643. *Some Physical Investigations of the Behaviour of Bacterial Surfaces. Part I. The Electrophoretic Mobility of* Aerobacter aerogenes.

By P. J. Barry and A. M. James.

The electrophoretic mobility of a strain of *Aerobacter aerogenes*, grown in a synthetic medium, has been determined in a phosphate buffer solution of constant ionic strength. The mobility, and hence the charge density, of a 24-hour culture was found to be reproducible on repeated sub-culture over a period of 6 months. Nevertheless, variation of either the ionic strength or the pH value of the test solution brought about marked changes in the mobility. Killing the organisms with formaldehyde had no effect, but steaming the culture for different times caused pronounced changes in the mobility. A preliminary investigation has shown that the mobility of the organism remained constant throughout the growth cycle.

The study of the electrophoretic mobility of a strain of *Aerobacter aerogenes* has been made in an attempt to correlate changes in growth characteristics with changes in mobilities and hence possible modifications of the cell surface. The literature contains many references to electrophoretic studies of different bacteria; but the variation of strain with the consequent change in growth conditions, and the different conditions of test, make any complete interpretation of the results difficult. *Aerobacter aerogenes* was chosen because, first, it has no exacting nutritional requirements, being readily cultured in a simple synthetic medium and, secondly, a careful study has already been made of its growth characteristics under different conditions (Hinshelwood, "Chemical Kinetics of the Bacterial Cell," Oxford, 1946).

The size and shape of bacteria vary considerably during the growth phase (Topley and Wilson, "Principles of Bacteriology and Immunity," 1946, E. Arnold & Co., London, p. 84) and we have observed a marked change in size with change of medium. Abramson and Michaelis (*J. Gen. Physiol.*, 1929, **12**, 587) and Abramson (*J. Phys. Chem.*, 1931, **35**, 289), however, found that the mobility of particles was independent of their size and shape within the limits of experimental error. This is in accord with the Helmholtz and the Smoluchowski theory that:

$$\text{electrophoretic mobility} = \bar{v} = v/E = \zeta\varepsilon/4\pi\eta$$

where ζ is the potential at the interface, and v the velocity of the particle under a field strength E in a medium of dielectric constant ε and viscosity η.

The mobility of *Escherichia coli* is independent of pH value over the pH range 4·0—7·0 in buffer solutions of constant ionic strength (Moyer, *J. Bact.*, 1936, **32**, 433), but a marked change of mobility accompanies variation of the ionic strength of the suspension

medium, similar to that observed for true colloidal particles (Robuschi, *Chem. Abs.*, 1947, 2114).

Moyer (*loc. cit.*) found no detectable change in the mobility of the organisms after repeatedly washing them with 0·0067M-phosphate buffer solution; Pedlow and Lisse (*J. Bact.*, 1936, **31**, 235), on the other hand, found some changes on washing *Esch. coli* grown in peptone broth containing added electrolytes.

This paper describes the standardisation of our technique and the determination of the mobility of a standard suspension. In this standardisation we have considered the possible variations of the mobility with the physical conditions of test, such as field strength, pH value, and ionic strength of the test solution, and with repeated washing.

The Mobility of Aerobacter aerogenes *grown in a Synthetic Medium.*—(a) *The mobility of a 24-hour culture.* The strain was sub-cultured regularly when full grown and in the first instance the mobility of a 24-hour-old culture was studied. The culture was harvested by centrifugation at 3000 r.p.m. and washed twice with 0·0067M-phosphate buffer solution (pH = 7·00; ionic strength = 0·013). The cells were re-suspended in the phosphate buffer to give a final suspension of approximately 10^8 organisms/ml., and the average velocity was determined under a constant field strength. The field strength was altered

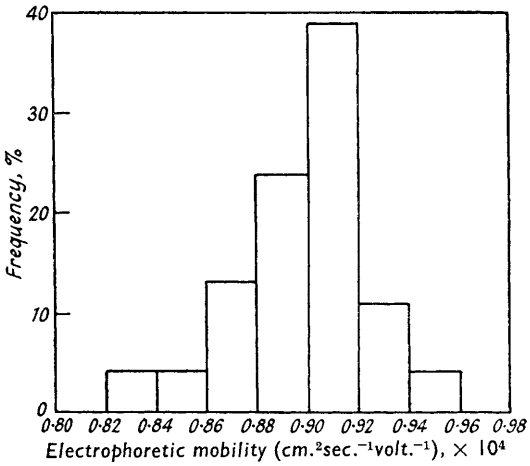


FIG. 1. *Histogram of the electrophoretic mobility of* Aerobacter aerogenes. *(All mobilities are to be understood as being towards the positive electrode.)*

and the velocity again determined, thus enabling a certain amount of personal error to be eliminated. From Table 1 it is seen that the mobility is independent of the field strength, within the limits of experimental error.

Repeated sub-culture of the organisms over a period of months showed that the mobility of a 24-hour culture was constant at $0{\cdot}90 \pm 0{\cdot}01 \times 10^{-4}$ cm.^{2}sec.$^{-1}$volt.$^{-1}$ (standard deviation $\pm 0{\cdot}03$). Fig. 1 shows the distribution of the mobilities in about 50 separate determinations carried out over a period of 6—7 months, each determination being the mean of 10 observations of an individual cell. It can be seen that the cultures investigated

TABLE 1. *Effect of field strength on the electrophoretic mobility.*

Field strength (volt/cm.)	10·95	12·77	13·68	13·85	15·66
Velocity at 25° (cm./sec.), × 10^4	10·12	11·53	12·14	12·38	14·30
Electrophoretic mobility (cm./sec./volt./cm.), × 10^4 ...	0·92	0·90	0·88	0·89	0·91

were of a single population, showing an approximate Gaussian distribution about the mean. The organisms carry a negative charge corresponding to a ζ potential of −0·011 v and a charge density of 860 e.s.u.

The mobility of such washed suspensions remained constant for periods of up to 24 hours when stored at 40°, at room temperature, or in the ice-chest, as previously shown by Dozois and Hachtel (*J. Bact.*, 1935, **30**, 473).

The effect on the mobility of repeatedly washing with phosphate buffer was studied to establish whether there is any change on the cell surface. The mobilities of 24-hour

cultures determined after each washing (Table 2) indicate that there is no modification of the cell surface under this treatment, in agreement with Moyer's observations on *Esch. coli* (*J. Bact.*, 1936, **32**, 433). Nevertheless, the standard technique adopted by us for all future work was to wash the cells twice with phosphate buffer solution.

TABLE 2. *Effect of washing on the electrophoretic mobility.*

Number of washings	0	1	2	3
Electrophoretic mobility (cm./sec./volt/cm.), × 10^4 ...	0·88	0·87	0·88	0·87

Killing the organisms with formaldehyde solution had no influence on the mobility, whilst boiling the culture had a pronounced but variable effect. A 24-hour culture was divided into five portions and treated as follows: (i) centrifuged and washed twice—control; (ii) formaldehyde added to give a final concentration of 5%, set aside for 10 minutes, centrifuged, and washed twice with phosphate buffer; (iii), (iv), and (v) culture heated in steam for 15, 60, and 120 minutes, respectively, centrifuged, and

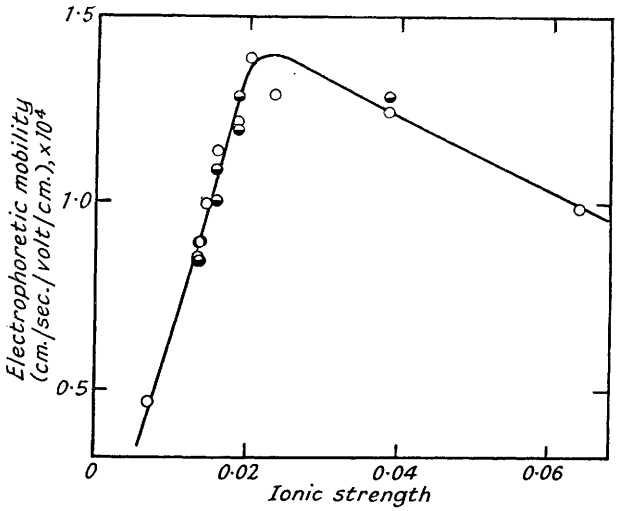


FIG. 2. *Variation of the electrophoretic mobility of* Aerobacter aerogenes *with the ionic strength of the test solution. (Open circles—24-hour culture, half-shaded circles—culture at the end of growth phase.)*

washed. Viability tests of the five portions were made on agar plates. The results (Table 3) are in agreement with those obtained for *Esch. coli* and *Corynebacterium diphtheriae* (Dozois and Hachtel, *loc. cit.*; Buggs and Green, *J. Bact.*, 1935, **30**, 453).

TABLE 3. *Effect of formaldehyde and steaming on the electrophoretic mobility.*

Treatment	Electrophoretic mobility (cm./sec./volt./cm.), × 10^4 observed	average	Viability
Control	0·89, 0·87, 0·89	0·88	+++
5% Formaldehyde	0·88, 0·87, 0·89	—	—
	0·90, 0·87, 0·88	0·88	—
15 Minutes' steaming	0·83, 0·80	0·82	—
60 Minutes' steaming *	0·67, 0·69	0·68	—
120 Minutes' steaming *	0·82	0·82	—

* Difficulty was encountered with these cultures as the organisms coagulated and were only re-suspended by shaking them for a long time.

(b) *Variation of the mobility with the ionic strength of the test solution.* A 24-hour-old culture was harvested, washed twice with 0·0067M-phosphate buffer solution, and suspended in phosphate buffer solutions (pH = 7·00) containing varying amounts of sodium chloride to increase the ionic strength. The mobilities determined in these solutions are shown in Fig. 2. The mobilities of cultures harvested at the end of the logarithmic growth phase were the same as those of older cells, indicating no detectable difference in the surface behaviour of old and young cells (Fig. 2). (Results obtained several months later were

in complete agreement with those of the main experiments, thus emphasising the reproducibility of the technique.)

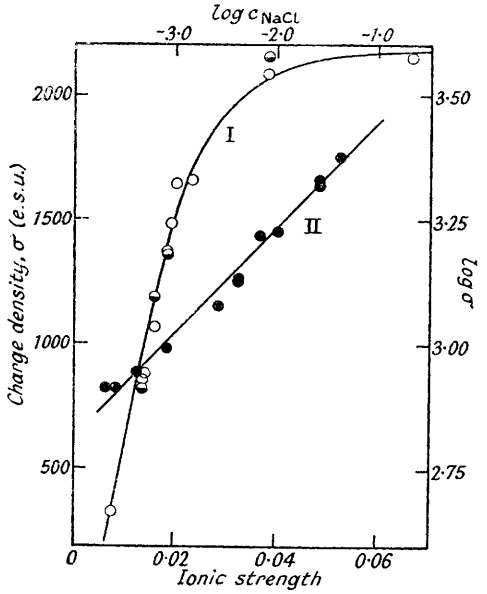
This behaviour was shown to be reversible, since cells washed free from the added salt and re-suspended in the normal phosphate buffer solution had the mobility of untreated cells.

The charge density on the organisms at different ionic strengths was calculated from the equation :

$$\sigma = 17{,}600[\Sigma c_i(e^{-z_i\zeta/0\cdot0256} - 1) + \Sigma c_j(e^{+z_j\zeta/0\cdot0256} - 1)]^{\frac{1}{2}}$$

where c_i and c_j are the ionic concentrations and z_i and z_j the valencies of the positive and negative ions, respectively (Abramson and Moyer, *J. Gen. Physiol.*, 1936, **19**, 601). Fig. 3, curve I, shows the charge density plotted as a function of the ionic strength.

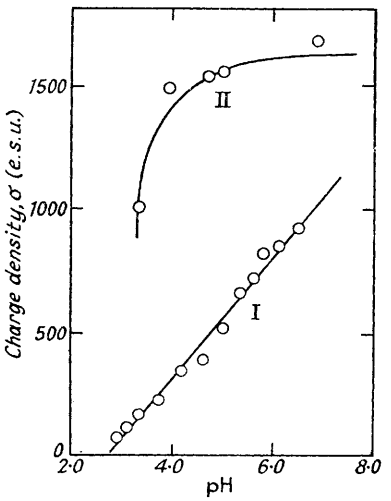
FIG. 3.



Curve I. *Variation of the charge density of* Aerobacter aerogenes *with the ionic strength of the test solution. (Open circles—24-hour culture, half-shaded circles—culture at end of growth phase.)*

Curve II. log σ *plotted against* log c_{NaCl} *(calculated from curve I).*

FIG. 4. *Variation of the charge density of* Aerobacter aerogenes *with the* pH *value of the test solution.*



Curve I. *At different ionic strengths.*
Curve II. *At constant ionic strength.*

(c) *Variation of the mobility with the* pH *value of the test solution.* The buffer solutions used, in the first instance, were made from suitable mixtures of 0·0067M-disodium hydrogen phosphate and 0·0033M-citric acid (McIlvaine, *J. Biol. Chem.*, 1921, **49**, 183), and their pH values determined. A 24-hour culture was centrifuged, washed twice with the appropriate buffer solution, and re-suspended in that solution for the mobility determination. The results, Fig. 4, curve I, show that below pH 7·5 the charge density is a linear function of pH; observations above pH 7·5 indicated little change. The experiment was repeated with cells harvested at the end of the logarithmic growth phase, but again, no significant difference could be detected between the behaviour of old and young cells.

The ionic strengths of these buffer solutions increased from approximately 0·0015 at pH 3·0 to 0·02 at pH 6·5, so the variation of charge density is possibly due to change of ionic strength as well as pH. The experiment was repeated with the addition of sodium chloride to bring the total ionic strength up to 0·02 (cf. normal value 0·013). The charge density at constant ionic strength is independent of pH between pH 7·0 and pH 4·0, but below pH 4·0 there is a marked decrease (Fig. 4, curve II).

Experimental

Cultures and Media.—The strain of *Aerobacter aerogenes*, originally obtained from the National Collection of Type Cultures (Hinshelwood and Lodge, *J.*, 1939, 1683), has been maintained by monthly sub-culture in veal bouillon. For test purposes the organism was grown in a sterile synthetic medium containing glucose (19·2 g./l.), potassium dihydrogen phosphate (3·46 g./l.), ammonium sulphate (0·96 g./l.), and magnesium sulphate (0·04 g./l.), adjusted to pH 7·12 with 4N-sodium hydroxide. The strain was sub-cultured daily in this synthetic medium at 40° in the presence of a slow stream of washed sterile air. In this medium the organism had a mean generation time (*i.e.* the time required for the number of cells to double during the logarithmic growth phase) of 32—34 minutes, which would indicate that the strain had undergone no apparent mutation during the past years. The growth was determined by measuring the turbidity of formaldehyde-killed samples with a Hilger photoelectric absorptiometer, which had been calibrated against hæmocytometer counts (Monod, "La Croissance des Cultures Bactériennes," Paris, Hermann & Cie., 1942).

The Electrophoretic Cell.—This was as described by Moyer (*J. Bact.*, 1936, **31**, 531). It consisted of two Hysil optically flat plates (25 × 40 × 0·5 mm.) mounted 0·5 mm. apart and connected to the electrode compartments by ground-glass joints, a modification that allowed the cell to be removed for cleaning. The plugs were prepared by packing a plaster paste tightly into the electrode limbs. These plugs were made to conduct by filling the apparatus with a saturated sodium sulphate solution and passing a current, with repeated reversal of the polarity until the current became constant. The electrode compartments were then filled with a saturated copper sulphate solution, which was sealed in by the rubber bungs carrying the copper electrodes.

The electrical circuit was as described by Moyer (*loc. cit.*) with the exception that the current through the cell was determined from the potential drop across a standard 200-ohm resistance. The field strength was calculated from the equation, $E = I/\kappa q$, where I is the current (amps.), q the cross-sectional area (sq. cm.), and κ the conductivity of the solution.

Determination of the Mobility.—The cell was mounted under a microscope fitted with a ×40 phase-contrast objective, used in conjunction with a ×10 eye-piece carrying a calibrated graticule. The cell temperature was measured by copper–constantin thermocouples attached to the two faces of the cell. The velocities of the particles under the applied potential were determined at room temperature and then corrected to 25° (Moyer, *loc. cit.*). In the flat cell, liquid flows along the walls of the cell in one direction and back through the centre in the other. According to Smoluchowski's theory, provided that the ratio of cell width to cell depth is not less than 20, stationary liquid layers occur at depth fractions of 0·21 and 0·79 down the cell. The microscope was adjusted by means of a calibrated fine adjustment to focus on the lower of the stationary levels. Any particle in focus at this level was timed over a known distance (usually 0·012 cm.) across the graticule first in one direction and then in the other, thus eliminating any slight differences between the two electrodes. At least ten particles were timed in both directions under a constant field strength to give an average velocity, from which the electrophoretic mobility was calculated.

Calibration of the Cell.—The electrophoretic mobility of mammalian erythrocytes is independent of the age, sex, colour, and blood group of the donor. The value of this mobility, which in 0·067M-phosphate buffer solution (pH 7·35) is $1{\cdot}31 \times 10^{-4}$ cm./sec./volt/cm. (Abramson, *J. Gen. Physiol.*, 1929, **12**, 711), was used to calibrate the electrophoresis cell.

Discussion

It has been demonstrated that the electrophoretic technique described above gives reproducible results when applied to bacterial suspensions prepared under standard conditions over a period of 6 months. Further, the mobility of the bacterial culture has remained constant over the same period when grown under the specified conditions. Washed-cell suspensions retained over a period of 24 hours also gave a constant mobility. On the other hand, cultures that have been steamed for 15 minutes showed a marked change in mobility, presumably due to cell disorganisation.

The charge density of the organisms has, however, been shown to be sensitive to changes in the ionic strength of the suspension medium, indicating that extreme care must be exercised to control this factor. Thus, as pointed out by Moyer (*J. Bact.*, 1936, **32**, 433), mobilities determined in water should be regarded as unsatisfactory since here the ionic

strength is an uncontrollable factor. The behaviour of the bacterial particles towards ionic-strength changes follows a curve similar to that expressed in the simple Langmuir adsorption isotherm $\sigma = kc^{1/n}$ where c is the electrolyte concentration, and k and n are constants. This is further borne out by Fig. 3, curve II, which shows a linear relation between log σ and log c; this is a general characteristic of colloidal particles.

The surface is relatively insensitive to changes in hydrogen-ion concentration over a wide range, indicating the absence of protein as a primary surface constituent. The observation that death incurred by treatment with formaldehyde is without effect on the mobility, further supports this inference. Abramson came to a similar conclusion regarding the surface of *Esch. coli*.

It has also been found that no detectable difference exists between young cells, harvested at the end of the growth phase, and those harvested several hours later. This observation suggests that the nature of the surface does not change either during active cell division or during ageing in the growth medium; this aspect is to be considered in more detail in a later paper.

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