mid-levels of each third of either the upper or lower half of the cell.

Although the form of the Helmholtz-Lamb equation is simple, its use in precise experimental work involves a number of difficulties because of the uncertainties attached to some of the terms involved. Thus, finite values for d and l are not at present known. But because there is some justification for considering that—under certain conditions, at least—the ratio $d:l$ approximates unity, it is sometimes the practice to omit these terms in calculating the P.D. (potential difference) between particles and a liquid. Another procedure is to refer to the calculated value of $(V \frac{l}{d})$ in volts as the potential difference. We are then dealing, however, with the Helmholtz and not the Helmholtz-Lamb equation. If one accepts as valid the theoretical considerations upon which Lamb's calculations were based and yet disregards $l:d$, obviously the P.D. calculated from experimental values is —in a *quantitative* sense—open to considerable question.

A more serious difficulty which is often overlooked, is the uncertainty which attaches to K, the dielectric constant for the solution. This constant should, theoretically, apply to the specific inductive capacity of the medium which separates the positive and negative layers of electricity on the particulate and liquid phase surfaces. When dealing with a pure menstruum free from solute and in which the substance of the suspended particles is imperceptibly soluble we can conceive that the dielectric constant of the liquid, as ordinarily determined, may be applied in the equation although the accuracy of this procedure is not clearly known. When dealing with a liquid in which the particles themselves are appreciably soluble or in which other substances have been dissolved, the case may be significantly different, depending upon whether the dissolved substances do or do not appreciably affect the dielectric constant of the liquid between the Helmholtz double layer. Even when the effect of the solute upon the inductive capacity of the bulk of the liquid is known, it must still remain an open question what the effect of the solute is upon the thin film of liquid which is conceived as lying between the electrical layers. Unfortunately the influences of electrolytes upon the dielectric constant of water are far from being clearly established. The subject was reviewed recently by Lattey (23). He points out that certain earlier investigators came to diametrically opposite conclusions on this

problem. Thus, Drude, Coolidge, and others were unable to distinguish between the dielectric constants of pure water and of solutions of copper sulfate. Smale, on the other hand, asserted that an 0.05 N solution of this salt showed a dielectric constant 15.5 per cent greater than that of water. Smale's view is supported (qualitatively, at any rate) by the data of Walden (42). For aqueous KCl solutions (1,000, 200, and 100 liters per mol concentration) the dielectric constants *increased* in his experiments from 81.7 for pure water to 82.8, 84.5, and 90.9, respectively. Also, Walden calculated the dielectric constants for the dissolved salts and obtained values far higher than those usually obtained for the solid, undissolved salt. Walden's conclusion was that with dissolution of electrolytes in solvents in which ionization occurs, the dielectric constant of the solvent is appreciably increased. Other investigators are similarly divided in their conclusions. Cohn, Yule, and others are in accord with Walden in having found *increases* in the dielectric constant of water with solution of electrolytes; Nernst, Palmer, and others, like Drude, found no significant changes with small concentrations of salts; and others have obtained results similar to those of Lattey, the most recent observer, who reports *decreases* in the dielectric constants of salt solutions. Lattey (23) himself presents a review of the methodology of earlier workers and finds ground for questioning the validity of many of their findings. For pure water he takes as a mean value for the dielectric constant the value 81.05. His data for aqueous solutions of potassium chloride (from 0.000755 to 0.00755 N) clearly show *depressions* of the dielectric constant. The depression is greater the higher the salt concentration and reduces the constant to 66.25 (81.5 per cent of the constant for water) in the most concentrated of these dilute KCl solutions. The data for more concentrated KCl solutions were not published because of uncertainty which attached to their accuracy. Similarly for aqueous copper sulfate solutions of 0.00114 to 0.00456 normality the dielectric constants were lower than for pure water. In the most concentrated solution the depression amounted to 10 per cent of the absolute value of the constant for pure water. In concluding, Lattey remarked that: "The electrolytes investigated appear to lower the dielectric constant of water, and are in this respect analogous to the majority of non-

electrolytes." All in all it appears that no unambiguous conclusion concerning the value of K in the Helmholtz-Lamb equation when studying electrophoresis in electrolyte solutions is possible. The changes in K produced by electrolytes which have been reported do not, in general, exceed $\pm$ 10 per cent of the K for water. They must, however, be taken with the precaution that only comparatively dilute solutions have thus far been carefully studied. The experimental difficulties inherent in studies upon concentrated solutions are not easily avoided. Until the values of K for various solutions are made known with some precision, the usefulness of the Helmholtz-Lamb equation in biological work will be markedly limited.

Besides K, η, the coefficient of viscosity of the fluid, is a variable in the Helmholtz-Lamb equation and its value demands some consideration here. In the papers reported by Northrop and his colleagues, K was taken as 80, η as 0.009 and because of the uncertainties attached to their evaluation, neither was treated as a variable although the solutions studied were of various hydrogen ion concentrations, and contained buffering salts and various concentrations of certain salts whose influences on P.D. were being investigated. (Northrop and Cullen, 28.)

According to the data cited in Tables annuelles de constantes (38), η increases markedly with salt concentration. Thus, for aqueous solutions of sodium chloride the coefficient of relative viscosity (η_{25} (rel))[4] increased from 1.118 to 1.862 with increasing concentrations of the salt from 1.25 to 5.0 mols per liter and for calcium chloride the absolute coefficient of viscosity at 15°C. (η_{15}) increased from 0.01528 to 0.11709 with increasing concentrations of the salt from 123.5 to 580 gm. per 1,000 cc. of solution. According to the data cited by Kaye and Laby (22) normal solutions of certain electrolytes show markedly increased viscosity as compared with pure water, others show no appreciable change, and others (*i.e.* NH_4Cl, KI) show diminished viscosity. In our studies we have given especial attention to the chlorides of sodium and calcium and it is therefore significant to note that the extent of the changes which these electrolytes cause is appreciable and varies with the concentration of salt. Obviously

[4] The coefficient of viscosity of the solution at 25°C. relative to the coefficient for water at the same temperature.

these effects of the electrolyte cannot be overlooked in calculating P.D. from v in the Helmholtz-Lamb equation. Burton (4, p. 145) has clearly shown the significance of variations in the viscosity of water in relation to the mobility of colloidal silver particles. The product ηV, he found, is sensibly constant. In conclusion from these considerations it seems to us that it is a highly questionable procedure to convert measured mobilities of particles (v) into P.D. $\left(V\frac{l}{d}\right)$ unless the variables involved are carefully controlled or measured.

On account of these uncertainties we have preferred to express our results in terms of observed velocity. If, however, we write $V\frac{l}{d}$ = P.D. and if we assume that the dielectric constant, K, is invariably 81 and that for pure water the coefficient of viscosity at 20° is equal to 0.010 our formula becomes

$$\text{P. D.} = \frac{4\pi}{81} \cdot \frac{0.010\, v}{X} \text{ (in C. G. S. units).}$$

The dimensions of our apparatus were such that the potential gradient per cm. (X) in the electrophoresis chamber was 0.106 times the impressed voltage (11.7 volts for the voltage of 110 generally used). To convert this value to C.G.S. units it must be divided by 300 and to express the final result in practical units we must multiply the whole fraction by 300.

Thus

$$\text{P. D.} = \frac{4\pi}{81} \cdot \frac{0.010\, v}{\frac{11.7}{300}} \cdot 300 \text{ (in volts).}$$

Whence

$$\text{P. D.} = 12\, v \text{ (in volts).}$$

Here v is in cm. per second. If v is expressed in micra per second,

$$\text{P. D.} = 1.2\, v \text{ (in millivolts).}$$

Our results in micra per second may be multiplied by this factor to give what may be considered to be a probable figure for absolute potential difference; and it may be noted that the figures thus derived are in reasonable accord with those given by Girard and Audubert

(16), Shearer (35, 36), and Northrop and De Kruif (29). They are somewhat lower, however, than the potential differences obtained by Northrop and De Kruif who report that a P.D. of 15 millivolts represents the critical point below which various bacteria agglutinate while it will be noted that our results indicate a charge in distilled water of neutral reaction equal to about 11 millivolts (averaging values in Table I for pH 6.0 to 7.9 and multiplying by 1.2). Whether this means that *Bacillus cereus* has a lower charge than the organisms studied by Northrop and his colleagues, or whether the difference is due to the complex reactions which occur in the zone surrounding a bacterial cell in an unbuffered solution, it is impossible to say.[5]

It should be noted that the P.D. value computed as above is the *electrokinetic* P.D. whose existence is evidenced in electrophoresis, or electro-osmose, or Poiseuille streaming experiments and which is most readily understood in terms of the Helmholtz electrical double layer concept. This must not be confused with the Nernst electromotive or so called *thermodynamic* P.D. between the inside of one phase and another in contact with it (*i.e.* water and glass) (17, 14, 15).

III.

Electrophoresis of Living Vegetative Cells at Various Hydrogen Ion Concentrations.

The general type of data obtained in the present study may first be indicated by citing a few typical experiments. Experiment 12, for example, gave the figures indicated below for a hydrogen ion range pH 1.0 to 10.0, inclusive.

Experiment 12.

pH.....	1.0	1.5	2.0	2.6	3.3	3.7	5.5	6.8	7.2	7.3	7.7	9.6	10.0
Velocities in micra per sec.....	0	+2.8	+2.5	+1.9	−1.8	−8.9	−9.7	−7.2	−10.6	−10.3	−10.3	−8.7	−10.0

+ signifies rate of movement toward cathode, − toward anode.

[5] The paper by Eggerth (Eggerth, A. H., *J. Gen. Physiol.*, 1923, vi, 63) published since this manuscript was prepared, suggests another possible explanation for this difference.

The suspension was at a pH of 6.8 when first tested. With decreasing hydrogen ion concentration (up to pH 10) no marked change in velocity of migration was apparent, the bacteria in each case moving toward the anode at a rate between 7 and 11 micra per second. An increase in acidity below pH 3.7 was on the other hand attended by a marked decrease in electrophoresis, with a reversal of movement (toward the cathode) at pH values between 1.5 and 2.6. An isoelectric point at about pH 3.0 is indicated and below pH 1.0 the velocity again returns to approximately zero.

Experiment 19 illustrates the phenomena observed within the alkaline range.

Experiment 19.

pH	8.8	9.8	10.4	10.9	11.5	11.6	12.2
Velocity in micra per sec.	−12.2	−20.0	−19.2	−23.8	−13.5	−6.3	−17.2

Here we see that at alkaline ranges between pH 9.8 and 10.9 the velocity of migration is markedly increased. Above pH 11 it falls again (in some experiments approximating zero) while in even more highly alkaline solutions it may perhaps show a second rise, although our data here are too few to warrant definite conclusions.

One more experiment may be cited in detail, Experiment 10, in which we modified our usual technique by washing off our original suspension in water at pH 2.9 and rewashing three times in water of the same pH in order to begin with a suspension of bacteria approximately isoelectric with their menstruum.

Experiment 10.

pH	1.0	1.5	2.2	2.9	3.4	3.7	4.6	5.1	6.2	8.1	10.0
Velocity in micra per sec.	+1.2	+2.7	+3.8	+2.7	+3.1	−0.7	−13.1	−9.4	−11.0	−13.6	−13.1

The original suspension (pH 2.9) was evidently not exactly isoelectric since the bacteria moved with a considerable velocity toward the cathode. At more acid reactions this velocity first increases and then diminishes. At less acid reactions the migration toward the

TABLE I.

Observed Velocities (in Micra per Second) of Living Vegetative Cells at Different Hydrogen Ion Concentrations (All Experiments).

pH	1.0 or less	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12 and over
No. of observations	4	4	8	8	3	7	13	5	8	5	11	8	5
Minimum velocity*	+1.2	+2.8	+3.8	+3.1	−6.6	−7.2	−3.6	−7.8	−7.7	−8.7	−7.0	0.0	0.0
Maximum velocity	−4.5	+0.1	−10.6	−8.9	−13.1	−15.4	−13.1	−10.6	−17.2	−20.0	−23.8	−13.5	−18.2
Average velocity	−0.8	+1.7	+0.3	−2.8	−8.8	−10.8	−8.7	−9.7	−12.0	−13.8	−15.6	−6.3	−10.2

* For convenience all velocities are here expressed with the anode as a point of reference. Therefore, at pH values below 4.0 the "minimum velocity" included in the table is the *maximum* velocity toward the cathode.

cathode is reversed and above pH 4.5 we observe a normal movement toward the anode.

The general relations observed may best be indicated by the averages in Table I.

As in the study of most biological phenomena differences were manifest in the results of different experiments, due to unavoidable pH variations in unbuffered solution, to slight differences in room temperature and in the biological characteristics of the oganisms, or to other unknown factors. On the whole, however, the lack of extreme variability was rather encouraging except in the case of the observations at the extreme alkaline end of the range. It seems on the whole clear that the bacteria studied moved toward the anode with a high and fairly uniform velocity at hydrogen ion concentrations on the alkaline side of pH 4.0. As we pass into the alkaline zone between pH 8.0 and 11.0 there is a definite increase in velocity and beyond pH 11.0 there is a second fall, reaching an isopotential point in individual instances. At the extreme alkaline end of the range (beyond pH 12.0) results are highly variable but we have not yet sufficient data to draw definite conclusions as to the course of phenomena in this zone.

At pH values below 4.0 there is a very sharp drop in velocity with an isoelectric point at about pH 3.0. At more acid reactions the direction of migration is reversed, being definitely toward the cathode between pH 1.0 and 2.0, with another trend toward diminished and variable migration at pH values below 1.0, tending again to approximate a condition in which the bacteria are isopotential[6] with their menstruum.

IV.

Electrophoresis of Killed Vegetative Cells at Various Hydrogen Ion Concentrations.

Two sets of experiments were made with suspensions prepared in the same manner described above but heated in an autoclave for 20 minutes at 17 pounds pressure to kill the bacteria. The results are indicated in Table II.

[6] The "isopotential" zones at the extreme acid and alkaline ranges probably are not true isoelectric points in the same sense as the pH 3.0 zero potential point of inflection in the electrophoresis curve. We therefore prefer to use the word "isopotential," suggested by Dr. Stuart Mudd.

With only two experiments random errors are likely to be considerable; but it is evident that the heat treatment has not materially affected the electrophoretic charge of the bacterial cells. The values are essentially the same as those recorded in Table I. The normal rate of migration toward the anode manifest near the neutral point rises as the solution is made more alkaline (pH 9.0 to 9.9) and falls as it is made more acid, almost disappearing at pH values near 3.0.

It is of considerable interest to note that the killing of bacterial cells by heat does not substantially alter their electrical charge since it suggests (if the phenomena of potential difference are to be explained on the theory of the Donnan equilibrium, to be discussed later on) that the permeability of the cell surface to those ions concerned in potential difference has not been materially affected by this treatment. The observations are in accord with the results of earlier workers

TABLE II.

Observed Velocities (in Micra per Second) of Killed Vegetative Cells at Different Hydrogen Ion Concentrations.

pH.....	1.0 or less	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	7.0–7.9	8.0–8.9	9.0–9.9
Velocity, Exp. 7*a*.....		−0.2		−4.0		−4.4		−6.0
Velocity, Exp. 9.....	−2.8		−1.7		−8.3	−14.2	−9.4	−17.0

(7, 32, 1, *a*, 33, 16, 31, 29) and with the familiar fact that the killing of bacterial cells by heat (as in the pasteurization of milk) does not alter their staining properties.

It should be noted that in our experiments on so called living vegetative cells described above many of the bacteria must actually have been killed by the extreme acidities and alkalinities used. It is probably of real significance that the hydrogen ion zone of migration toward the anode is the zone within which bacterial life is ordinarily possible and that the zones of isopotential and migration toward the cathode (whether conditioned by acids, alkalies, or salts) are beyond the limits of normal viability. In other words marked deviation from ordinary potential differences is very probably incompatible with viability. On the other hand the death of the cells need not alter their electrophoretic reaction as evidenced not only by our

studies of heat-killed cells but also by the fact that bacterial cells whose migration has been reversed in direction by exposure to pH 1.0 exhibit a quite normal migration toward the anode when brought back to the neutral zone.

V.

Electrophoresis of Bacterial Spores at Various Hydrogen Ion Concentrations.

In studying the electrophoresis of the spores of *Bacillus cereus* two different methods were used. In our first experiments old agar slants, already rich in spores, were washed off and tested directly. In later work 18 hour agar cultures were washed off and held in water suspension, until sporulation had occurred. Both methods proved satisfactory, vegetative cells being rarely observed in either case.

The general results of these experiments are indicated in Table III.

TABLE III.

Observed Velocities (in Micra per Second) of Spores at Different Hydrogen Ion Concentrations.

pH.....	1.0-1.9	3.0-3.9	5.0-5.9	7.0-7.9	8.0-8.9	9.0-9.9	10.0-10.9	11.0-11.9	12.0 and over
Velocity, Exp. 20.....				−5.0	−10.9	−11.8	−16.0	−13.5	+10.9
Velocity, Exp. 38.....	+2.9	−0.7	−3.4	−5.9	−6.8	−8.4	−9.4		
Velocity, Exp. 39.....	+1.7	0.0	−2.8	−4.7	−6.6	−5.8	−9.8		
Average velocity.....	+2.3	−0.3	−3.1	−5.2	−8.1	−8.7	−11.7	−13.5	+10.9

These results are even more strikingly concordant than those obtained with the vegetative cells and the spores behave on the whole exactly as the vegetative cells did, although the absolute velocity appears to be slightly less at all pH values. That the velocity of spores and vegetative cells is so nearly identical and if anything slower, in the case of spores, is in accord with the theoretical physical considerations of the Quincke-Helmholtz-Lamb analysis which postulates that electrophoretic velocity is independent of the mass of the moving particle.

Again we note an isoelectric point in the neighborhood of pH 3.0, with migration toward the cathode at more acid reactions and with migration toward the anode at less acid reactions, increasing in

velocity up to pH 11.0. The positive charge manifest in a single experiment at pH 12.0 may or may not be significant. This is a point to which we are now giving further study.

VI.

Influence of NaCl upon Electrophoresis of Bacterial Cells.

Our next experiments were designed to test the influence of sodium chloride upon the electrophoretic charge. The material was prepared as described above except that the bacteria after suspension in water were centrifuged and resuspended two or three times in the salt solution in which their migration was to be observed. When the reaction of the salt solution diverged appreciably from neutrality

TABLE IV.

Observed Velocities (in Micra per Second) of Living Vegetative Cells at Different Hydrogen Ion Concentrations and in the Presence of Different Concentrations of NaCl.

pH	1.0–2.0	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9
0.145 M NaCl	+0.9	+0.8		−3.4		−4.9		−4.0	0.0	0.0
0.363 M NaCl	0.0		−2.8		−6.5		−4.9	−1.8		0.0
0.725 M NaCl	−0.5	−2.7	−2.6	−3.8	−3.9	−5.1	−4.9	−2.4	0.0	0.0
1.450 M NaCl	+1.0		+0.4	−3.2		−5.6	−4.9		0.0	0.0

it was brought back to pH 7.0 before use. The results presented in Table IV are based on a single experiment at the 0.145 M, 0.363 M, and 1.450 M concentrations. For the 0.725 M concentration four separate experiments have been averaged. The correspondence between these four experiments was remarkably close.

It is evident, as pointed out by earlier workers (39, 32, 20, 16, 35, 36, 31, 29) that the presence of salts has a marked depressing effect upon electrophoretic velocity. Within the limits here studied (0.145 M to 1.450 M) this effect in the case of NaCl appears to be independent of concentration. At reactions near neutrality the velocity of migration is but little more than half as great as that observed in the absence of the salt (*cf.* Table I). The isoelectric point at about pH 3.0 is again manifest. On the alkaline side of the neutral point the most marked

effect is apparent. The rise to a maximum velocity near pH 10.0 observed in aqueous solution is here replaced by a complete absence of migration. This observation in regard to the narrowing by salts of the pH zone within which electrophoresis occurs is, so far as we are aware, a new one. Decreased rate of movement in the alkaline range may be in part explained by increase in viscosity and decrease in inductive capacity but it seems improbable that complete loss of movement can be wholly attributed to these effects of NaCl upon the menstruum without assuming a real decrease in electrokinetic potential difference.

VII.

Influence of $CaCl_2$ upon the Electrophoresis of Bacterial Cells.

We have conducted precisely similar experiments using $CaCl_2$ solutions of varying concentrations for our menstrua, the results

TABLE V.

Observed Velocities (in Micra per Second) of Living Vegetative Cells at Different Hydrogen Ion Concentrations and in the Presence of Different Concentrations of $CaCl_2$.

pH	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9
0.014 M $CaCl_2$	+6.8		−3.0		−5.5	−6.8		−3.7	0.0	0.0
0.072 M $CaCl_2$	+1.6		−3.6		−5.3		−5.6	−5.0	0.0	0.0
0.145 M $CaCl_2$	+0.8	+0.3	−1.4	−1.8	−2.3	−3.8	−3.8	−1.8	−0.4	0.0
0.725 M $CaCl_2$		0.0		0.0		0.0		0.0		

being summarized in Table V. A single experiment was made at each concentration except 0.145 M where the figures in the table represent the results of four experiments.

It appears from Table V that the effect of $CaCl_2$ in concentrations of 0.014 M and 0.072 M is almost precisely the same as that of NaCl in concentrations up to 1.450 M. An increase of $CaCl_2$ to 0.145 M however, cuts down the migration velocity very materially and a concentration of 0.725 M completely abolishes movement at all pH values. These findings are in accord with the general fact that calcium salts are more potent physiologically than sodium salts (12).

VIII.

Influence of NaCl and $CaCl_2$ When Present Together upon Electrophoresis of Bacterial Cells.

Finally, we have observed the effect of NaCl and $CaCl_2$ together when present in a ratio of four parts of NaCl to one part of $CaCl_2$, the ratio which has generally been found most favorable to bacterial viability (43, 44, 12). The actual concentrations used were 0.580 M NaCl and 0.145 M $CaCl_2$ and the average results of four experiments are presented in Table VI in comparison with pure NaCl (0.725 M) and $CaCl_2$ (0.145 M) solutions.

TABLE VI.

Observed Velocities (in Micra per Second) of Living Vegetative Cells at Different Hydrogen Ion Concentrations and in the Presence of NaCl Alone, $CaCl_2$ Alone, and Both Salts Together.

pH	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9
0.725 M NaCl	−0.5	−2.7	−2.6	−3.8	−3.9	−5.1	−4.9	−2.4	0.0	0.0
0.145 M $CaCl_2$	+0.8	+0.3	−1.4	−1.8	−2.3	−3.8	−3.8	−1.8	−0.4	0.0
0.580 M NaCl + 0.145 M $CaCl_2$	−0.5	−1.2	−2.5	−3.3	−3.8	−4.4	−2.8	−1.6	0.0	0.0

The differences are very slight but there does appear to be a certain antagonistic action manifest at pH values between pH 3.0 and 6.9. Within this range a mixture of 0.580 M NaCl + 0.145 M $CaCl_2$ appears to exert a distinctly less marked depression of migration velocity than does a 0.145 M $CaCl_2$ solution alone. This finding suggests a plausible mechanism for the efficacy of antagonizing ions in stabilizing spontaneously agglutinable suspensions of bacteria in salt solution as reported by Mellon (25). It is conceivable that the antagonizing ion reduces the effectiveness of the Na^+ ion in depressing the potential difference below the critical point for agglutination of the bacteria. The theoretical possibilities involved are too complex to be discussed at the present time.

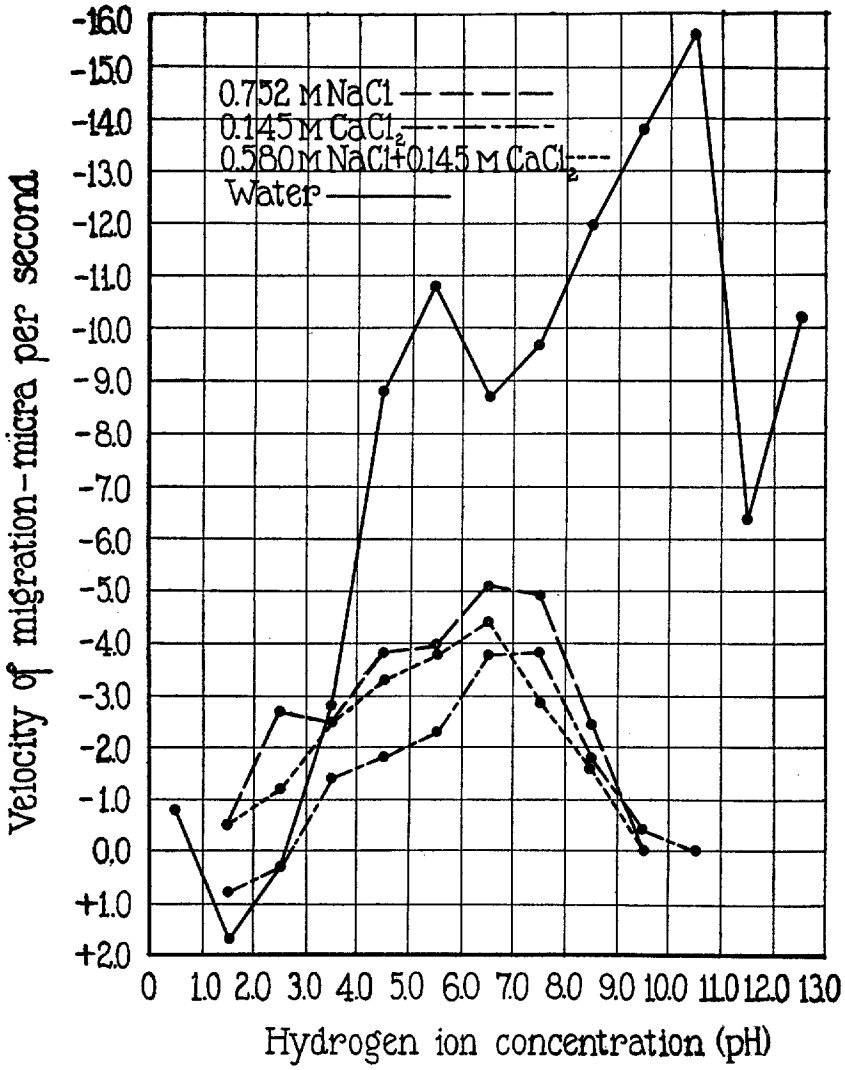

FIG. 1. The relation of the hydrogen ion concentration to the velocity of migration of living cells in water and salt solutions.

The migration velocity of −6.3 recorded at pH 11 to 12 is the average of widely varying individual observations ranging from 0 to −13.5. In this zone of hydrogen ion concentration there appears to be a sharp change from high to low velocity and probably to high velocity again, and slight variations in hydrogen ion concentration will show marked differences in result. It seems probable that, if observations were made at sufficiently close pH intervals, a point of approximate isopotential would be found in all experiments, as it was in approximately half of them.

IX.

General Theoretical Considerations.

The most plausible explanation of the differences in potential between a living cell and its surrounding menstruum is that which has been developed by Loeb (24) on the basis of the Donnan equilibrium and its application to protein systems by Proctor and Wilson.

Donnan's theory demands that when a membrane separates two solutions, the membrane being permeable to all the ionic constituents of one solution and to a part of the ionic and molecular constituents of the other solution, a definite potential difference should appear between the solutions when at equilibrium, depending on the concentrations and the charge of the various constituents. Using gelatin and other proteins as the non-diffusible constituent and various electrolytes as the diffusible constituents, Loeb shows that the quantitative results obtained in terms of potential difference are exactly what would be predicted on the basis of the Donnan equilibrium. If for example the influence of H ions on potential differences be computed, the probable variations in the dissociation constants of the substances involved taken in connection with the Donnan formula should give us a curve whose characteristics are defined by: (*a*) an isoelectric point which in the case of gelatin is near pH 4.7; (*b*) as we pass outward on the acid side of this point an area of increasing positive charge, later decreasing (with still greater acidity) toward electrical neutrality; (*c*) on the alkaline side of the isoelectric point, as we pass to less and less acid solutions the potential difference becomes increasingly negative to a maximum point and then falls toward electrical neutrality in the extreme alkaline range.

Furthermore according to the Donnan principle the addition of neutral salts should be expected, like marked excess of acid or alkali, to depress potential difference and this conclusion, too, Loeb has confirmed for compounds of gelatin and of other proteins.

As pointed out in an earlier section of this report we cannot transform our data in regard to velocity of migration into terms of potential difference between the bacterial cells and their suspending menstruum without making the assumption that viscosity and dielectric constants remain substantially constant within the range of our experimental

conditions. From computations of the known changes in viscosity in salt solutions of the strengths studied we are convinced that viscosity effects can be disregarded. That the dielectric constant also remains reasonably constant we, like other workers in this field, may for the present assume. That the phenomena involved when bacterial cells are suspended in water and salt solutions of the types studied are essentially similar to those observed by Loeb for gelatin and other types of particles and are in general accord with the postulates of the Donnan equilibrium would seem to be indicated by the general parallelism between our results and those obtained by Loeb. This parallelism has already been shown for bacterial cells by Northrop and De Kruif (29) for solutions between pH 1.0 and 9.0 but we find that in the extreme alkaline range the same parallelism is clearly manifest.

We have also confirmed the observations of Loeb and of Northrop and De Kruif that the presence of salts depresses velocity of migration, just as it should depress electrokinetic potential according to the Donnan theory and we have shown that this depression is particularly effective on the alkaline side of the pH range. We have also confirmed the findings of Northrop and De Kruif in regard to the relatively higher depressing effect of $CaCl_2$ as compared with NaCl which is another corollary of the Donnan equation.

X.

SUMMARY OF CONCLUSIONS.

1. We have confirmed the results of earlier workers particularly of Northrop and De Kruif in regard to the following points:

(*a*) the general tendency of the bacterial cell when suspended in distilled water near the zone of neutrality to move toward the anode of an electrical field;

(*b*) the fact that the migration of bacterial cells in the electrical field is a function of the reaction of the menstruum. The curve obtained by plotting velocity of migration against pH passes through an isoelectric point at about pH 3.0, at greater acidity the direction of migration becomes reversed (toward the cathode) and in still more acid solution (pH = 1.0) again disappears; while at reactions less acid than pH 3.0 the velocity is toward the anode and increases with increasing alkalinity;

(*c*) the fact that neutral salts depress the velocity of migration, calcium salts being much more effective than sodium salts of the same concentration.

2. We further find:

(*a*) that on the extreme alkaline side of the curve of velocity of migration plotted against pH a maximum value is reached at about pH 10 with a fall at about pH 12.0 which in many experiments reaches an isopotential point;

(*b*) that the depressing effect of salts is accompanied by a general shifting of the curve of migration velocity so that a maximum velocity (of course absolutely less than that manifest in the absence of salts) appears at about pH 7.0 and an abolition of velocity at pH 9.0 to 10.0;

(*c*) that an apparent "antagonistic" effect is indicated between $CaCl_2$ and NaCl, the presence of a certain concentration of the latter salt diminishing to a slight but definite degree the depressing effect produced by the former;

(*d*) that heat-killed bacterial cells exhibit essentially the same curve of migration velocity as that of the living cells;

(*e*) that bacterial spores exhibit the same general curve of migration velocity as vegetative cells, although the actual velocity is apparently slightly less.

3. All of the observed phenomena appear to be in accord with the assumption that marked differences in dielectric constants did not appear under the conditions studied and if this assumption be granted the results are in accord with the fundamental postulates of the Donnan equilibrium as applied to the explanation of the origin of potential difference between a bacterial cell and its enveloping menstruum. It is possible but not at all certain that the phenomenon of antagonism may require the introduction of additional assumptions for its explanation.

Professor Donnan and other investigators have clearly understood the importance of applying the concept of membrane equilibria in the elucidation of physiological phenomena. Our findings add to the numerous vindications favoring this view and emphasize the importance of further study of membrane equilibria in bacterial suspensions. We have pointed out that certain potential differences between

bacteria and their menstrua are apparently associated with some of the phenomena of viability. Viability and potential differences may, however, under certain conditions vary quite independently as evidenced by the fact that normal rates of migration are demonstrable after the cells have been killed by heat. Thus, considerable caution must be exercised in relating the existence of these charges to the metabolism of the cell.

BIBLIOGRAPHY.

1. Abbott, J. F., 1908, Galvanotropism of bacteria, *Science*, xxvii, 910.

1, *a*. Arkwright, J. A., 1914, On the presence in an emulsion of *Bacillus typhosus* of two different substances which are agglutinable by acids, and their relation to serum agglutination, *Z. Immunitätsforsch., Orig.*, xxii, 396.

2. Bechhold, H., 1904, Die Ausflockung von Suspensionen bzw. Kolloiden und die Bakterienagglutination, *Z. physik. Chem.*, xlviii, 385.

3. Beniasch, M., 1911–12, Die Säureagglutination der Bakterien, *Z. Immunitätsforsch., Orig.*, xii, 268.

4. Burton, E. F., 1921, The physical properties of colloidal solutions, London and New York, 2nd edition.

5. Burton, E. F., 1922, Cataphoresis: the motion of colloidal particles in an electrical field. Fourth report on colloid chemistry and its general and industrial applications, London, 23–33.

6. Buxton, B. H., and Shaffer, P., 1906–07, Die Agglutination und verwandte Reaktionen in physikalischer Hinsicht. I, *Z. physik. Chem.*, lvii, 47.

7. Cernovodeanu, P., and Henri, V., 1906, Détermination du signe électrique de quelques microbes pathogènes, *Compt. rend. Soc. biol.*, lxi, 200.

8. Clark, W. M., 1920, 1922, The determination of hydrogen ions, Baltimore, 1st and 2nd editions.

9. Eggerth, A. H., and Bellows, M., 1921–22, The flocculation of bacteria by proteins, *J. Gen. Physiol.*, iv, 669.

10. Ellis, R., 1911–12, Die Eigenschaften der Ölemulsionen. I. Die elektrische Ladung, *Z. physik. Chem.*, lxxviii, 321.

11. Ellis, R., 1912, Die Eigenschaften von Ölemulsionen. II. Beständigkeit und Grösse der Kügelchen, *Z. physik. Chem.*, lxxx, 597.

12. Falk, I. S., 1923, The rôle of certain ions in bacterial physiology. A review (Studies on salt action. VII), *Abstr. Bact.*, vii, 33, 87, 133.

13. Falk, I. S., and Shaughnessy, H. J., 1922–23, Effect of certain electrolytes on the buffering power of *Bacterium coli*, *Proc. Soc. Exp. Biol. and Med.*, xx, 426.

14. Freundlich, H., 1921, The effective potential difference of electro-osmosis and allied phenomena. The physics and chemistry of colloids and their bearing on industrial questions, London, 146.

15. Freundlich, H., and Gyemant, A., 1922, Thermodynamischer und elektrokinetischer Potentialsprung an der Grenzfläche zweier Flüssigkeiten, *Z. physik. Chem.*, c, 182.
16. Girard, P., and Audubert, R., 1918, Les charges électriques des microbes et leur tension superficielle, *Compt. rend. Acad.*, clxvii, 351.
17. Haber, F., and Klemensiewicz, Z., 1909, Über elektrische Phasengrenzkräfte, *Z. physik. Chem.*, lxvii, 385.
18. Hardy, W. B., 1899, On the coagulation of proteid by electricity, *J. Physiol.*, xxiv, 288.
19. Hardy, W. B., 1899–1900, A preliminary investigation of the conditions which determine the stability of irreversible hydrosols, *Proc. Roy. Soc. London*, lxvi, 110.
20. Höber, R., 1914, Physikalische Chemie der Zelle und der Gewebe, Leipsic, 4th edition.
21. Höber, R., 1914, Beitrag zur physikalischen Chemie der Vitalfärbung, *Biochem. Z.*, lxvii, 420.
22. Kaye, G. W. C., and Laby, T. H., 1921, Tables of physical and chemical constants, London.
23. Lattey, R. T., 1921, The dielectric constants of electrolytic solutions, *Phil. Mag., Series 6*, xli, 829.
24. Loeb, J., 1922, Proteins and the theory of colloidal behavior, New York.
25. Mellon, R. R., 1922, Spontaneous agglutination of bacteria in relation to variability and to the action of equilibrated solutions of electrolytes, *J. Med. Research*, xliii, 345.
26. Neisser, M., and Friedemann, U., 1904, Studien über Ausflockungserscheinungen. II. Beziehungen zur Bakterienagglutination, *Münch. med. Woch.*, li, 465, 827.
27. Northrop, J. H., 1921–22, The stability of bacterial suspensions. I. A convenient cell for microscopic cataphoresis experiments, *J. Gen. Physiol.*, iv, 629.
28. Northrop, J. H., and Cullen, G. E., 1921–22, An apparatus for macroscopic cataphoresis experiments, *J. Gen. Physiol.*, iv, 635.
29. Northrop, J. H., and De Kruif, P. H., 1921–22, The stability of bacterial suspensions. II. The agglutination of the bacillus of rabbit septicemia and of *Bacillus typhosus* by electrolytes, *J. Gen. Physiol.*, iv, 639.
30. Powis, F., 1914-15, Der Einfluss von Elektrolyten auf die Potentialdifferenz an der Öl-Wassergrenzfläche einer Ölemulsion und an einer Glas-Wassergrenzfläche, *Z. physik. Chem.*, lxxxix, 91.
31. Putter, E., 1921, Untersuchungen über Bakterienkataphorese, *Z. Immunitätsforsch., Orig.*, xxxii, 538.
32. Russ, C., 1909, The electrical reactions of certain bacteria, and an application in the detection of tubercle bacilli in urine by means of an electric current, *Proc. Roy. Soc. London, Series B*, lxxxi, 314.

33. Salus, G., 1917, Die Bakterienadsorption durch Bolus, *Biochem. Z.*, lxxxiv, 378.
34. Schmidt, P., 1913, Physikalisch-chemische Untersuchungen über die Serum-Agglutination, *Arch. Hyg.*, lxxx, 62.
35. Shearer, C., 1919, The action of electrolytes on the electrical conductivity of the bacterial cell and their effect on the rate of migration of these cells in an electric field, *Proc. Cambridge Phil. Soc.*, xix, 263.
36. Shearer, C., 1922, Studies on the action of electrolytes on bacteria. Part II. The influence of the trivalent positive salts on the rate of migration of bacteria in an electric field, and their effect on growth and virulence of pathogenic organisms, *J. Hyg.*, xxi, 77.
37. von Szent-Györgyi, A., 1921, Kataphoreseversuche an Kleinlebewesen. Studien über Eiweissreaktionen. III, *Biochem. Z.*, cxiii, 29.
38. Tables annuelles de constantes et données numeriques de chimie, de physique, et de technologie, Paris, 1910–1916; Chicago, 1912–1922.
39. Teague, O., and Buxton, B. H., 1906–07, Die Agglutination in physikalischer Hinsicht. III. Die von den suspendierten Teilchen getragene elektrische Ladung, *Z. physik. Chem.*, lvii, 76.
40. Thornton, W. M., 1909–10, The opposite electrification produced by animal and vegetable life, *Proc. Roy. Soc. London, Series B*, lxxxii, 638.
41. Traube, J., 1915, Bemerkungen zu der Mitteilung von R. Höber: Beitrag zur physikalischen Chemie der Vitalfärbung, *Biochem. Z.*, lxix, 309.
42. Walden, P., 1913, The dielectric constants of dissolved salts, *J. Am. Chem. Soc.*, xxxv, 1649.
43. Winslow, C.-E. A., and Falk, I. S., 1923, Studies on salt action. VIII. The influence of calcium and sodium salts at various hydrogen ion concentrations upon the viability of *Bacterium coli*, *J. Bact.*, viii, 215.
44. Winslow, C.-E. A., and Falk, I. S., 1923, Studies on salt action. IX. The additive and antagonistic effects of sodium and calcium chlorides upon the viability of *Bacterium coli*, *J. Bact.*, viii, 237.
45. Winslow, C.-E. A., Falk, I. S., and Caulfield, M. F., 1923, The influence of certain electrolytes upon the electrical charge of bacteria, *Proc. Soc. Exp. Biol. and Med.*, xx, 428.