the formation of an inactive complex of reduced enzyme having lower valency state molybdenum with alloxanthine, a metabolic product of allopurinol. At present, it is difficult to determine the exact nature of enzyme inhibition. Synthesis of new compounds and kinetic studies to evaluate their inhibitory activity using purified enzyme preparations could possibly elucidate the mechanism of action of such selective inhibition of liver xanthine oxidase.

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Electrokinetic Studies of Bacteria I: Effect of Nature, Ionic Strength, and pH of Buffer Solutions on Electrophoretic Mobility of *Streptococcus faecalis* and *Escherichia coli*

HANS SCHOTT▲ and C. Y. YOUNG

Abstract □ The characteristics of the exterior surface of bacteria are important because it is the first region of the cell with which dissolved nutrients and drugs come into contact. The electrical properties of the outer layer of the cell wall were studied in detail for the Gram-positive *Streptococcus faecalis* by microelectrophoresis. A few experiments were made with the Gram-negative *Escherichia coli*. Incubation time had only a minor effect on the electrophoretic mobility, even when comparing bacteria during the exponential growth phase and during the stationary phase. Increasing concentrations of buffer electrolytes reduced the mobility. When the mobility of *S. faecalis* was measured as a function of pH between 2.5 and 10.8 at constant ionic strength with phosphate, acetate, and biphthalate buffer systems, all points fell on a single curve, indicating the absence of specific ion effects. The curve had a plateau between the pH values of 4 and 7. It dropped sharply at lower pH and rose slowly at higher pH. The extrapolated isoelectric point was at pH 2.3. *S. faecalis* was negatively charged over the entire pH range studied. According to the apparent ionization constant, the group responsible for that charge was the carboxyl group. The charge density at the outer portion of the cell wall was one carboxyl group per (48 Å)^2. For *E. coli*, the value was one carboxyl group per (31 Å)^2.

Keyphrases □ Bacteria—electrokinetic studies □ Electrokinetics—bacteria □ *Streptococcus faecalis*, electrophoretic mobility—effect of nature, ionic strength, and pH of buffer solutions □ *Escherichia coli*, electrophoretic mobility—effect of nature, ionic strength, and pH of buffer solutions □ Electrophoresis—bacteria

From the colloid-chemical viewpoint, bacteria, like all small particles, can be characterized by size, shape, hydration, and charge. The latter two properties are determined by the characteristics of the bacterial surface, specifically, of the outer layer of the cell wall. The nature of the exterior of bacteria is of interest because it is the first region of the cell with which nutrients and antibacterial agents come into contact. Their interac-

tion with and/or penetration of the cell wall depend on its hydrophilicity, on the sign and magnitude of its electric charge, and on the ionic groups responsible for that charge.

The present study deals with the charge of the Gram-positive bacterium *Streptococcus faecalis* as measured by microelectrophoresis. *S. faecalis* was selected because it is prevalent in sewage and because the structure of its cell wall is comparatively simple (1). A few measurements were made with the Gram-negative *Escherichia coli*.

A wide variety of bacteria have been studied by electrophoresis, including the Gram-negative *E. coli* (2–4) and *Brucella abortus* (5). Among the Gram-positive bacteria are a variety of staphylococci (6), *Streptococcus pyogenes* (7), *Bacillus subtilis* (4, 8, 9), and *Micrococcus lysodeikticus* (4, 10).

EXPERIMENTAL

Instrumentation—The microelectrophoresis apparatus was a commercial instrument[1] consisting of the following components: a variable d.c. power supply equipped with voltmeter and microammeter; a dark-field stereoscopic microscope; a cylindrical cell made of clear acrylic plastic, equipped with a platinum–iridium strip as the cathode and a platinum–iridium rod or a molybdenum cylinder as the anodes. The anode compartment of the cell is closed with a perforated rubber stopper bearing the electrode. The molybdenum anode combines with the oxygen liberated by electrolysis to form an oxide. Since the anode compartment is closed, the evolution of oxygen bubbles causes spurious displacement of the particles during migration velocity measurements. At buffer concentrations of 0.01 *M* and higher, oxygen bubbles formed at the platinum–iridium anode. Therefore, if it were not for the molybdenum electrode capable of binding the anodic oxygen, reversible electrodes would be required.

Focussing of the microscope on the stationary level is accomplished by superimposing the reflection of the optical front wall of the cell with a line on the ocular micrometer. The stationary level is a cylindrical layer at a distance of 0.146 of the cell diameter from the cell wall, at which the electroosmotic flow of the liquid near the wall and the return flow in the center portion of the cell cancel.

Bacteriological Aspects—A few experiments were carried out with *E. coli* B/r (11); most of the work was done with *S. faecalis* (ATCC 9790). The growth medium for *E. coli* was described previously (11). *S. faecalis* was grown at 37° in a liquid growth medium of 1% bacto-tryptone[2], 1% yeast extract[2], 0.1% dextrose, and 0.04% K_2HPO_4. The dextrose solution and the solution containing the other ingredients were sterilized separately by autoclaving for 15 min. at 15 p.s.i. (121°).

The bacteria were inoculated at 1:100 dilution in a 300-ml. culture flask with side arm. The turbidity of the suspension was measured at 640 nm. by inserting the side arm into a spectrophotometer[3]. The inside diameter of the side arm was 1.2 cm. The relation between optical density and bacterial concentration, expressed as milligrams bacterial dry weight per cubic centimeter, is not linear (1). When the bacteria reached the required age, they were harvested by centrifuging their suspension for 10 min. at 9000 r.p.m. or 5500 times the acceleration of gravity. The bacteria were freed of adhering nutrients by resuspending the sediment three times in the appropriate buffer solution and centrifuging the suspensions for 10 min. at 9000 r.p.m. each time. The final suspension in the buffer solution was about 12 times more dilute than the suspension in the liquid growth medium.

No lysis of bacteria by washing, centrifugation, or electrophoresis could be detected from absorption measurements at the 260-nm. wavelength, which is characteristic for nucleic acids.

Materials—Except when stated otherwise, all chemicals were ACS reagent grade. Distilled water was redistilled in a seasoned all-glass apparatus; it was boiled out when used for preparing buffer solutions.

Treatment of Data—To translate current intensity in microamperes (I) into specific conductance (L) in micromhos per centimeter, the cell factor (K) was determined with KCl solutions of 0.001, 0.01, and 0.1 *M* concentrations, which have specific conductances of 147, 1413, and 12,886 μmhos/cm. at 25°, respectively. The relationship is:

$$L = KI/V \qquad \text{(Eq. 1)}$$

where V is the applied voltage. The values found for K were 67.6 ± 0.3 cm.$^{-1}$ for the molybdenum anode and 67.8 ± 1.1 cm.$^{-1}$ for the platinum–iridium anode.

The electrophoretic mobility (U) is the velocity of migration (v), expressed for convenience in microns per second, under unit field strength or potential gradient. The distance between the tips of the two electrodes was 12.0 cm. Thus:

$$U = v\left(\frac{\text{micrometer divisions}}{\text{sec.}}\right)\left(\frac{\text{microns}}{\text{division}}\right)\left(\frac{\text{12 cm.}}{\text{volts}}\right) \quad \text{as} \quad \frac{\text{microns/sec.}}{\text{volts/cm.}} \qquad \text{(Eq. 2)}$$

With an applied voltage of 100 v. and using the 8× objective, where each division in the ocular micrometer represents 120 μ, Eq. 2 becomes:

$$U = v\left(\frac{\text{divisions}}{\text{sec.}}\right)\left(\frac{120\ \mu}{\text{division}}\right)\left(\frac{\text{12 cm.}}{\text{100 v.}}\right) = 14.40\ v\ \frac{\text{microns/sec.}}{\text{volts/cm.}} \qquad \text{(Eq. 3)}$$

In the case of large, approximately spherical, and nonconducting particles, where the thickness of the double layer is much smaller than the radius of curvature, the Smoluchowski equation applies (12, 13) and the ζ-potential is given by:

$$\zeta = \frac{4\pi\eta U}{D} \qquad \text{(Eq. 4)}$$

where D is the dielectric constant of the dispersion medium and η is its viscosity. Using the values for water at 25° in Eq. 4, namely, $D = 80$ and $\eta = 0.0091$ poise, results in:

$$\zeta\ \text{(millivolts)} = 12.9\ U\ \frac{\text{microns/sec.}}{\text{volts/cm.}} \qquad \text{(Eq. 5)}$$

since there are 300 practical volts to the e.s.u.-C.G.S. volt or statvolt.

The surface charge density (σ) is calculated (12, 13) according to:

$$\sigma = \sqrt{\frac{2NkTD}{1000\pi}}\ \sqrt{c}\ \sinh\frac{ze\zeta}{2kT} \qquad \text{(Eq. 6)}$$

provided that the anion and cation of the added buffer salt are of the same valence (z). The concentration of the buffer salt (c) is in moles/liter; k is the Boltzmann constant, N is Avogadro's number, and ϵ is the charge of one electron. If ζ is expressed in practical millivolt units at 25°, this equation simplifies to:

$$\sigma = 3.53 \times 10^4\ \sqrt{c}\ \sinh 0.0197z\zeta \qquad \text{(Eq. 7)}$$

where σ is given in e.s.u./cm.^{2}. The factor 2.08×10^9 converts e.s.u./cm.^{2} into electric charges per square centimeter.

Strictly speaking, since the ζ-potential refers to the plane of shear or slip separating the layer of hydration bound to the bacterium from free water, rather than to the bacterial surface proper, so does σ. In view of the relatively low charge density and the low ionic strength of the buffer solutions used, however, the difference is likely to be quite small. The ψ_0 and the ζ-potentials of emulsion droplets of petroleum ether in aqueous solutions of an anionic and a cationic surfactant were found to converge for charge densities of 3×10^4 e.s.u./cm.^{2} and less (14). The potential ψ_0 is the surface or thermodynamic potential.

[1] Zeta-Meter, Zeta-Meter, Inc., New York, N. Y.
[2] Difco Laboratories.
[3] Spectronic 20, Bausch & Lomb Co.

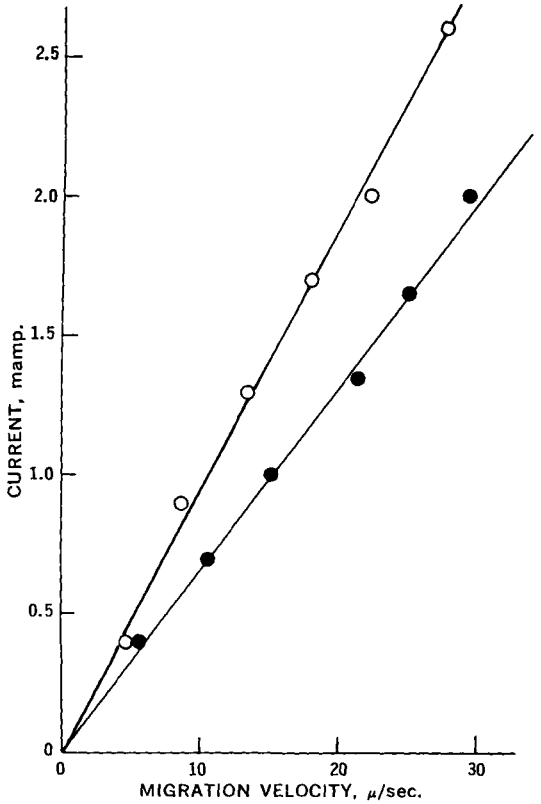

Figure 1—*Velocity of migration of* S. faecalis *as a function of current intensity. Key:* ○, *pH 7.2,* L = *1150 μmhos/cm.; and* ●, *pH 3.0,* L = *940 μmhos/cm.*

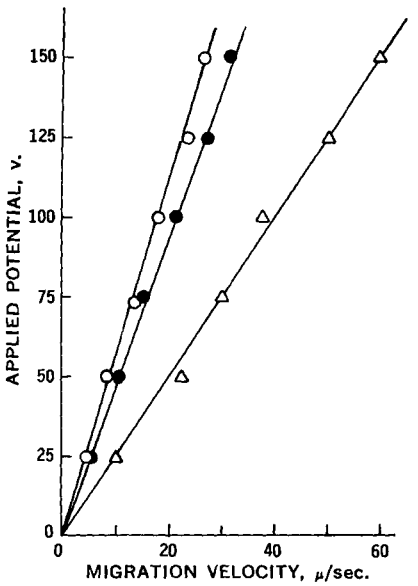

Figure 2—*Velocity of migration of bacteria as a function of applied voltage. Key:* Δ, E. coli, *pH 7.2,* L = *1490 μmhos/cm.;* ○, S. faecalis, *pH 7.2,* L = *1150 μmhos/cm.; and* ●, S. faecalis, *pH 3.0,* L = *940 μmhos/cm.*

The ionogenic groups which give rise to the surface charge of the bacteria are part of the cell wall, bound to it by primary valence forces, rather than merely adsorbed ions. The charge density σ thus remains constant. Increases in the concentration c of the buffer salts cause ζ to decrease. The product $\sqrt{c}\sinh\zeta$ remains reasonably constant.

RESULTS

Instrumental Variables—Every value quoted below is the average of 10 measurements; fresh suspension was used after the first five measurements. The ± sign precedes the standard deviation of the average. The units are [(microns/sec.)/(volts/cm.)] for electrophoretic mobility and millivolts for ζ-potential. Minus signs indicate that the bacterium is negatively charged and migrates to the anode.

Measurements of the mobility at different magnifications gave the same values. For instance, the mobility values measured with *E. coli* (incubation time 67 hr.) in 0.0062 *M* phosphate buffer (0.0040 *M* K_2HPO_4 + 0.0022 *M* KH_2PO_4) at pH 7.12 were −3.790 ± 0.060 for the 4 × objective, −3.960 ± 0.058 for the 6× objective, and −3.888 ± 0.048 for the 8 × objective, all measured with the same 15× ocular.

At the low buffer concentrations used, the mobilities measured with the molybdenum and the platinum–iridium anodes were identical, but the values obtained with the former had a higher precision. Under the conditions of the previous example, where the molybdenum anode had been used, the platinum–iridium anode gave the mobility of −3.780 ± 0.088 with the 6× objective. The specific conductance of the medium was 1490 μmhos/cm. In view of the smaller standard deviation for measurements with the molybdenum anode and because of the evolution of oxygen bubbles at the platinum–iridium anode in more concentrated salt solutions, the molybdenum anode was used for all subsequent experiments. The oxide layer was removed frequently with a metal polish.

One way to ensure that the bacteria being measured are at the stationary level is to vary the applied voltage and to plot velocity of migration against current intensity. A straight line going through the origin is obtained at the two stationary levels only (Fig. 1). The fact that the plots of velocity of migration *versus* voltage are also straight lines going through the origin provides an indication that serious electrode polarization was absent (Fig. 2).

Effect of Bacterial Treatment on Mobility—The standard procedure for purifying the bacteria was to wash them three times with buffer solutions consisting of phosphoric, acetic, or phthalic acid and/or their potassium salts. Potassium chloride was added when

Table I—Electrophoretic Mobility of *S. faecalis* as a Function of Pretreatment

	Buffer 1	Buffer 2	Buffer 3	Buffer 4
Composition (molarities)	0.00177 KH_2PO_4 +0.00274 K_2HPO_4	0.00177 NaH_2PO_4 +0.00274 Na_2HPO_4	0.00600 KH_2PO_4 +0.00067 H_3PO_4 +0.00339 KCl	0.00600 NaH_2PO_4 +0.00067 H_3PO_4 +0.00339 NaCl
pH	7.2	7.2	3.5	3.5
L, μmhos/cm.	810	680	1150	950
Washing Medium	Mobility ± *s*[a], Determined in: Buffer 1	Buffer 2	Buffer 3	Buffer 4
Water	2.28 ± 0.06	2.29 ± 0.07	2.18 ± 0.04	2.29 ± 0.02
Buffer 1	2.31 ± 0.05	—	—	—
Buffer 2	—	2.41 ± 0.04	—	—
Buffer 3	—	—	2.19 ± 0.03	—
Buffer 4	—	—	—	2.23 ± 0.04

[a] Units of −(microns/sec.)/(volts/cm.); each number represents the average of 10 readings ± the standard deviation *s* of the average.

Table II—Electrophoretic Mobility of *S. faecalis* in 1/150 *M* Phosphate Buffer as a Function of Incubation Time

Incubation Time, hr.	Mobility ± s^a, at pH Values of 3.0	5.1	7.2	8.1
4	2.40 ± 0.07	2.49 ± 0.02	—	—
5	—	—	2.09 ± 0.04	1.93 ± 0.05
17	—	—	2.19 ± 0.05	1.94 ± 0.03
17.5	2.37 ± 0.05	2.58 ± 0.02	—	—
27	2.54 ± 0.05	2.59 ± 0.03	2.26 ± 0.04	2.11 ± 0.05
41	—	—	2.24 ± 0.05	2.22 ± 0.04
48	2.32 ± 0.06	2.85 ± 0.06	—	—
51	—	—	2.29 ± 0.02	2.10 ± 0.03
65	2.59 ± 0.04	2.72 ± 0.05	2.26 ± 0.04	1.97 ± 0.03
	Specific Conductance, μmhos/cm.			
	950	680	1220	1490

[a] Units of −(microns/sec.)/(volts/cm.); each number represents the average of 10 readings ± the standard deviation *s* of the average.

necessary to adjust the ionic strength. This pretreatment may have replaced some cations on the cell wall by potassium and possibly altered the electrokinetic properties of the bacteria.

To verify this possibility, a single batch of *S. faecalis* was divided into three parts. One part was washed three times with water, and the electrophoretic mobility was measured in sodium phosphate buffers and in potassium phosphate buffers. A second part was washed three times with sodium phosphate buffers, and the mobility was measured in the same buffers. The third portion was washed three times in potassium phosphate buffers, and the mobility was measured in those buffers. All buffer solutions had an ionic strength of 0.01 *M*.

The results are summarized in Table I. Application of the *F* test to the four sets of mobility measurements at pH 7.2 and to the four sets of mobility measurements at pH 3.5 indicates that the different pretreatments and the change from potassium to sodium buffers produced no significant differences in mobility at either of the two pH values. The two calculated *F* values were smaller than the critical *F* value for the 5% level. The same conclusion was reached by comparing pairs of averages determined at the same pH value by means of the *t* test.

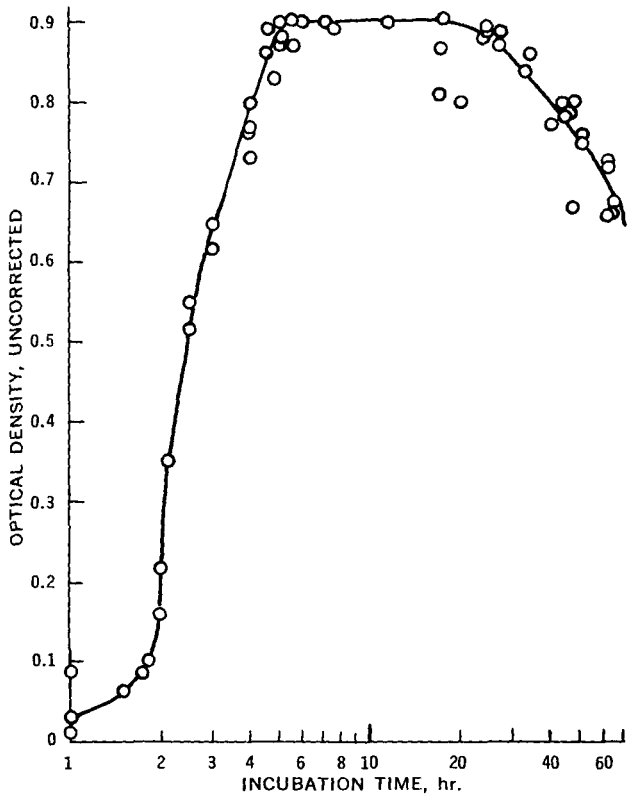

Figure 3—*Growth curve of* S. faecalis: *uncorrected optical density as a function of incubation time.*

Effect of Incubation Time on Mobility—Eight batches of *E. coli* were harvested after the following incubation times: two after 29 hr., two after 48 hr., two after 72 hr., and two after 96 hr. Even the shortest of these incubation times was longer than the exponential growth period. Electrophoretic mobilities were measured in the 0.0062 *M* phosphate buffer. The pH of the medium was 7.15, its specific conductance was 1490 μmhos/cm. When the electrophoretic mobilities of each pair of batches of the same incubation time were averaged together, the averages of the 20 readings and their standard deviations were −4.67 ± 0.038, −3.63 ± 0.067, −3.57 ± 0.109, and −3.37 ± 0.053, corresponding to the incubation times of 29, 48, 72, and 96 hr., respectively. There was a slight but consistent and significant decrease in the mobility of *E. coli* with incubation time.

The growth curve of *S. faecalis* is plotted as optical density or absorbance at 640 nm. *versus* hours of incubation in Fig. 3. Optical density is not proportional to the number of bacteria nor to the bacterial dry weight per unit volume but increases somewhat more slowly than the latter (1). Moreover, the intrinsic absorbance of cells [(optical density)/(bacterial concentration × optical path length)] changes somewhat with the phases of the growth cycle (15). However, the following conclusions regarding the bacterial growth pertinent to the present work can be drawn from Fig. 3: the lag period terminates after 2 hr. or less of incubation, the logarithmic or exponential growth phase extends from less than 2 to 5 hr., the stationary phase extends from 5 to about 26 hr., and the period of decline sets in after 26 hr.

S. faecalis was harvested after different incubation times, and electrophoretic mobilities were measured as a function of age in four phosphate buffer solutions. These solutions had a total phosphate concentration of 1/150 *M* and consisted of mixtures of $K_2HPO_4 + KH_2PO_4$ or $KH_2PO_4 + H_3PO_4$. Since the ionic strengths of these solutions are not the same, the results of Table II can only be compared within a given column but not between columns. The mobilities determined with each of the four buffer solutions show remarkably little dependence on incubation time; this includes the initial values which refer to bacteria during the exponential growth phase. At the pH values of 5.1 and 7.2, mobilities increased slightly with incubation time; while at pH 8.1, there was a slight increase followed by a slight decrease.

A 22% increase in mobility with incubation time was observed for *S. faecalis* measured at pH 7.3 in acetate buffer (Table III).

Table III—Electrophoretic Mobility of *S. faecalis* in 0.02 *M* Potassium Acetate Buffer as a Function of Incubation Time

Incubation Time, hr.	Mobility ± s^a in pH Range of 7.2–7.5
5	1.32 ± 0.07
24	1.54 ± 0.04
28	1.61 ± 0.06
51	1.62 ± 0.05
	Specific Conductance, μmhos/cm.
	2030

[a] Each number represents the average of 10 readings ± the standard deviation *s* of the average; units are −(microns/sec.)/(volts/cm.).

Table IV—Effect of Electrolyte Concentration on Electrophoretic Mobility of *S. faecalis*[a]

	Potassium Acetate of Molarity			
	0.005	0.010	0.015	0.020
U[b]	2.73 ± 0.06	2.28 ± 0.06	1.75 ± 0.06	1.61 ± 0.06
pH	7.0	7.0	7.1	7.3
L[c]	540	1080	1620	2030
	Phosphate Buffers of pH Range			
	3.0–3.2	5.1–5.4	7.1–7.3	8.0–8.1
U for 1/150 M	2.54 ± 0.05	2.59 ± 0.03	2.26 ± 0.04	2.11 ± 0.05
L for 1/150 M	950	680	1220	1490
U for 1/600 M	3.38 ± 0.06	4.00 ± 0.08	—	2.83 ± 0.12
L for 1/600 M	590	200	—	410

[a] Incubation time = 24–26 hr. [b] Mobility in units of −(microns/sec.)/(volts/cm.); average of 10 or more measurements ± standard deviation of the average. [c] Specific conductance in μmhos/cm.

Effect of Ionic Strength on Electrophoretic Mobility—Electrolytes tend to reduce the thickness of the electric double layer surrounding bacteria and the absolute value of the ζ-potential (13). Thus, electrophoretic mobilities should be compared only at constant ionic strength. The effect of ionic strength on the mobility of bacteria at constant incubation time and nearly constant pH is shown in Table IV. The mobility depended more on ionic strength than on pH in the range of variables studied. A fourfold dilution or a fourfold reduction in ionic strength at constant pH increased U by about 50%.

Effect of pH on Electrophoretic Mobility—To study the effect of pH on mobility, the incubation period of *S. faecalis* was maintained constant at 23–25 hr. and the ionic strength of the buffer solutions at 0.010 M. Phosphate buffers consisted of mixtures of K_2HPO_4 + KH_2PO_4 or of KH_2PO_4 + H_3PO_4, or of K_3PO_4, with KCl added when necessary to bring the ionic strength up to the constant value. Acetate buffers consisted of potassium acetate and/or acetic acid, with or without KCl. In one instance, KBr was used in place of KCl without noticeably affecting the results. Potassium acid phthalate was the third buffer used, as shown in Table V.

As can be seen from Fig. 4, mobilities obtained with the three buffer systems fell approximately on the same curve, indicating that there was no specific interaction between buffer ingredients and the bacterial cell wall. Specific ion effects were absent in these buffer systems.

The absolute value of the mobility drops abruptly at pH values below 3. Extrapolation to $U = 0$ places the isoelectric point in the vicinity of pH 2.3. This finding is in agreement with the reported value of 1.90 obtained at an ionic strength of 0.1 M (16).

Table V—Effect of pH on Electrophoretic Mobility of *S. faecalis* at Constant Ionic Strength[a]

pH	Mobility, − (microns/sec.)/(volts/cm.) ± s[b]	Specific Conductance, μmhos/cm.
	Acetate Buffer	
3.2[c]	2.04 ± 0.04	1490
3.4	2.09 ± 0.02	1550
4.7	2.20 ± 0.09	1350
6.9	2.10 ± 0.03	1080
7.1	2.15 ± 0.04	1090
	Phosphate Buffer	
2.45	0.72 ± 0.03	2440
2.55	1.31 ± 0.03	2030
2.70	1.65 ± 0.04	1620
3.55	2.07 ± 0.04	1080
4.05	2.11 ± 0.03	1080
4.85	2.22 ± 0.02	1080
6.1	2.21 ± 0.03	1010
7.2	2.30 ± 0.03	950
8.0	2.50 ± 0.04	750
8.5	2.72 ± 0.05	740
10.7	2.94 ± 0.09	810
	Potassium Acid Phthalate	
4.2	2.22 ± 0.03	950

[a] Ionic strength constant at 0.01 M; incubation time constant at 23–24 hr. [b] Average of 10 or more measurements ± standard deviation of the average. [c] Using KBr instead of KCl.

Viability of Bacteria—The following experiment was made to test the effect of the washing and centrifugation treatment in media of different pH values on the viability of *S. faecalis*. After incubating at 37° for 24 hr., the bacteria were harvested by centrifugation, resuspended in buffer media and centrifuged three times. The bacteria were then maintained suspended in the buffer media for 30 min. After suitable dilution, counts were made by the pour plate method.

The three buffers consisted of: 1/150 M H_3PO_4 + 0.00580 M KCl (pH 2.46, ionic strength 0.010 M); 1/600 M K_3PO_4 (pH 10.8, ionic strength 0.010 M); and 0.00261 M KH_2PO_4 + 0.00405 M K_2HPO_4, total phosphate concentration 1/150 M (pH 7.1, ionic strength 0.015 M). The relative ratios of the plate counts for these three buffers were 1:2:5. Since approximately 75% of the bacteria in the buffer solution of pH 7.1 were living, 15% of the bacteria in the acid medium and 30% of the bacteria in the alkaline medium were living.

The standard deviations of the average electrophoretic mobility values were about the same for all three systems, indicating that viable and nonviable bacteria have approximately the same mobilities. This conclusion is supported by the data of Table II, where the differences in mobility at a given pH between bacteria in the exponential growth phase and in the period of decline were a mere 1–8%.

DISCUSSION

The electrophoretic mobility of *S. faecalis* at different pH values is shown in Fig. 4. The plot up to a pH of 7 resembles the potentiometric titration curve of an acid. The initial rise in mobility reflects the progressive ionization of acidic groups of the cell wall. To identify these groups through their apparent ionization constant, it is necessary to know the pH at the interface between the cell wall and water This surface pH is lower than the bulk pH because the negative groups of the charged surface attract hydrogen ions from the surrounding aqueous medium and repel hydroxyl ions. If the potential near the cell wall is greater than the average potential for the whole solution by an amount ζ, the hydrogen-ion concentration near the cell wall will be $[e^{-(\epsilon\zeta/kT)}]$ times the hydrogen-ion concentration in bulk (17). Therefore:

$$pH_{surf.} = pH_{bulk} + \frac{\epsilon\zeta}{2.303kT} \qquad \text{(Eq. 8)}$$

Using the electrophoretic mobility U expressed as (microns/sec.)/(volts/cm.) and setting T = 298°K, this equation becomes for uni-univalent buffer salts:

$$pH_{surf.} = pH_{bulk} + \frac{U}{4.65} \qquad \text{(Eq. 9)}$$

The value of pH_{bulk} corresponding to the midpoint of the mobility step in Fig. 4 (18) is approximately 2.5. This is the pH_{bulk} corresponding to one-half of the mobility of the plateau region (3), namely, to −2.2/2 = −1.1. Entering these values of pH_{bulk} = 2.5 and U =

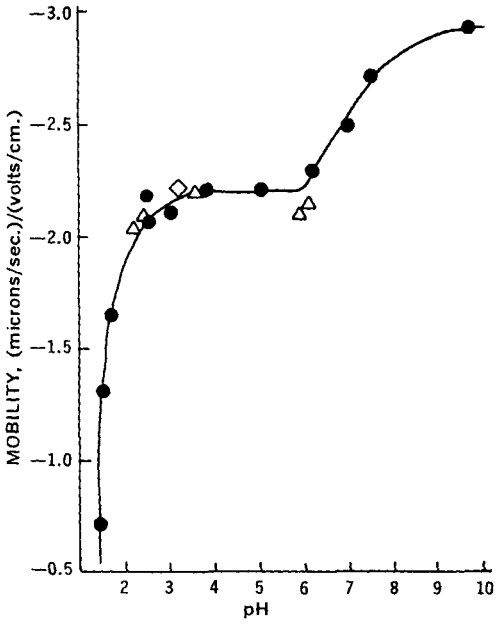

Figure 4—*Effect of pH on electrophoretic mobility of* S. faecalis *at constant ionic strength. Key:* ●, *phosphate buffer;* △, *acetate buffer; and* ◇, *potassium acid phthalate.*

−1.1 into Eq. 9 gives the value of $pH_{surf.} = 2.3$. This is the apparent pK value for the acidic groups of the cell wall. This value is in good agreement with the apparent pK value of 2.5 found by electrophoresis of droplets of mineral oil coated with adsorbed arabic or pectic acids or with synthetic copolymers of maleic acid, all of which have carboxyl groups (18). Phosphate groups have approximate pK values of 1.8 (3). This indicates that the acidic groups on the cell wall of *S. faecalis* are carboxylate groups only.

The second rise in the plot of mobility *versus* pH, beyond the plateau, suggests the existence of basic groups on the bacterial cell wall (3). This rise is much less pronounced than the rise preceding the plateau. Therefore, there are far fewer basic groups than acid groups on the cell exterior, not enough to invalidate the estimate of the apparent ionization constant of the latter (18). The carboxyl groups probably belong to the mucopeptide component of the cell wall. Amino sugars have been found in the cell wall of *S. faecalis* and, in lesser amounts, of *E. coli* (19).

The surface charge density of *S. faecalis* calculated by means of Eq. 7 for an ionic strength of 0.01 *M* and the plateau *U* of −2.2 is −2080 e.s.u./cm.^{2}. For *E. coli* at pH 7.1 and an ionic strength of 0.0142 *M*, $U = -3.88$ and $\sigma = -4890$ e.s.u./cm.^{2}. Some published surface charge density values at comparable ionic strengths are −4000 for *E. coli* (20), −3390 for *Typhoid bacilli* (12), −2900 for *Aerobacter aerogenes* (21), and −3700 (22) and −2500 (23) for human and guinea pig erythrocytes, respectively. The present σ values indicate that there is on the average one carboxyl group per 2310 Å^2 or (48 Å)^2 on the outer cell wall of *S. faecalis* and one per 980 Å^2 or (31 Å)^2 on that of *E. coli*.

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