252. *Some Physical Investigations of the Behaviour of Bacterial Surfaces. Part II.* The Variation of the Electrophoretic Mobility of* Aerobacter aerogenes *with the Age of the Culture and the Nature of the Culture Medium.*

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

No change in the electrophoretic mobility of *Aerobacter aerogenes* has been detected during growth in a range of completely synthetic media, despite marked differences in cell metabolism. Growth in more complex media, such as meat broth and agar, produces no change in the cell surface as revealed by the constancy of the mobility.

Previous investigations (Part I *) indicated little difference in the nature of the surface of young and old cells. A more detailed study of the electrophoretic mobility during the growth of *Aerobacter aerogenes* under a wide range of conditions is now reported.

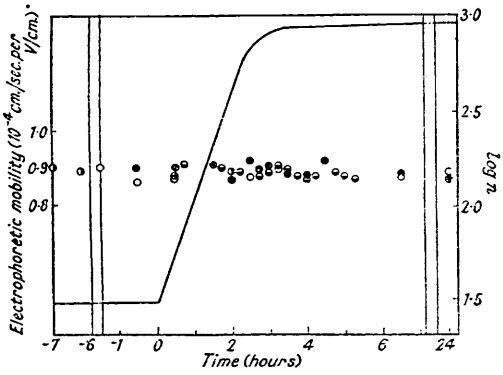
Moyer (*J. Bact.*, 1936, **32**, 433) and Shibley (*J. Exp. Med.*, 1924, **40**, 453) working with different strains of bacteria have found that in all cases young cells have a lower mobility

* Part I, *J.*, 1952, 3340.

than older ones, whilst, on the other hand, Pedlow and Lisse (*J. Bact.*, 1936, **31**, 235) and Buggs and Green (*ibid.*, 1935, **30**, 453) have shown that *Escherichia coli* grown either in peptone or on agar has a constant mobility for up to 10 days' incubation. The variation of the mobility of the *typhoid bacillus* with age is dependent on the growth medium, some media causing a decrease, others an increase (Watrous, *J. Infect. Dis.*, 1937, **60**, 47). Selective removal of lipid and amphoteric materials from the bacterial cell surface has led Dyar (*J. Bact.*, 1948, **56**, 821) to conclude that the surface composition depends on the growth medium.

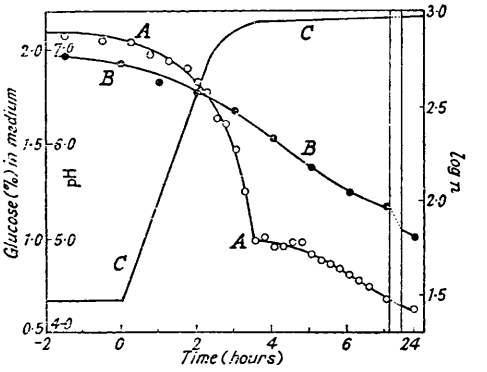
In all these previous experiments, however, growth has occurred in complex media, and the change from one medium to another is made without detailed knowledge of the changes in chemical environment concerned. We have overcome this by using simple synthetic media, their chemical composition being subjected to rigid control. In addition to the electrophoretic mobility a study was made of the differences in the rates of growth, and of hydrogen-ion production and glucose utilisation during growth in these different media, in an attempt to correlate surface changes with changes in certain metabolic functions.

FIG. 1. *Variation of electrophoretic mobility of* Aerobacter aerogenes *during growth in glucose–ammonium sulphate medium.*



(The differently shaded circles represent different experiments; the solid line is the average growth curve.)

FIG. 2. *Variation of* pH *and glucose content of the medium during the growth of* Aerobacter aerogenes.



(*A*, pH *values.* B, *Glucose concentration.* C, *Growth curve.*)

Variation of the Electrophoretic Mobility.—(a) Growth in glucose–ammonium sulphate medium. The relation between the electrophoretic mobility and age for cells grown in the normal medium is shown in Fig. 1. This includes observations made in several independent experiments, and since the length of the lag phase was variable, the graph has been plotted so that the end of the lag phase is zero; *i.e.* observations appearing in the lag are at negative time. The mobility remains constant, within the limits of experimental error, during the

TABLE 1. *Growth in media containing different amino-acids.*

	Electrophoretic mobility ($cm.^2\ sec.^{-1}\ v^{-1} \times 10^4$)					
	Glycine	Alanine	Serine	Aspartic acid	Glutamic acid	Tyrosine
Lag phase	0·91	0·91	0·91	0·87	0·88	0·88
Logarithmic growth phase	0·92 (3) *	0·90 (4)	0·89 (7)	0·88 (6)	0·90 (6)	0·90 (5)
Stationary phase	0·87	0·87	0·87	0·87	0·88	0·87
M.g.t.	120′	48′	60′	108′	84′	108′

* Figures in parentheses show the number of observations made in the growth phase.

complete growth cycle, the average being $0{\cdot}89 \times 10^{-4}$ $cm.^2\ sec.^{-1}\ volt^{-1}$, towards the positive electrode. This result is further supported by our previous observation that the behaviour of old and young cells towards change of ionic strength and pH was sensibly the same.

(*b*) *Growth in glucose amino-acid media.* The following amino-acids were used to give a

representative selection : glycine, alanine, serine, aspartic acid, glutamic acid, and tyrosine. The mobility was constant throughout the growth cycle and numerically the same as in the previous series (Table 1).

(*c*) *Growth in ammonium sulphate media containing various sources of carbon.* In the normal medium a monosaccharide was used, and we considered any possible variations which might accompany growth in a medium containing a disaccharide (sucrose), a polysaccharide (starch), or a simple carbon source (glycerol). In all these experiments, cells adapted for growth in the different media were used and, in addition, cells trained and grown in glucose were used to inoculate a glycerol medium. In the last case the growth curve revealed that initially the organisms grew on the glucose carried over in the inoculum, and later, when this was exhausted, there was an arrest in the growth accompanying the utilisation of a different carbon source. Again the mobility was constant throughout the growth cycle and not significantly different from that recorded in Fig. 1 (Table 2).

TABLE 2. *Growth in media containing different carbon sources.*

	Electrophoretic mobility (cm.^{2} sec.$^{-1}$ v^{-1} × 10^4)				
	Glycerol (untrained)	Glycerol (trained)	Glucose	Sucrose	Starch
Lag phase	0·90	0·89	0·90	0·88	0·89
Logarithmic growth phase	0·90	0·88	0·88	0·90	0·89
	(4)	(5)	(6)	(5)	(4)
Stationary phase	0·90	0·87	0·90	0·87	0·89
M.g.t.	* 42′, 100′	55′	33′	45′	53′

* Composite growth curve.

(*d*) *Complex media.* In an attempt to repeat earlier work, we considered a wider range of growth conditions by the use of a richer medium. The constancy of the electrophoretic mobility of the organism, already demonstrated with amino-acids, was maintained during growth (without aeration at 37°) in a meat broth (Lab-Lemco, Oxoid preparation) both in the presence and in the absence of glucose, and also, on agar slopes (Table 3).

TABLE 3. *Mobility* (cm.^{2} sec.$^{-1}$ v^{-1} × 10^4) *of* Aerobacter aerogenes *during growth in Lab-Lemco medium, in the presence and absence of glucose, and on agar slopes.*

Time (days)	7 hr.	1	2	4	5	7	9	11
Lab-Lemco (+ glucose)	—	0·87	0·90	0·84	—	0·86	—	0·89
Lab-Lemco (− glucose)	—	0·89	0·92	0·88	—	0·89	—	0·92
Agar	0·90	0·91	0·83	—	0·91	—	0·86	—

Variation of Metabolism during Growth in Various Media.—Variations of certain metabolic functions during growth in different media are apparent from the different rates of growth, hydrogen-ion production, and glucose utilisation, and also from previous work on the different enzyme systems in the cell (dehydrogenase, James and Hinshelwood, *Trans. Faraday Soc.*, 1948, **44**, 967; catalase, Cole and Hinshelwood, *ibid.*, 1947, **43**, 266). The pH values and the glucose concentrations [estimated by a modification of the Shaffer–Hartmann method (*J. Biol. Chem.*, 1920, **45**, 365)] at different times during the growth of the organism in the normal medium are plotted in Fig. 2. Similar determinations were made during growth in the other culture media. However, despite differences in the metabolism revealed by these observations the mobility remained constant.

EXPERIMENTAL

The culture of *Aerobacter aerogenes* was grown in a standard culture medium which consisted of 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.

Variation of the Nitrogen and Carbon Sources.—In one series of experiments the ammonium sulphate was replaced by amino-acids in concentrations giving an equivalent supply of nitrogen. In the other series the glucose was replaced by glycerol (7·7 g./l.), sucrose (9·6 g./l.), or starch

(0·75 g./l.). In all cases the growth rate was determined turbidometrically as previously described.

The cells were adapted for growth in each medium by at least 10 previous sub-cultures. 25 Ml. of a 24-hour old culture, suitably trained, were used as the inoculum for a litre of fresh medium. This was aerated at 40° and samples were withdrawn at convenient intervals for the determination of electrophoretic mobility (Part I), pH value, glucose concentration, and bacterial count. Samples withdrawn for mobility determination were centrifuged, washed twice with 0·0067M-phosphate buffer solution at pH 7·03, and finally re-suspended in that menstruum.

Discussion

It has been shown that the electrophoretic mobility of *Aerobacter aerogenes* determined under the given conditions is constant and independent of both the age of the culture and the nature of the culture medium. The individual observations are identical within the specified limits of experimental error and in no case has an observation varied from the mean by an amount comparable with that reported by other workers using different organisms (Moyer and Watrous, *locc. cit.*).

Growth is accompanied by the production of acid (cf. the rapid fall in pH shown in Fig. 2). Such variations would undoubtedly give rise to a change in the ζ-potential (Fig. 4, Part I), but that growth in wide ranges of hydrogen-ion concentration is not accompanied by irreversible changes in the nature of the surface responsible for electrokinetic phenomena is shown by the constancy of the mobility after the organisms had been washed in the phosphate buffer solution. Similarly, both growth intermediates and toxins will be present in the medium during growth, but these also fail to give rise to irreversible changes in the nature of the surface.

Whereas the electrophoretic mobility of the organisms constitutes a satisfactory standard of reference for future work, we have, however, concluded that, at least with reference to *Aerobacter aerogenes*, electrophoretic determinations are unlikely to throw further light on the problems of the bacterial cell surface during growth.

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