The Electrokinetic Properties of *Aerobacter aerogenes*

A COMPARISON OF THE PROPERTIES OF NORMAL AND CRYSTAL VIOLET-TRAINED CELLS

By J. H. B. LOWICK and A. M. JAMES
Department of Chemistry, Chelsea Polytechnic, London, S.W. 3

(*Received* 18 *June* 1956)

In a previous communication (Lowick & James, 1955) we reported marked changes in the electrophoretic behaviour of a strain of *Aerobacter aerogenes* during growth in the presence of crystal violet. The alteration of the surface, as followed electrophoretically, depended on the age of the untrained parent culture used for inoculation; further, it occurred only during actual cell division in the presence of the drug. Once a population of type C (i.e. extreme) behaviour was produced, it was stable on subsequent subculture in both the presence and the absence of the dye. In the present paper we report an investigation of this strain in an attempt to explain these altered electrokinetic properties.

It seemed reasonable that the electrokinetic properties of different strains of bacteria on treatment with suitable reagents, e.g. surface-active agents, under carefully controlled conditions of pH and ionic strength would be mainly dependent on the nature of the surface constituents. Dyar & Ordal (1946) have shown the sensitivity of bacteria of widely different taxonomic groups to treatment with both anionic and cationic surface-active agents. In general these workers found that the presence of sodium tetradecyl sulphate produced no change, or a slight or a marked increase in the electrophoretic mobility. The cationic surface-active agent, cetylpyridinium chloride, always produced the same general pattern of decrease of charge—reversal of charge and finally stabilization of charge. A 'fattened' strain of *Micrococcus aureus*, known to contain a greater amount of fatty substance than the normal strain, was more sensitive to both the anionic and cationic detergents. This was attributed to an increase in the amount of lipid material in the surface of these cells. Later Dyar (1948) described a method of characterizing cell surfaces in which chemical and enzyme treatments were employed to remove parts of the cell surface. In this way both lipid and amphoteric material were detected and removed from the surface of *M. aureus*.

Using the same technique, Douglas (1955) concluded that the absence of any effect of sodium dodecyl sulphate on *Bacillus subtilis* spores showed surface lipid to be absent, whereas with *Bacillus megatherium* spores the increase in mobility in the presence of this detergent indicated the presence of some lipid. Further evidence in support of the difference between the surfaces of these two spores was obtained from a study of the variation of their mobilities in solutions of different pH values, under conditions of constant ionic strength.

In the light of this previous work we considered that the difference of electrophoretic behaviour between the normal cells of *Aero. aerogenes* and those trained to crystal violet must be due to a change of surface constituents. Accordingly, we have investigated the effect of surface-active agents on the mobilities of these two strains. In addition, we have attempted to correlate changes of mobility on treatment with various organic solvents with the fractions extracted, as measured by ultraviolet-absorption spectra.

EXPERIMENTAL

Cultures. Aero. aerogenes was grown at 40° with aeration in a simple synthetic medium containing glucose, potassium dihydrogen phosphate, ammonium sulphate and a small quantity of magnesium sulphate (Lowick & James, 1955). The organisms were freeze-dried from aqueous suspension (rather than from serum suspension, to avoid contamination of the surface with protein) and were resuspended in the buffer solution when required. Cells stored in this way remained viable during the period of observation. This drying technique had no effect on the mobility of either the normal bacteria or those trained to crystal violet.

Cells trained to crystal violet with type C electrokinetic properties were chosen for the detailed study of altered surface characteristics for two reasons: first, these cells with very low mobility values have undergone the greatest change in the surface properties incurred by training to the dye. Secondly, cell populations of this type exhibit a homogeneous distribution of mobilities, enabling an average mobility to be determined (compare type B, Lowick & James, 1955). It is more convenient to follow changes in the average mobility of a strain produced by different chemical treatments than to detect changes in histograms which depict a heterogeneous population (type B).

Electrophoretic measurements. Suspensions for electrophoretic measurements were prepared by shaking a sample of the freeze-dried organisms with mixed phosphate buffer solution (1 part of M/150 KH_2PO_4 to 1 part of M/150 $Na_2HPO_4,12H_2O$) of ionic strength 0·013 and pH 7·00. The technique for determining the mobility and mobility

distribution of cell suspensions has already been described (Lowick & James, 1955; Moyer, 1936). The micro-electrophoresis cell was calibrated with human red-blood cells for which the mobility is 1·31 μ./sec./v/cm. in M/15 phosphate buffer solution, pH 7·35 (Abramson, 1929). Cells of *Aero. aerogenes* have an average mobility of 2·37 $\pm$ 0·04 μ./sec./v/cm. (coefficient of variation, 8%) in phosphate buffer solution of ionic strength 0·013, pH 7·00 (Lowick, Loveday & James, 1956).

RESULTS

Variation of electrophoretic mobility accompanying the first sub-culture in medium containing crystal violet

When *Aero. aerogenes* is first grown in synthetic medium containing 5·6 mg. of crystal violet/l., three different types of mobility distribution can be obtained, according to the age of the parent inoculum: for a parent of age less than 8 hr., the population at the end of the growth phase has a histogram which is typically Gaussian and similar to that for cells grown in drug-free medium (type A). A parent of age 8–36 hr. gives rise to a heterogeneous population with mobility values varying from 0·4 to 2·70 μ./sec./v/cm. (type B). A parent culture over 48 hr. old gives rise to a population of cells whose mobilities have a Gaussian distribution centred round very low mobility values (type C). Fig. 1 shows the three types of behaviour and gives the corrected absolute values for the electrophoretic mobility (Lowick *et al.* 1956). It can be seen, by comparing this diagram with Fig. 2 of our earlier paper (Lowick & James, 1955), that the new values have not affected the mobility distributions, but merely displaced the histograms towards higher values.

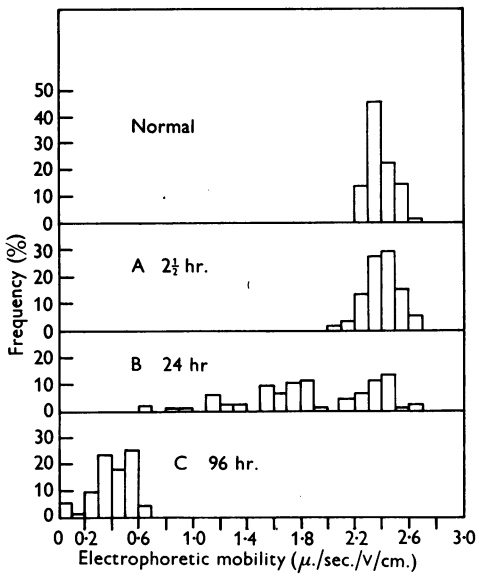

Fig. 1. Effect of the age of the inoculum of untrained cells on the mobility distribution after growth in media containing 5·6 mg. of crystal violet/l. The times 2½, 24 and 96 hr. refer to the age of inoculum; A, B and C refer to the types of behaviour.

Effect of pH *on the mobility of normal and trained cells*

The buffer solutions used in the range pH 2·8–6·9 were prepared from M/300 citric acid and M/150 disodium hydrogen phosphate adjusted to constant ionic strength (0·02) by the addition of sodium chloride. The extreme acid solution was 0·01 N hydrochloric acid, also at constant ionic strength (0·02).

The mobility–pH curves for the two strains are shown in Fig. 2. The response of the two types is considerably different; the normal cells give a curve similar to that obtained for inert surfaces, and the trained cells a curve (with an isoelectric point at pH 3·5) similar to that of some protein and lipid surfaces (Price & Lewis, 1933; Abramson, 1933).

The act of suspension of the normal cells in acid solution caused no surface damage, since when washed after this treatment and resuspended in normal phosphate buffer solution at pH 7·00 their mobility returned to the normal value. The mobility

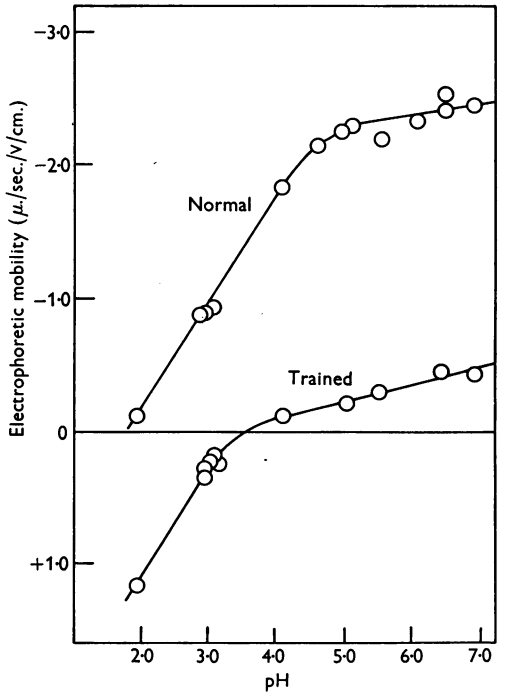

Fig. 2. Mobility–pH curves of normal and crystal violet-trained cells of *Aero. aerogenes*.

Table 1. *Effect of washing on the electrophoretic mobility of the organisms after treatment at low* pH *values*

Treatment of cells	pH of test soln.	Ionic strength	Mobility (μ./sec./v/cm.) Normal	Trained
Suspended in M/300 phosphate buffer (control)	7·00	0·013	−2·37*	−0·39
Suspended in 0·01 N-HCl	1·94	0·02	−0·12	+1·17
Suspended in 0·01 N-HCl, washed and suspended in M/300 phosphate buffer	7·00	0·013	−2·44	−0·54
Suspended in citrate–phosphate buffer (control)	6·89	0·02	−2·41	−0·31
Suspended in citrate–phosphate buffer	2·93	0·02	−0·87	+0·28
Suspended in citrate–phosphate buffer at pH 2·93, washed and suspended in citrate–phosphate buffer	6·89	0·02	−2·43	−0·43

* The sign indicates the charge carried.

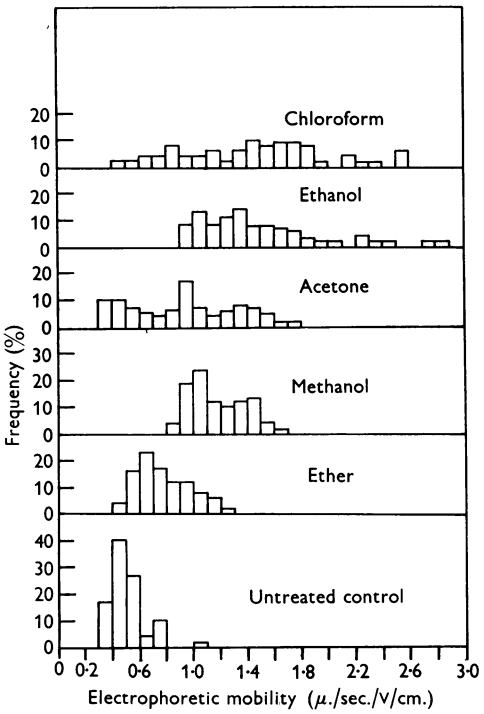

Fig. 3. Effect of solvents on the mobility distribution of trained cells of *Aero. aerogenes*.

of the trained cells, however, after similar treatment, returned to a more negative value than the control (Table 1).

Effect of organic solvents on Aerobacter aerogenes

A known weight of the dried bacteria (generally 0·01 g.) was shaken for 4 days with 40 ml. of ether, methanol, ethanol, acetone or chloroform. The organisms were removed on the centrifuge and the supernatant liquid was saved (see below). The cells were then washed twice and resuspended in the normal phosphate buffer solution.

Electrophoretic mobility. The mobility distribution of normal cells was unaltered after shaking with any of these solvents. When the same treatment was applied to trained cells, however, the histograms were greatly altered (Fig. 3). Ether and methanol caused a slight increase in the average mobilities of these cells, but the distribution ranges were not significantly increased. Acetone and ethanol produced considerable increases in mobility values and the final histograms showed a heterogeneous population. A very widespread heterogeneous population was obtained on treatment with chloroform or a mixture of chloroform and methanol, and many mobility values were in the same range as those for normal untrained cells. The final histogram obtained with any solvent was independent of the time of shaking, for periods of 2–96 hr.

Absorption spectra of the extracts. After the cells had been treated with the different solvents, the supernatant liquid was evaporated to dryness. The residue was then dissolved in absolute alcohol and the ultraviolet-absorption spectrum determined with a Unicam SP. 500 spectrophotometer. When the same weight of bacteria was shaken for different periods, ranging from 2 to 96 hr., it was observed that the absorption increased with shaking time until a maximum was attained. Extracts of both types of bacteria with ethanol, ether and acetone gave absorption spectra with similar intensities when equal amounts of bacteria were taken. Methanol was, however, a much poorer solvent.

The absorption spectra of the extracts from the two types of cells were essentially the same in the ultraviolet range (Fig. 4), consisting of an intense peak at 225–230 mμ., presumably due to the presence of lipid-like material (Miller, Brown & Burr, 1938), and a lesser peak at 250–252 mμ. due to the presence of purines or pyrimidines (possibly nucleic acids). A comparison of the relative intensities of these absorption spectra shows that the trained cells contain approximately the same amount of these materials as an equal weight of normal cells. The trained cells are considerably

smaller than the normal cells; 1 mg. of normal cells contains $32{\cdot}5 \times 10^8$ organisms, whereas 1 mg. of trained cells contains $81{\cdot}5 \times 10^8$ organisms.

Crystal violet is soluble in all the solvents used (except ether) and was therefore extracted by this shaking procedure. This was shown by the additional peak in the absorption spectra of the extract of trained cells at 580 mμ. due to the dye. From the intensity of this peak the amount of crystal violet extracted from 1 mg. of bacteria was calculated as being 470 μg. After such extraction the bacteria were colourless.

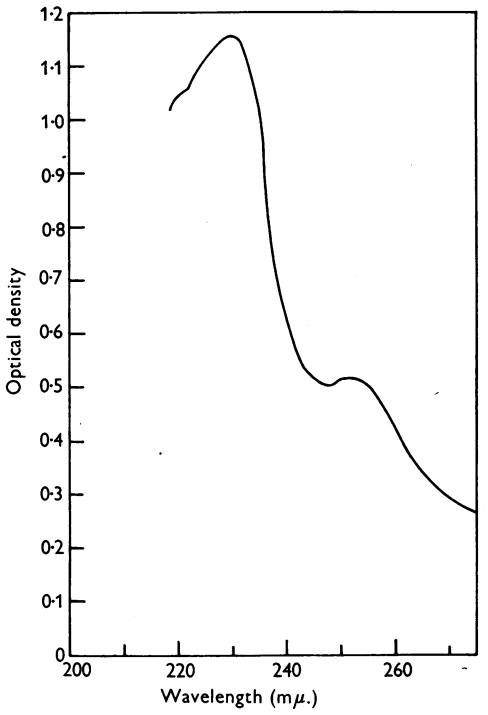

Fig. 4. Absorption spectrum in absolute ethanol of the ether extract of normal cells of *Aero. aerogenes*. 15 mg. of bacteria was shaken for 4 days with 30 ml. of ether, the solvent was evaporated and the residue dissolved in 200 ml. of absolute ethanol.

Although the solvents have generally removed the dye from the trained organism, their mobility distribution never returns to that characteristic of untrained cells.

Effect of surface-active agents on the mobility of normal and trained cells

To characterize the surface of normal and trained bacteria their mobilities were determined in buffer solution containing a surface-active agent. Cetylpyridinium chloride was chosen as the cationic and sodium dodecyl sulphate as the anionic surface-active agent. Concentrations up to 0·02 M of each of these were prepared in buffer solution and mixed with the bacterial suspension in buffer solution to give the appropriate concentration of the detergent. The final concentration of bacteria in each suspension was 3×10^8 organisms/ml.

The normal cells were insensitive to sodium dodecyl sulphate, there being no change in the electrophoretic mobility at concentrations up to mM. In cetylpyridinium chloride, however, the mobility became less negative at a concentration of about 50 μM, and at higher concentrations the cells actually possessed a positive charge. The cells of the trained strain were much more sensitive to sodium dodecyl sulphate, the mobility becoming more negative at 0·1 mM. At a concentration of mM the mobility showed an increase of 800% over the value in the absence of surface-active agent (Table 2). In addition the trained cells were slightly more sensitive to cetylpyridinium chloride than the normal cells, the curve crossing the concentration axis at a much lower concentration (60 μM for trained compared with 600 μM for normal) (Fig. 5).

The physical effect of these surface-active agents on the surface was investigated by washing the organisms, after treatment with the detergent, twice with phosphate buffer solution and then determining their mobility in this medium. The results (Table 3) show that this washing does not

Table 2. *Effect of surface-active agents on the electrophoretic mobility of* Aerobacter aerogenes

Strain and treatment	Surface-active agent	Electrophoretic mobility (μ./sec./v/cm.) Control	Electrophoretic mobility (μ./sec./v/cm.) In mM	Change from control (%)	Concn. at which departure from control occurs
Normal	SDS†	−2·35	−2·39	2	Above mM
Trained	SDS	−0·46	−4·18	810	0·1 mM
Normal treated with ether	SDS	−2·31	−2·52	9	0·5 mM
Trained treated with ether	SDS	−0·88	−5·50	525	0·1 mM
Trained treated with $CHCl_3$ + methanol	SDS	−2·32*	−4·18	80	0·1 mM
Normal	CPC	−2·36	+0·80	134	50 μM
Trained	CPC	−0·48	+0·89	286	10 μM

* Population heterogeneous.

† In this and subsequent tables SDS represents sodium dodecyl sulphate and CPC represents cetylpyridinium chloride.

Table 3. *Effect of washing on the electrophoretic mobility of organisms after treatment with surface-active agents*

Treatment of the cells	Electrophoretic mobility (μ./sec./v/cm.) Normal	Trained
Suspended in M/300 phosphate (control)	−2·48	−0·39
Suspended in buffer + mM SDS	—*	−3·8
Suspended in buffer + mM SDS, washed and suspended in M/300 phosphate	—*	−4·10
Suspended in buffer + mM CPC	+0·35	+1·23
Suspended in buffer + mM CPC, washed and suspended in M/300 phosphate	−1·90	−0·66

* Not tested owing to the lack of effect of SDS on normal cells.

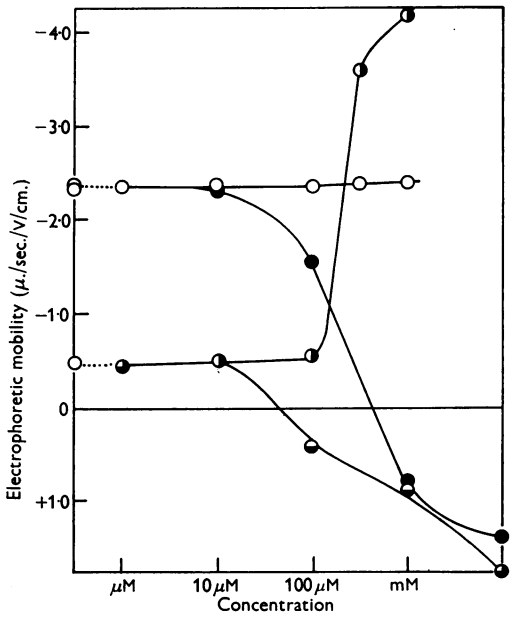

Fig. 5. Electrophoretic mobility of normal and trained cells of *Aero. aerogenes* in the presence of surface-active agents. ○, Normal cells in SDS; ●, normal cells in CPC; ◑, trained cells in SDS; ◒, trained cells in CPC.

completely remove the cetylpyridinium chloride from the surface of normal cells. The effect of sodium dodecyl sulphate on the trained cells is irreversible, possibly due to the formation of a stable lipid–detergent complex. Treatment of the trained cells with cetylpyridinium chloride followed by washing results in an increased negative mobility value over the control.

The effect of sodium dodecyl sulphate was tested on bacteria which had been treated with ether for 4 days. Normal cells were now slightly more sensitive, and the trained cells were less sensitive, to this reagent, but still showed a considerable increase in their mobility with mM sodium dodecyl sulphate (Table 2). Trained bacteria treated with other solvents gave similarly high mobility values in the presence of mM sodium dodecyl sulphate. The distributions which were widespread in the controls (e.g. chloroform–methanol) became homogeneous in the presence of the surface-active agent.

The differences in behaviour of normal and trained strains towards surface-active agents can most probably be attributed to a higher content of lipid-like material in the surface of the trained organisms. This material is partly extracted by the ether.

Effect of lipase on the surface properties of normal and trained cells

A sample of each of the freeze-dried strains (0·01 g.) suspended in 25 ml. of phosphate buffer solution at pH 7·00 was treated with lipase (Sol-media Ltd.; final concentration, 1 % w/v) at 40° for about 18 hr. Controls, in which the enzyme had previously been inactivated by heat treatment, were always included. The suspension was centrifuged and the organisms were washed twice with phosphate buffer solution and finally resuspended in that menstruum. The mobility distribution of each suspension was determined in the presence and absence of mM sodium dodecyl sulphate.

The average values (Table 4) show that the active lipase is without effect on the electrophoretic mobility of the normal cells in the presence and absence of sodium dodecyl sulphate. In contrast, lipase brings about a marked increase in the mobility of the trained cells to a value characteristic of normal untrained and untreated cells. Further, these cells are no longer sensitive to the presence of sodium dodecyl sulphate. Inactivated lipase is without effect on the mobility of normal cells. It increases the average mobility of trained cells by some 40 % and reduces their sensitivity to sodium dodecyl sulphate by approximately the same amount. These changes are, however, negligible when compared with those resulting from the use of the active enzyme.

Table 4. *Effect of lipase on the electrophoretic mobility of normal and trained cells of* Aerobacter aerogenes

	Electrophoretic mobility (μ./sec./v/cm.)	
	Normal cells	Trained cells
Untreated cells (controls)		
Suspended in buffer solution	2·40	0·45
Suspended in buffer solution + mM SDS	2·39	4·44
Percentage increase in the presence of SDS	0	890
Cells treated with active lipase		
Suspended in buffer solution	2·45	2·31
Suspended in buffer solution + mM SDS	2·29	2·34
Percentage increase in the presence of SDS	−6*	0
Cells treated with inactive lipase		
Suspended in buffer solution	2·43	0·63
Suspended in buffer solution + mM SDS	2·31	4·00
Percentage increase in the presence of SDS	−5*	540

* These values have little significance, as they are only just outside the limits of experimental error.

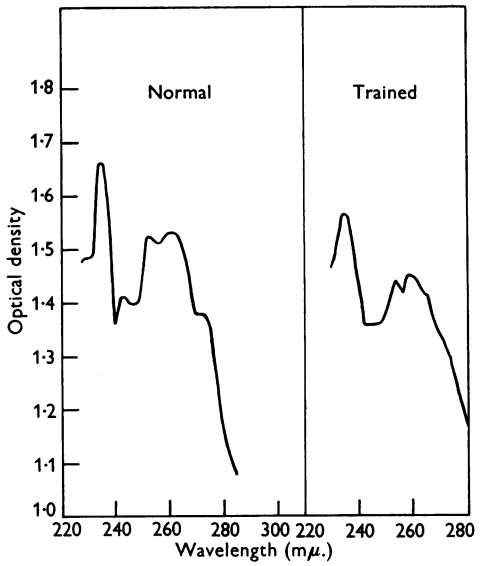

Fig. 6. Ultraviolet-absorption spectra of aqueous suspensions of normal and trained cells of *Aero. aerogenes*.

The histograms obtained from the trained cells after treatment with the active enzyme tended to be bimodal in form, there being a small percentage (about 10%) of the population with the original low mobility value. A similar enzymic treatment of killed cells (2 hr. at 55°) with active lipase produced the same result as for viable cells.

Absorption spectra of aqueous suspensions of normal and trained cells

The ultraviolet-absorption spectra of cells of the normal and trained strains were determined in aqueous suspension by the method of Shibata, Benson & Calvin (1954) (Fig. 6). The absorption spectra are similar, maxima occurring at 235, 254 and 260 mμ. The maxima at 260 mμ. may be due to deoxypentosenucleic acid. The other maxima probably correspond to those observed on the solvent extracts of the bacteria.

DISCUSSION

The electrophoretic behaviour of cells of *Aerobacter aerogenes* trained to crystal violet is markedly different from that of untrained cells. It is evident from the colour of the trained organisms that the dye has been absorbed. The amount absorbed on the surface from the low dye concentration in the growth medium is insufficient to produce the change in mobility (Lowick & James, 1955). The dye is not removed by washing with phosphate buffer solution, and the mobility is unchanged by this treatment (Lowick & James, 1955). The differences in the electrokinetic properties are due to an alteration in the nature of the cell surface and not to the effect of the dye combined with surface components of the trained organism. This is supported by two independent observations: First, repeated and prolonged growth of the trained cells (type C behaviour) in the absence of the dye caused no alteration in the mobility distribution (Lowick & James, 1955). The large increase of surface area which occurred during growth in the absence of the dye precludes the possibility of any significant amount of crystal violet remaining on the surface. The organisms were colourless and no dye was ever detected by examination of ethanolic extracts of the organisms. Secondly, organic solvents (except ether) extract the dye from the trained organisms, but the mobility distribution, although in some cases undergoing a change, never returns to the value for normal untrained cells. A further type of training to crystal violet-type A (reported pre-

viously) is worth recalling. Cells of this training series, although containing considerable amounts of the dye, possessed a mobility distribution which was indistinguishable from that of untrained cells.

Thus it can be concluded that the outermost surface layers of normal and trained cells (type C) are of different chemical or physical structure, or both. This fact is amply illustrated by the large quantitative differences observed in the effect of pH and surface-active agents on the mobility values (Figs. 2, 5). Some idea of the nature of these two cell surfaces can be obtained by comparing these results with those for model surfaces, e.g. polysaccharide, protein and lipoprotein, obtained by previous workers.

For the cells of *Aero. aerogenes* trained to crystal violet, the form of the mobility–pH curve (with an effective isoelectric point at pH 3·5) is similar to those of protein (Abramson, 1933) and lecithin (Price & Lewis, 1933) surfaces—ionogenic, amphoteric—and to those of some sterols (Moyer, 1934). In addition these cells show a marked increase in mobility in the presence of the anionic surface-active agent sodium dodecyl sulphate. Droplets of a hydrocarbon and of lipids acquire a greater negative charge in the presence of these surface-active agents, a phenomenon attributed to the solution of the hydrocarbon ends of the detergent molecules in their surface with the negatively charged polar groups oriented outwards (Dyar, 1948). Thus the material in the cell surface responsible for the increase in mobility is most probably lipid. Treatment of these trained cells with organic solvents, particularly ether, makes them slightly less sensitive to sodium dodecyl sulphate. This may be due to the removal of some of the fatty lipid material from the surface, resulting in a surface in which less of the sodium dodecyl sulphate can dissolve.

In contrast the mobility–pH curve for the untrained cells, which is essentially independent of pH above pH 5·0, is quite unlike those for protein or lipid surfaces. Rather does it resemble in form the curve for an inert surface (Abramson, Moyer & Gorin, 1942), and those for the smooth forms of bacteria which almost certainly have a polysaccharide surface. The anionic surface-active agent has no effect on the mobility [cf. cellulose and glass particles (Dyar, 1948)], but the cationic agent (cetylpyridinium chloride) results in neutralization and reversal of charge, as would be expected.

Further evidence about the nature of the surfaces was obtained from a study of the effect of lipase on the cells. This enzyme has a pronounced effect on the mobility of trained cells. After treatment, these cells had a mobility characteristic of untrained cells; further, like untrained cells, they were insensitive to sodium dodecyl sulphate. In contrast, lipase had no effect on the mobility of normal bacteria either in phosphate buffer suspension or in the presence of sodium dodecyl sulphate, suggesting the absence of lipid in the surface of these cells.

Bacterial cell walls, particularly those of the Gram-negative bacteria, are known to contain a considerable amount of lipid material (Salton, 1956). The ultraviolet-absorption spectra of the normal and trained organisms and the solvent extracts are consistent with this observation. The spectra show that both strains contain approximately the same amounts (w/w) of the different materials, but as the trained are of approximately one-third the size of the normal cells it would appear that there would be less material (lipid, nucleic acids, etc.) per cell. This lipid in the trained cells is exposed and is readily detected. This is in contrast to that in the normal cell, where the lipid probably constitutes the deeper layers in the cell wall. Treatment of normal cells with solvents does not affect their mobility, but renders them slightly more sensitive to sodium dodecyl sulphate, indicating the removal of an outer layer, or layers, and the consequent laying bare of (at least) a portion of the lipid material.

We believe, from the evidence reported, that cells of *Aero. aerogenes* which have been trained to grow in the presence of crystal violet contain lipid material exposed on the surface, whereas the surface of the normal cells is predominantly polysaccharide. Further work on the effect of specific enzymes on the electrical properties of these surfaces is in progress.

SUMMARY

1. The mobility–pH curve of the normal strain of *Aerobacter aerogenes* is similar to that of a polysaccharide surface; that of the trained strain is similar to the curve obtained for protein surfaces.

2. The greater sensitivity of the trained cells towards both anionic and cationic surface-active agents suggests that a greater amount of lipid material is present in their surface.

3. Treatment with organic solvents does not affect the mobility distribution of normal cells, but histograms obtained from the trained cells were altered to varying degrees. Ultraviolet-absorption spectra of the extracts from normal and trained cells were similar.

4. Extraction with solvents renders the normal cells slightly more sensitive and the trained cells less sensitive to sodium dodecyl sulphate.

5. Lipase has no effect on the mobility of normal cells, but increases the value for trained cells to that of a normal untrained strain. The trained cells, so treated, are insensitive to sodium dodecyl sulphate.

6. The experimental observations, discussed in the light of model surfaces of known structure, are consistent with the hypothesis that the cells of *Aero. aerogenes* trained to crystal violet have a surface which is lipid in nature, in contrast to that of the normal cells, which is polysaccharide.

The authors acknowledge with thanks continued financial support from the British Empire Cancer Campaign.

REFERENCES

Abramson, H. A. (1929). *J. gen. Physiol.* **12**, 711.
Abramson, H. A. (1933). *Cold Spr. Harb. Symp. quant. Biol.* **1**, 39.
Abramson, H. A., Moyer, L. S. & Gorin, M. H. (1942). *Electrophoresis of Proteins*, chaps. 1 and 4. New York: Reinhold Publ. Corp.
Douglas, H. W. (1955). *Trans. Faraday Soc.* **51**, 146.
Dyar, M. T. (1948). *J. Bact.* **56**, 821.
Dyar, M. T. & Ordal, E. J. (1946). *J. Bact.* **51**, 149.
Lowick, J. H. B. & James, A. M. (1955). *Biochim. biophys. Acta*, **17**, 424.
Lowick, J. H. B., Loveday, D. E. E. & James, A. M. (1956). *Biochim. biophys. Acta*, **20**, 392.
Miller, E. S., Brown, W. R. & Burr, G. O. (1938). *Oil & Soap*, **15**, 62.
Moyer, L. S. (1934). *Biochem. Z.* **273**, 122.
Moyer, L. S. (1936). *J. Bact.* **31**, 531.
Price, C. W. & Lewis, W. C. M. (1933). *Trans. Faraday Soc.* **29**, 775.
Salton, M. R. J. (1956). *Bacterial Anatomy, 6th Symp. Soc. gen. Microbiol.* p. 81.
Shibata, K., Benson, A. A. & Calvin, M. (1954). *Biochim. biophys. Acta*, **15**, 461.

The Metabolism in the Rat of Naturally Occurring (+)-14-Methylhexadecanoic Acid

By MURIEL LIVINGSTON, MURIEL E. BELL
Nutrition Research Department, University of Otago, Medical School, Dunedin

F. B. SHORLAND, T. GERSON and R. P. HANSEN
Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand

(*Received* 6 *September* 1956)

The recent isolation of branched-chain fatty acids from animal fats (Hansen, Shorland & Cooke, 1952) has aroused interest in the fate of such acids as dietary components. Hitherto, feeding experiments with branched-chain acids were carried out mainly in relation to the effects of such acids present in synthetic fat. Thus Appel, Böhm, Keil & Schiller (1947) fed glycerides of the higher branched-chain fatty acids with methyl, ethyl or propyl side chains to goats, whereas Weitzel (1951) fed dogs with α-substituted myristic and stearic acid triglycerides. The metabolism of the acids was followed by determining the nature of the ether-soluble breakdown products in the urine. In addition, without giving experimental evidence, Appel *et al.* (1947) stated that 13·6 % of the branched-chain acids fed in synthetic fat to goats was found in the depot fats. Weitzel (1951) estimated that 90 % of the α-substituted myristic and stearic acids fed to dogs was metabolized, but he did not determine the storage of these acids in the body.

In the present study the branched-chain acid (+)-14-methylhexadecanoic acid, naturally occurring up to approximately 0·5 % in ruminant fat, has been fed to rats to determine its storage, excretion, breakdown and effect on growth.

Because of the difficulty of isolation of traces of branched-chain fatty acids from animal fats, metabolic studies without the use of isotopically labelled compounds are not normally feasible. However, as the acid used in this work is optically active it has been possible to utilize this property as a sensitive method of estimation.

EXPERIMENTAL

Rats of the Wistar strain maintained as an inbred colony for the past 22 years were used. They were fed on the experimental diet from weaning (40–50 g.) until they reached 160 g.

The 'fat-free' basal diet used was similar to that described by Longenecker (1939). It consisted of (%): casein, 20; corn starch, 60; sucrose, 9; salt mixture, 4; agar-agar, 2; dried yeast, 5. The diet was computed to contain not more than 1·36 % of fat.

The animals were divided into groups, each with two subgroups consisting of five bucks and five does. Group A (controls) were given the basal 'fat-free' diet; group B received the same diet but with a supplement of 0·1 g. of C_{17} *anteiso*-acid (from a preparation obtained by Hansen *et al.* 1952)/rat/week. Altogether seven such supplements were given, after which the bucks returned to the basal diet for 3 weeks, and the does for 7 weeks. The animals were then killed The faeces of bucks and does were collected for 48 hr.