THE INFLUENCE OF ELECTROLYTES UPON THE ELECTROPHORETIC MIGRATION OF BACTERIA AND OF YEAST CELLS.*

By C.-E. A. WINSLOW and ELIZABETH H. FLEESON.

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

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Previous Studies of the Influence of Electrolytes upon Electrophoretic Charge.

The migration of unicellular organisms in the electrical field has been studied by many observers since Kühne (1864) first noted the effect of an electrical current upon protozoa. The early work on this subject is well summarized by Dale (1900–01). Of the biologists who investigated the problem prior to 1900 only Lortet (1896) appears to have worked with bacteria and, curiously enough, he reports no influence of a direct current upon these organisms.

The work of Hardy (1899, 1899–1900) upon the reaction of protein particles to electrical stimuli gave new impetus and direction to the study of this subject and in particular indicated the important effect of the reaction of the menstruum upon the direction of migration. Bechhold (1904) was the first to observe the phenomena of bacterial electrophoresis. Neisser and Friedemann (1904), Teague and Buxton (1906–07), Cernovodeanu and Henri (1906), Abbott (1908), and Russ (1909) worked on bacterial electrophoresis; while Thornton (1909–10) and Hardy and Harvey (1911–12) observed the migration of yeast cells.

Hardy and Harvey (1911–12) first employed non-polarizable electrodes in work of this kind; and Ellis (1911–12) in studying oil emulsions introduced correction for electrical endosmose. Powis (1914–15),

* Thesis presented by the junior author in candidacy for the degree of Doctor of Philosophy at Yale University.

also working with oil emulsions, concluded that the cation is chiefly effective in governing the influence of electrolytes upon negatively charged particles and that the extent of the effect is proportional to valency.

Girard and Audubert (1918) report an extensive study on the electrical charge of organisms of many types, indicating among other things that lanthanum nitrate markedly reduces the charge on the cell but that this effect can be partially neutralized by the addition of a salt with a trivalent anion. Shearer (1919; 1922) also used lanthanum nitrate in a series of studies of electrical conductivity and electrophoresis of *Bacterium coli* and meningococci. The migration velocities observed by him are in general in accord with those recorded in this laboratory. von Szent-Györgyi (1921) contributed studies on bacteria and protozoa. Northrop (1921–22) designed a new and admirable type of cell for electrophoretic work and Northrop and De Kruif (1921–22) made a contribution of the first importance in a study of the effect of electrolytes upon *Bacterium typhosum* and upon the bacillus of rabbit septicemia. These authors present their results in graphic form with almost no comment, but their graphs show very clearly (*a*) that the normal negative charge on the bacterial cell is diminished by the presence of electrolytes, (*b*) that the effect of the electrolyte increases with the valency of the cation although hydrogen is more effective than other monovalent ions; (*c*) that with hydrogen and with trivalent ions in considerable concentration the charge is reversed and becomes positive; and (*d*) that with still further increase, to very high concentrations, the positive charge decreases again.

More recent work along somewhat similar lines has been done by Loeb (1922) on collodion particles, by Eggerth (1923–24, *a* and *b*) with *Bacterium coli* and with red blood cells, by Winslow, Falk, and Caulfield (1923–24) and Winslow and Shaughnessy (1924) with *Bacillus cereus* and *Bacterium coli*, by Northrop and Freund (1923–24) with red blood cells, and by Oliver and Barnard (1924–25, *a* and *b*) with collodion particles and with red blood cells. Additional references to the literature may be found in the bibliography of Winslow, Falk, and Caulfield (1923–24) and in the special bibliography on the rôle of electrolytes in bacterial physiology by Falk (1923).

Scope of the Present Study and Description of Methods Used.

The object of the present investigation was to make a more detailed study of the influence of various electrolytes, in varying concentration, and at different pH values, upon the migration of unicellular organisms in the electrical field.

Two species of unicellular plants, *Bacterium coli* and *Saccharomyces apiculatus* were used as test organisms. The colon bacillus was a strain isolated in 1916 from a polluted stream and used in many earlier studies of viability and electrophoresis in this laboratory. The yeast was a strain of *Saccharomyces apiculatus* sent to us through the courtesy of the Department of Botany of the Michigan Agricultural College. The work on these organisms was checked by observations made on particles of silica dust provided by Dr. Leonard Greenburg of this laboratory, as representative inert inorganic suspensoids. In the course of the work with *Bacterium coli* the strain appeared to undergo a modification affecting the size of cells and the vigor of growth. The smaller and less vigorous cells which characterized the strain toward the end of our work showed a lower migration velocity than was noted in earlier experiments. Practically all the work here reported was done with the organism in its earlier and more typical state.

Cultures of *Bacterium coli* were grown on nutrient agar in Kolle flasks for 24 hours at 37°, while *Saccharomyces apiculatus* was cultivated on glucose agar for 48 hours at room temperature. The growth from a series of such flasks was washed off in a small amount of distilled water, then centrifuged and resuspended three times in the particular menstruum to be studied. Finally the last suspension was shaken with glass beads to break up clumps and filtered through cotton.

The effects of the following substances were tested, either alone or in combination, as indicated in the description of the results obtained, HCl, NaOH, NaCl, KCl, $CaCl_2$, $AlCl_3$, Na_2SO_4, Na_3 citrate, glucose, glycerol, and saponin. Lanthanum chloride was tried and discarded because of the presence of an insoluble impurity which we were unable to remove; and the use of sodium phosphate was abandoned because of precipitation at the point of contact with the zinc sulphate electrode.

The reaction of the distilled water used was about pH 6.0 but the

addition of the bacterial cells caused an immediate fall to pH 5.2 to 5.8. The initial reaction in the salt solutions will be discussed in a succeeding paragraph.

In our first preliminary series of experiments a single concentration of salt was used (.145 M) and the reaction was adjusted to various points between pH 1.5 and pH 13.5. Although no special buffer was added the plant cells themselves exerted a considerable buffering effect and all pH values were checked by observations made on the suspension as it left the apparatus. Observations of pH were ordinarily made by the standard method of Clark and Lubs (Clark, 1922) but in the extreme alkaline range the indicators of Prideaux (1917) were used with the standards in glycine sodium phosphate, sodium acetate buffer solution described by Northrop and De Kruif (1921–22). These standards proved somewhat unstable and unsatisfactory but were capable of yielding results close enough for our purposes.

Observations of migration velocity were made in a glass cell of the general type devised by Northrop (1921–22). The observation cell used in this laboratory was, however, detachable, joining the adjacent side-arms by means of tapered connections of ground glass. The electrode chambers of the side-arms were provided with lateral glass tubes closed by stop-cocks for adding the zinc sulphate solution.

The observation cell was connected with zinc sulphate electrodes through which was passed a current of 115 volts and 12 milliamperes, giving a potential gradient of 12 volts per cm. Considerable trouble was experienced as a result of the action of the more highly alkaline solutions upon the de Khotinsky cement with which the parts of the cell were held together and a whole glass cell (such as we understand is now on the market) would be preferable. All observations were made by timing with a stop-watch the passage of a given particle across a definite space on the eyepiece micrometer.

It was found most essential for accurate results to keep the cell and its connections scrupulously clean. At the close of each day's work the apparatus was therefore taken to pieces and its parts stored in cleaning solution (potassium dichromate and sulphuric acid). The observation cell was also cleaned daily with soap solution and filter paper.

In an apparatus of the type used there is a current of liquid toward

the cathode in the vicinity of the glass surfaces, due to electrical endosmose between the electronegative glass and the electropositive water. This is of course balanced by a streaming toward the anode in the central zones of the cell. The true migration velocity of suspended particles will therefore be accelerated at mid-depths and retarded or reversed in the extreme upper and lower layers, as a result of such streaming. In the beginning of our work we corrected for this factor (as most recent investigators have done) by observing the velocity of the particles at the central points of the three lower sixths of the cell and taking the average of these three readings as the true migration velocity. In working with the large cells of *Saccharomyces apiculatus* in particular, we were, however, struck with the marked irregularities in readings taken in the lower sixth of the cell depth. The change in velocity (from positive to negative) is very rapid near the glass and wide variations may occur between duplicate observations at this point. Putter (1921) has suggested that a reading of velocities at either or both of two levels respectively .2 and .8 of the distance from the top to the bottom of the cell should give a theoretically correct picture of true migration velocity. In order to see how closely the two procedures corresponded we compared our figures, based on the average of readings in the three lower sixths of the cell, with the figure obtained in the middle sixth alone (.25 of the distance from bottom to top,—nearly corresponding to Putter's .2). We found that on comparing the results of a set of 100 observations the average velocity at level .25 alone, was 1.2 times the average of the measurements at levels .08, .25, and .42.

The correlation between the single and the triple readings in the individual case was very close, $.89 \pm .02$. The velocity at level .25 would naturally be just about 20 per cent in excess of the velocity at Putter's level of .20, so that either Putter's level or the average of the three lower sixths would seem to be theoretically correct. In view, however, of the practical errors involved in observing velocities in the lower sixth of the cell we have based our conclusions for the earlier experiments on the .25 level alone and for the later experiments on the average of the .25 and the .75 levels. These values, expressed in the tables and charts as velocities in micra per second, are therefore about 20 per cent above the theoretical mean migration velocity. We have

left the figures as they stand, however, because they represent actual velocities as measured (at the .25 and .75 levels) and because they happen, by a curious coincidence, to correspond directly to the probable potential difference between particle and menstruum. As pointed out by Winslow, Falk, and Caulfield (1923–24) the conversion of migration velocities into potential differences involves certain assumptions which are probable, but not absolutely certain. If these assumptions (as to dielectric constants, etc.) be made, it happens that with the cell dimensions and current strength used by us, the figure for potential difference works out at 1.2 times the mean migration velocity in micra per second. Thus, since our velocities as measured were 1.2 times the theoretical mean migration velocity our results can be transposed directly into millivolts of potential difference between particle and menstruum,—if the assumptions ordinarily made in regard to the Helmholtz-Lamb equation are accepted as justified.

Migration Velocity of Bacterium coli in Isotonic Salt Solution at Various pH Values.

Our first experiments were made in .145 M solutions of six different pure salts and in a Ringer-Locke solution, the pH values being adjusted in each solution to various points between pH 1.5 and 12.5. The average results obtained are presented in Table I and in Figs. 1 and 2, each figure in the table representing the average of from 2 to 8 experiments (or from 10 to 80 individual observations). We have included in this table for comparison the curve of migration velocities obtained by Miss M. F. Upton in this laboratory for the same strain of *Bacterium coli* in distilled water at different pH values.

Inspection of Table I and of Figs. 1 and 2 shows very clearly the depressing effect of .145 M salt solutions upon the normal negative charge of the bacterial cell at all pH values between pH 3.5 and 11.5.[1] It will be noted that in the presence of salt of this concentration the migration velocity is practically unaffected by alkali, the curves above pH 7.5 running almost level; while increase in acid causes a

[1] The phenomena which occur in the simultaneous presence of salts and high concentrations of either acid or alkali will be discussed in a forthcoming contribution by Winslow and Upton (Winslow, C.-E. A., and Upton, M. F., *J. Bact.*, 1926, xl (in press).

progressively greater loss of velocity. It will be noted from Fig. 1, where compounds of chlorine with various cations are compared, that Na is least effective and Ca most effective, K falling about halfway between. Aluminum could be studied only in the extreme acid range because beyond pH 3.5 the salt is converted to aluminum hydroxide, with corresponding formation of NaCl. Ringer-Locke solution, as might be expected from its composition, falls between the curves for Na and Ca.

Fig. 2, which includes the curves for three different anions combined with sodium, shows that the valency of the anion exerts a much less

TABLE I.

Migration Velocity of Bacterium coli in .145 M *Salt Solutions at Various pH Values.* (*Velocities in Micra per Second.*)

pH	NaCl	KCl	$CaCl_2$	$AlCl_3$	Na_2SO_4	Na_3 citrate.	Ringer-Locke solution.	Water.
1.5	−3.4	−0.5	−2.4	−1.7	−1.2	−0.9	−1.5	0.0
2.5	−5.6	−0.8	−0.9	−1.9	−1.5	−1.9		−1.9
3.5	−4.4	−2.2	−0.6	−2.6	−2.9	−1.4	−1.4	−6.0
4.5	−6.0	−2.8	−0.7		−5.8	−2.1		−12.4
5.5	−8.2	−4.5	−1.5		−5.2	−3.2	−3.9	−11.8
6.5	−9.8	−4.8	−1.5		−6.5	−4 9	−4.5	−14.5
7.5	−7.0	−5.6	−2.5		−5.8	−4 0	−4.5	−14.9
8.5	−8.1	−4.1	−2.5		−5.1	−3.9	−3.2	−14.1
9.5	−7.9	−5.8	−2.3		−6.0	−5.7	−3.9	−11.9
10.5	−4.7		−2.0		−6.5	−5.8	−4.9	
11.5	−7.2		−1.4		−8.0	−5.1		−7.3
12.5	−7.7		−0.0		−6.0	−5.2		−5.1

marked effect than that of the cation. On the alkaline side all three curves are nearly coincident and even on the acid side the curves for the bivalent and trivalent salts nearly coincide, although both show a somewhat greater depression than is caused by the NaCl.

These results are in accord with those of Northrop and De Kruif (1921–22) who found that NaCl and Na_2SO_4 gave identical results; and with those of Oliver and Barnard (1924–25, *b*), who found that the valency of the cation is the chief influence which determines the effect of a menstruum upon the migration velocity of negatively charged red blood cells.

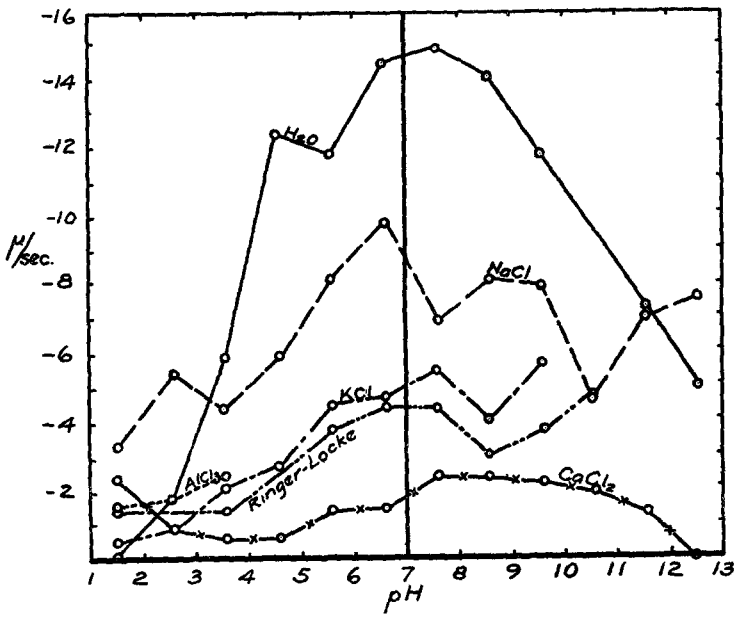

FIG. 1. Migration velocity of *Bacterium coli* in .145 M salt solutions at various pH values.

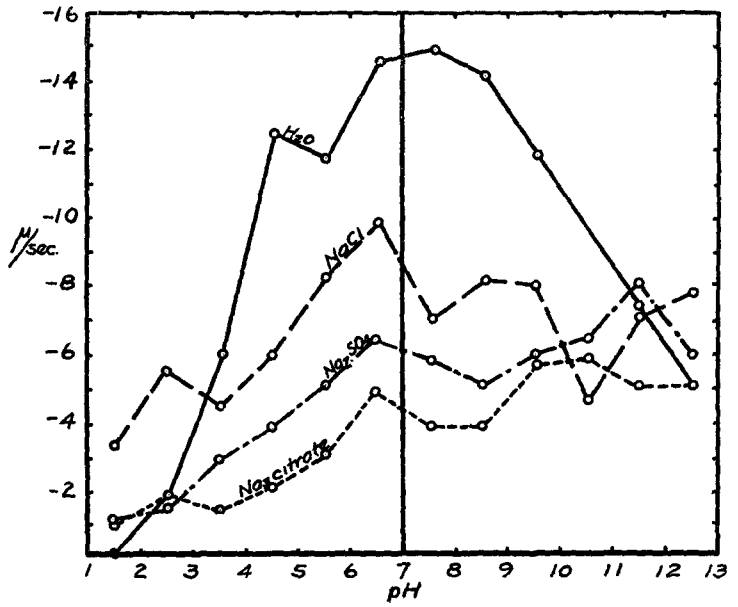

FIG. 2. Migration velocity of *Bacterium coli* in .145 M salt solutions at various pH values.

Migration Velocity of Bacterium coli in Solutions of Various Salts of Varying Concentration.

After this preliminary study of electrophoretic velocity in fixed salt concentration at varying pH values, we proceeded to a more detailed study of the effect of variation in the concentration of the salts themselves. In all subsequent work the pH of the solutions was left unadjusted to avoid the complications due to addition of ions other than those primarily studied, but the pH of the suspensions was of course always carefully recorded. With both *Bacterium coli* and *Saccharomyces apiculatus* the reaction of water and NaCl solutions, and solutions of glucose and saponin after addition of the organism, was buffered by the cells to a point between pH 5.0 and 5.8, generally close to pH 5.4. With $CaCl_2$ the same was true up to .01 M solutions. In .1 M $CaCl_2$, however, the median pH value was 6.4 for *Bacterium coli* and 6.8 for the yeast. In Na_2SO_4 the conditions were much the same,—pH values between 5.0 and 6.0 being recorded at concentrations below .1 M, with values of 6.0 to 7.5 for .1 M solutions and of 7.0 to 9.0 for molar solutions. The $AlCl_3$ solutions had a pH of 4.0 to 5.8 in less than molar concentration and of 3.2 to 3.6 in molar strength. Finally, in sodium citrate solution the pH values were between 5.5 and 7.5 in dilute solution, while for .1 M strength they were between 7.0 and 8.0 and for molar strength 8.0 to 8.6. Glycerol suspensions had a pH of 6.0 to 6.4. The practical effect of these variations in reaction is not particularly significant except in the case of the $AlCl_3$ and perhaps the sodium citrate. In all other solutions the reaction varied about a pH of 5.5, except in solutions of .1 M or molar strength and in the latter case the effect of the slightly more acid or slightly more alkaline reaction was more than balanced by the great increase in the effect of the salt itself. The $AlCl_3$ solutions, however, were much more acid than the rest (median pH under 5.0) while the sodium citrate solutions had a median pH between 6.0 and 6.5.

The average results of the experiments with *Bacterium coli* are presented in Table II and Figs. 3 and 4.

In Fig. 3 are presented the data for *Bacterium coli* in solutions of the three chlorides studied. The velocity observed in pure water at pH 5.5, the reaction corresponding to that of the NaCl and

$CaCl_2$ solutions was 11.8 micra. This value is the average of about 50 observations and is therefore of a high degree of reliability. It is obvious that all of the three solutions studied tend to depress the migration velocity of the bacteria, Na producing the least effect and Al the greatest. The depressing influence increases with increasing salt concentrations except that in the highest concentrations of $AlCl_3$ the velocity tends to rise once more. It must be remembered in considering this curve that a part of the inhibitive effect of the $AlCl_3$ solution must no doubt be due to its greater acidity.

Fig. 4 shows that when one compares the various anions in combination with sodium a very different picture is presented. NaCl and

TABLE II.

Migration Velocity of Bacterium coli in Salt Solutions of Varying Concentration.
(Velocities in Micra per Second.)

Concentration.	NaCl	$CaCl_2$	$AlCl_3$	Na_2SO_4	Na_3 citrate.
10^{-7} M	−6.5	−4.8	−7.3		−7.8
10^{-6} M	−6.6	−6.4	−6.5	−12.2	−15.4
10^{-5} M	−11.8	−8.2	−8.8	−9.0	−11.0
10^{-4} M	−8.1	−8.0	+2.7	−10.7	−12.3
10^{-3} M	−7.8	−5.5	+6.1	−9.3	−19.2
10^{-2} M	−8.6	−2.9	+6.1	−7.1	−10.9
10^{-1} M	−6.0	−0.8	+1.1	−3.7	−6.9
M	−2.8	0.0	0.0	−4.5	

Na_2SO_4 exhibit an almost identical influence, while Na_3 citrate at low concentrations appears actually to increase the velocity of migration. This latter phenomenon we believe to be due to the fact that the citrate solution had a more alkaline reaction (about pH 6.5), which would in itself account for the higher velocity observed.

While all the cations studied (except in 10^{-5} M NaCl) show a velocity less than the basic value of 11.8 micra, characteristic of *Bacterium coli* in water at pH 5.5, it is of interest to note, in view of the results with yeast cells to be discussed later, that almost all the curves in Fig. 3 show a somewhat greater velocity at a 10^{-5} M or 10^{-6} M concentration than at a 10^{-7} M concentration—suggesting that there is a certain optimum point of salt concentration, more favorable to electrophoresis than either a higher or a lower concentration.

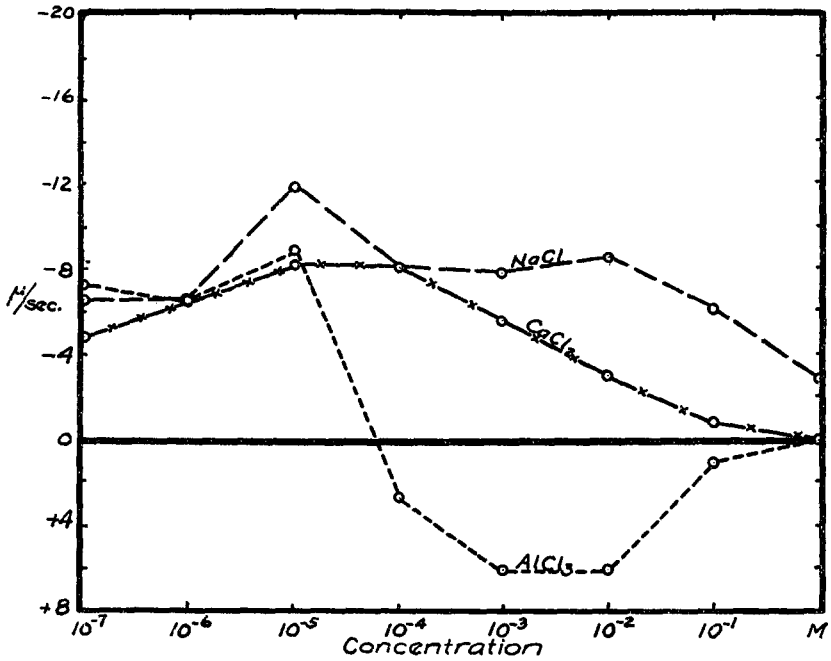

FIG. 3. Migration velocity of *Bacterium coli* in salt solutions of varying concentrations.

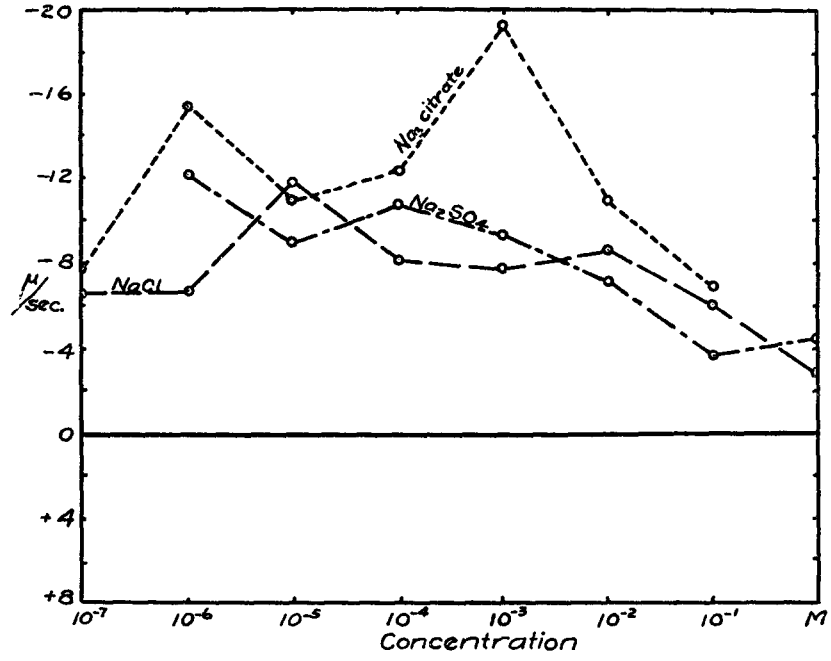

FIG. 4. Migration velocity of *Bacterium coli* in salt solutions of varying concentrations.

Migration Velocity of Saccharomyces apiculatus in Solutions of Salts of Varying Concentration.

Our results for *Saccharomyces apiculatus* are presented in Table III and in Figs. 5 and 6. In addition to the six salts studied with *Bacterium coli* we observed the effect of HCl and NaOH in similar concentrations; and we also, for purposes of control, studied three organic

TABLE III.

Migration Velocity of Saccharomyces apiculatus in Salt Solutions of Varying Concentration. (Velocities in Micra per Second.)

Concentration.	NaCl	$CaCl_2$	$AlCl_3$	Na_2SO_4	Na_3 citrate.
10^{-8}M	−9.5	−20.8			
10^{-7}M	−11.0	−27.3	−12.6	−14.8	−14.2
10^{-6}M	−10.8	−21.2	−17.0	−15.8	−16.5
10^{-5}M	−10.8	−21.2	−7.7	−18.6	−13.3
10^{-4}M	−12.5	−15.7	−3.5	−19.7	−18.9
10^{-3}M	−10.1	−12.9	0.0	−11.2	−13.2
10^{-2}M	−8.5	−7.6	0.0	−7.3	−5.9
10^{-1}M	−3.5	−3.0	0.0	−0.8	−1.5
M	0.0	−1.2	0.0	0.0	
	HCl	NaOH	Glucose.	Glycerol.	Saponin.
10^{-8}M					−15.1
10^{-7}M	−14.8	−15.0	−10.4	−11.9	−11.8
10^{-6}M	−11.9	−15.1	−14.4	−10.7	−11.5
10^{-5}M	−11.1	−17.0	−10.5	−9.1	−8.5
10^{-4}M	−8.6	−17.9	−13.0	−11.2	−14.6
10^{-3}M	−6.1	−17.4	−11.1	−10.7	−11.8
10^{-2}M	0.0	−10.3	−11.1	−9.4	−8.8
10^{-1}M	0.0	−4.5	−7.1	−10.0	
M			−2.2	−8.2	

compounds which are undissociated or only slightly dissociated: glucose, glycerol, and saponin.

A study of migration velocity in about 50 suspensions in water at pH 5.5, gave, as in the case of *Bacterium coli*, an average velocity of 11.8 micra.

The effect of the various cations is shown in Fig. 5 which exhibits a general relationship very much like that shown for *Bacterium coli* in Fig. 3. Na is least effective in depressing the charge, and Al is most

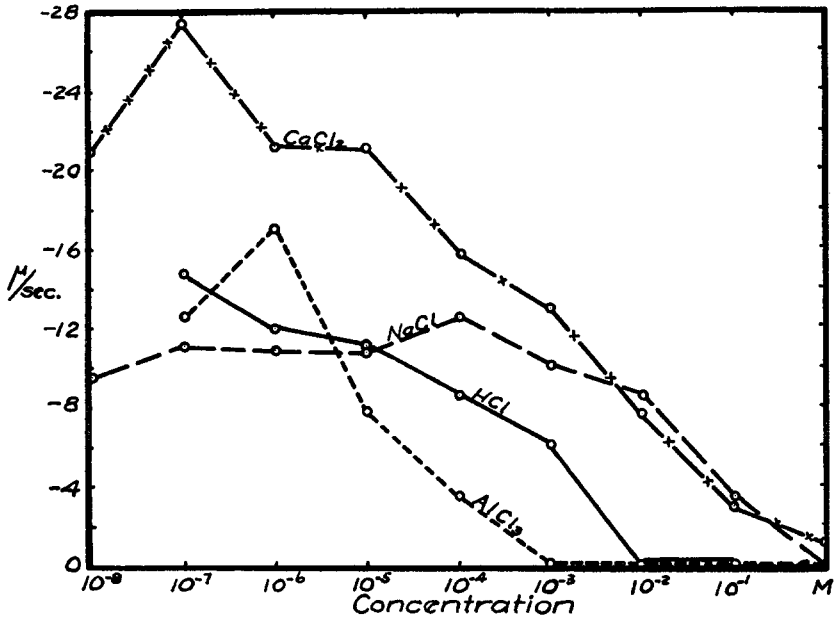

FIG. 5. Migration velocity of *Saccharomyces apiculatus* in salt solutions of varying concentrations.

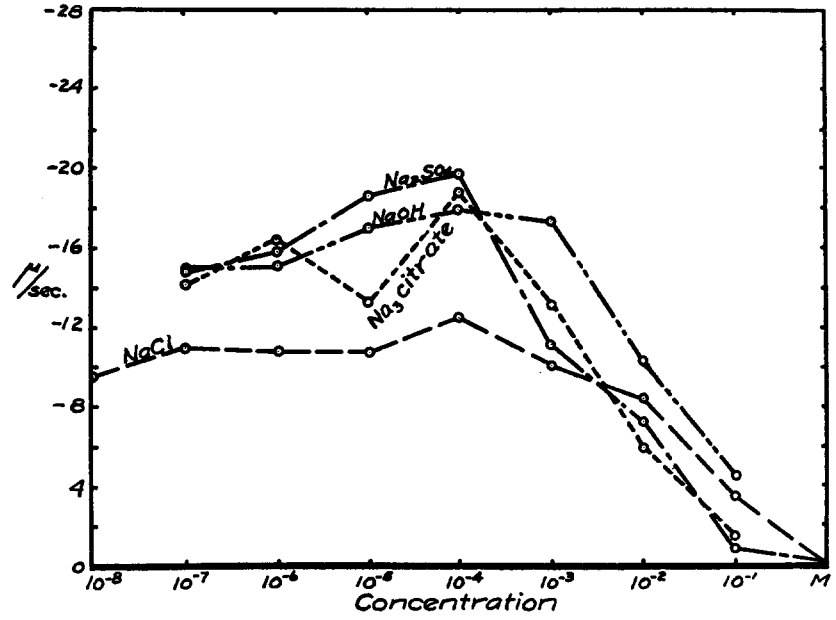

FIG. 6. Migration velocity of *Saccharomyces apiculatus* in salt solutions of varying concentrations.

effective, while the curve for H lies between the curves for $CaCl_2$ and $AlCl_3$. This is in accord with the general rule that hydrogen, in keeping with its greater migration velocity, is usually more effective than other monovalent ions in its physicochemical effects.

The most striking thing about these curves, however, is that they are all shifted upward on the velocity scale,—that is, the migration of *Saccharomyces apiculatus* in the presence of a given salt is more rapid than that of *Bacterium coli*. Since the velocity of the two organisms in water at pH 5.5 is the same (11.8), this means that the yeast cells are more resistant than the bacterial cells to the depressing influence of electrolytes upon electrophoretic charge. In all of the substances studied (except NaCl) the charge on the cells is actually increased by concentrations below 10^{-5} M and in $CaCl_2$ this increased negative charge is manifest up to a 10^{-3} M concentration.

The effect of the four anions studied (Fig. 6), as in the case of *Bacterium coli*, is practically identical in respect to decrease of charge at high concentrations. At one point (10^{-2} M) the trivalent anion is most effective, followed respectively by the bivalent ion and the univalent ions, but the differences are slight and inconstant. At low concentrations NaCl is less effective in increasing charge than the other electrolytes; but NaOH, Na_2SO_4 and Na_3 citrate all show a definite increase at concentrations below 10^{-3} M.

The three undissociated organic compounds, as might be expected, showed (Table III) no marked effect on electrophoretic velocity, except in molar glucose solution where there was a marked fall in velocity probably due to increased viscosity. With this exception all the velocities for these suspensions fall between 7.1 and 15.1 micra and 17 out of 22 averages fall between 8 and 12 micra.

Migration Velocity of Silica Dust in Various Salts of Varying Concentration.

Finally, to see how far the phenomena observed were due to biological, and how far to purely physical phenomena, we made a series of tests on particles of silica dust, of a size intermediate between that of the yeast cells and that of the bacteria studied. The results for NaCl, $CaCl_2$, and $AlCl_3$ are presented in Table IV and Fig. 7.

The reaction of the dust suspensions in water was between pH 5.4

TABLE IV.

Migration Velocity of Silica Dust in Salt Solutions of Varying Concentration.
(Velocities in Micra per Second.)

Concentration.	NaCl	$CaCl_2$	$AlCl_3$
10^{-7}M	−16.9	−17.9	−14.7
10^{-6}M	−20.1	−16.0	−15.6
10^{-5}M	−14.7	−13.2	−5.5
10^{-4}M	−19.6	−15.2	+6.3
10^{-3}M	−19.7	−16.1	+19.3
10^{-2}M	−16.1	−12.8	+21.5
10^{-1}M	−11.4	−6.1	+14.4
M	0.0	0.0	+2.9

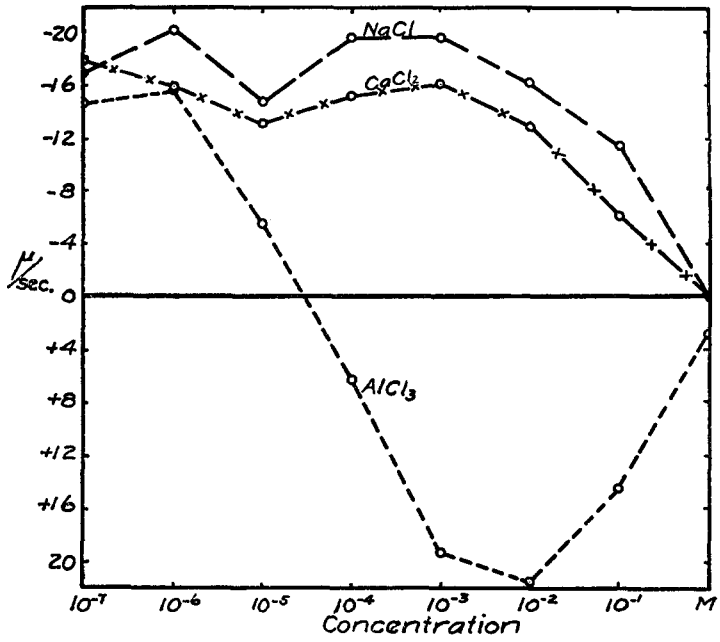

FIG. 7. Migration velocity of silica dust in salt solutions at varying concentrations.

and 6.0; in NaCl between 5.2 and 5.8; in $CaCl_2$ it varied from 5.2 to 10.0 with increasing concentration; in $AlCl_3$ from 6.4 to 3.4, with increasing concentration. The velocity observed in water at pH 5.6 was 16 micra.

Comparison of Fig. 7 with Figs. 3 and 5 will show that the general effects of electrolytes upon the migration velocity of dust, of yeast cells, and of bacteria are essentially the same. Again we note a tendency to increase the rate of migration toward the anode in dilute solutions of electrolytes, the reduction of velocity with further increase in concentration, and the fact that Ca is more effective than Na and much less effective than Al in producing such results. The curves for the dust, the yeast, and the bacteria in NaCl are almost identical. Calcium affects *Bacterium coli* and silica dust in the same way but causes a more marked increase in charge at low concentrations with *Saccharomyces apiculatus*. Aluminum in high concentrations affects the yeast cells least and the dust most markedly, reducing the velocity of the yeast only to zero but causing a reversal of the bacterial charge (corresponding to a maximum velocity of 6.1 micra toward the cathode) and of the dust charge (corresponding to a maximum velocity of 21.5 micra toward the cathode). As in the case of *Bacterium coli*, extremely high concentrations of $AlCl_3$, beyond 10^{-2} M, show a second decrease in positive charge.

SUMMARY AND DISCUSSION OF RESULTS.

1. It seems first of all clear from our results that the effect of electrolytes upon electrophoretic charge is essentially the same, whether one is dealing with silica dust, bacteria, or yeast cells, although certain quantitative differences appear which will later be discussed.

2. The normal negative charge on the suspended particles appears to be slightly increased by very low concentrations of electrolytes, markedly so in the case of yeast cells. Increase in charge due to minimal concentrations of electrolytes has been recorded by Loeb (1922) for collodion particles.

3. Higher concentrations of electrolytes cause a marked and progressive decrease in negative charge, sometimes leading to an isopotential condition and sometimes to a complete reversal of charge with active migration toward the cathode. This effect is apparently due to the cation alone and increases with the valency of the cation, except that the H ion shows specially marked activity, between that of bivalent and trivalent ions. Since NaOH behaves like an ordinary univalent salt, increased alkalinity of a solution does not further depress the

charge already depressed by salts; but, since the H ion is much more active than other univalent or bivalent ions, increased acidity does cause a further progressive depression of charge, even in salt solutions. Certain electrolytes appear to show individual peculiarities due to something else than their valency. Thus KCl for example is distinctly more effective than NaCl. Sodium chloride in general appears to exert less influence upon electrophoretic charge, either in low or high dilution, than do other compounds of univalent ions studied.

This depressing effect of moderately high concentrations of electrolytes is much less marked with yeast cells than with *Bacterium coli*. Silica dust is still less affected by monovalent and bivalent ions than are the yeast cells but appears to be more affected than either yeast or *Bacterium coli* by $AlCl_3$.

4. Very high concentrations of $AlCl_3$ (above 10^{-2} M) show a third effect, a decrease of the positive charge produced by concentrations of moderate molar strength. This is analogous to phenomena observed for trivalent salts by Northrop and De Kruif (1921–22) and for acid by Winslow, Falk, and Caulfield (1923–24).

5. Organic substances, such as glucose, glycerol, and saponin produce no effect on electrophoretic velocity until they reach a concentration at which viscosity changes are involved.

6. The first two results observed,—(*a*) the increase in charge as a result of slight additions of electrolytes, and (*b*) the marked decrease in charge with further concentration of electrolytes, depending on the valency of the cation, so far as vegetable cells are concerned, are entirely in accord with the theory of the Donnan equilibrium as worked out by Loeb (1922).

We might assume in explaining such phenomena that the plant cell contains a certain proportion of unbound protein material and that the first modicum of cation which enters the cell is bound by the protein, leading to an increase in the relative negative charge of the cell as compared with its menstruum, while subsequent increments of cation remain unbound in the cell and thus lower its charge. When we find, however, that the same phenomena are apparent with collodion particles, as shown by Loeb, and with silica dust, it seems difficult to apply such a theory, involving the conceptions of a permeable mem-

brane and unbound organic compounds. Loeb (1923–24) suggests that the primary increase may be due to an aggregation of anions in the part of the electrical double layer adjacent to the suspended particles; but why there should be first an aggregation of anions and later (with increasing concentration) an aggregation of cations, is not easy to conceive.

The third result,—the reversion to a more negative charge in the presence of a marked excess of trivalent ions,—is again difficult to explain. Loeb, in this connection, postulates the existence of complex ion-protein compounds, which can scarcely be assumed in the case of the silica particles.

BIBLIOGRAPHY.

1. Abbott, J. F., 1908, Galvanotropism of bacteria, *Science*, xxvii, 910.
2. Bechhold, H., 1904, Die Ausflockung von Suspensionen bzw. Kolloiden und die Bakterienagglutination, *Z. physik. Chem.*, xlviii, 385.
3. Cernovodeanu, P., and Henri, V., 1906, Détermination du signe électrique de quelques microbes pathogènes, *Compt. rend. Soc. biol.*, lxi, 200.
4. Clark, W. M., 1920, 1922, The determination of hydrogen ions, Baltimore, 1st and 2nd editions.
5. Dale, H. H., 1900–01, Galvanotaxis and chemotaxis of ciliate infusoria. Part 1, *J. Physiol.*, xxvi, 291.
6. Eggerth, A. H., 1923–24, *a*, Changes in the stability and potential of cell suspensions. I. The stability and potential of *Bacterium coli*, *J. Gen. Physiol.*, vi, 63.
7. Eggerth, A. H., 1923–24, *b*, Changes in the stability and potential of cell suspensions. II. The potential of erythrocytes, *J. Gen. Physiol.*, vi, 587.
8. Ellis, R., 1911–12, Die Eigenschaften der Ölemulsionen. I. Die elektrische Ladung, *Z. physik. Chem.*, lxxviii, 321.
9. 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.
10. Girard, P., and Audubert, R., 1918, Les charges électriques des microbes et leur tension superficielle, *Compt. rend. Acad.*, clxvii, 351.
11. Hardy, W. B., 1899, On the coagulation of proteid by electricity, *J. Physiol.*, xxiv, 288.
12. Hardy, W. B., 1899–1900, A preliminary investigation of the conditions which determine the stability of irreversible hydrosols, *Proc. Roy. Soc. London*, lxvi, 110.
13. Hardy, W. B., and Harvey, H. W., 1911–12, Note on the surface electric charges of living cells, *Proc. Roy. Soc. London, Series B*, lxxxiv, 217.
14. Kühne, 1864, cited by Dale, H. H. (1900–01).

15. Loeb, J., 1922, Proteins and the theory of colloidal behavior, New York and London.
16. Loeb, J., 1923–24, The influence of the chemical nature of solid particles on their cataphoretic P.D. in aqueous solutions, *J. Gen. Physiol.*, vi, 215.
17. Lortet, L., 1896, Influence des courants induits sur l'orientation des bactéries vivantes, *Compt. rend. Acad.*, cxxii, 892.
18. Neisser, M., and Friedemann, U., 1904, Studien über Ausflockungserscheinungen. II. Beziehungen zur Bakterienagglutination, *Münch. med. Woch.*, li, 465, 827.
19. Northrop, J. H., 1921–22, The stability of bacterial suspensions. I. A convenient cell for microscopic cataphoresis experiments, *J. Gen. Physiol.*, iv, 629.
20. 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.
21. Northrop, J. H., and Freund, J., 1923–24, The agglutination of red blood cells, *J. Gen. Physiol.*, vi, 603.
22. Oliver, J., and Barnard, L., 1924–25, *a*, The influence of electrolytes on the stability of red blood corpuscle suspensions, *J. Gen. Physiol.*, vii, 99.
23. Oliver, J., and Barnard, L., 1924–25, *b*, The effect of valency of cations and anions on negatively and positively charged red blood cells, *J. Gen. Physiol.*, vii, 225.
24. 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.
25. Prideaux, E. B. R., 1917, The theory and use of indicators, London, 1917.
26. Putter, E., 1921, Untersuchungen über Bakterienkataphorese, *Z. Immunitätsforsch., Orig.*, xxxii, 538.
27. 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.
28. 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.
29. 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.
30. von Szent-Györgyi, A., 1921, Kataphoreseversuche an Kleinlebewesen. Studien über Eiweissreaktionen. III, *Biochem. Z.*, cxiii, 29.
31. 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.

32. Thornton, W. M., 1909–10, The opposite electrification produced by animal and vegetable life, *Proc. Roy. Soc. London, Series B*, lxxxii, 638.
33. Winslow, C.-E. A., Falk, I. S., and Caulfield, M. F., 1923–24, Electrophoresis of bacteria as influenced by hydrogen ion concentration and the presence of sodium and calcium salts, *J. Gen. Physiol.*, vi, 177.
34. Winslow, C.-E. A., and Shaughnessy, H. J., 1923–24, The alkaline isopotential point of the bacterial cell, *J. Gen. Physiol.*, vi, 697.