THE BACTERIAL SURFACE

I. EFFECT OF CETYL-TRIMETHYL-AMMONIUM BROMIDE ON THE ELECTROPHORETIC MOBILITY OF CERTAIN GRAM-POSITIVE BACTERIA

by

KENNETH McQUILLEN

Medical Research Council Unit for Chemical Microbiology, Biochemical Laboratory, University of Cambridge (England)

The microscopic method of electrophoresis is a useful tool in the elucidation of the changes which occur, subsequent to various treatments, at the interface between bacteria and their external environment. It has the advantage of giving some measure of the differences from one cell to the next in a supposedly homogeneous collection of organisms since observations are made on individual bacteria. The electrophoretic mobility of a particle in an electric field is determined both by the nature of the suspending medium and by the net surface density of charge of the particle. This latter parameter is an integral of all the components of what for the present purpose is termed the surface. Here is an immediate limitation of the technique but, as DYAR[1] has pointed out, it can to some extent be overcome by the concomitant use of chemical or enzymic reagents which combine with or displace specific constituents of the surface. At the same time information may be gained as to the mechanism of interaction between the reagent and the bacterial cell. BRADBURY AND JORDAN[2] used a micro-electrophoresis method to analyse the effect of *p*-aminobenzoic acid and sulphonamides on the surface charge of *Escherichia coli*. Active substances showed a similar pattern of the time course of charge change, while inactive compounds behaved differently. It was concluded that the active substances associate with the organisms *via* their aromatic amino-groups.

The effects of surface-active substances on bacteria have been studied by a variety of techniques and many workers have suggested that the primary site of action is at the cell surface. BAKER, HARRISON AND MILLER[3] examined the effect of anionic and cationic detergents on the metabolic properties of bacteria and proposed that disorganisation of the cell membrane occurs first, followed by denaturation of certain essential proteins. HOTCHKISS[4] reported that tyrocidin and various ionic detergents cause a rapid release of soluble cell constituents from staphylococci and streptococci and concluded that the first effect of these substances is an alteration in the cellular membrane or surface. GALE AND TAYLOR[5] showed that tyrocidin, phenol and various other surface-active substances including the cationic detergent, cetyl-trimethyl-ammonium bromide (CTAB), cause the loss to the medium of the free amino-acids from inside Gram-positive

References p. 471.

bacteria. Changes in the appearance of the surface of cells treated in this way may be seen in the electron micrographs taken by MITCHELL AND CROWE[6] and others (MITCHELL AND MCQUILLEN[7]; SALTON[8]). Studies on the effect of surface-active agents on the electrophoretic mobility of bacteria have been carried out by DYAR AND ORDAL[9] and DYAR[1]. Bacteria from widely different taxonomic groups, on treatment with a cationic detergent (1–1000 μM cetylpyridinium chloride), were found to exhibit a similar pattern of behaviour: in the absence of the compound the cells were negatively charged, and as the concentration of detergent was raised the charge decreased, became neutralized, reversed and finally became stabilized at some positive value. The differences between species related to initial charge and the concentrations at which the various changes occurred rather than to the general picture. The charge acquired in a given concentration of detergent did not change with time nor was it affected by the suspension density of bacteria.

In the course of the present investigations the effects of CTAB on the electrophoretic mobilities of certain bacteria have been studied. Some of the findings differ from those reported by DYAR AND ORDAL[9] and are of interest in relation to results from the use of other techniques.

METHODS

Bacteria: Escherichia coli H (isolated in this laboratory), *Streptococcus faecalis ST* (N.T.C.C. no. 6782), and two strains of *Staphylococcus aureus* (*Micrococcus pyogenes var. aureus*) DUNCAN and 21.5 (the latter kindly given by Mr M. R. J. SALTON) were grown in a medium containing casein digest, 1% glucose and 0.1% marmite for 16 h at 37° C.

Reagents: All experiments were carried out at 25° C in phosphate buffer of ionic strength 0.01 and p_H 7.0. Solutions of cetyl-trimethyl-ammonium bromide were made up in this buffer and ranged in concentration from 0.36–90.0 μg/ml, that is, from 1–250 μM.

Micro-electrophoresis: A horizontal, cylindrical cell of internal diameter 2.55 mm and length 20 cm was used and observations were made in the stationary layer, the position of which was determined after the manner of HENRY[10]. Usually platinum electrodes were used but in some experiments these were replaced by silver/silver chloride electrodes. The potential gradient down the cell was between 5 and 10 volts/cm and was calculated from the specific resistance of the bacterial suspension (measured in the electrophoresis cell) and the current as recommended by MOYER[11]. The cell was designed and made by Mr P. D. MITCHELL and full details of the technique will be published shortly (MITCHELL AND MCQUILLEN[12]).

The standard procedure was to harvest the organisms from the growth medium, wash three times on the centrifuge with phosphate buffer, and re-suspend in buffered CTAB at a final dry-weight concentration of bacteria of 0.1 mg/ml. The suspension was then run into the micro-electrophoresis cell and observations of the mobility begun within 2 mins of the addition of CTAB to the cells. 20 bacteria were timed over a distance of 100 microns with the current passing first in one and then in the reverse direction. A set of observations was completed in 10 mins, and from the average velocity and the potential gradient the electrophoretic mobility was calculated. This is expressed as microns/sec. per volt/cm, and at any given p_H and salt concentration is directly proportional to the surface density of charge of the bacteria.

Adsorption Isotherms: The method of SALTON AND ALEXANDER[13] for estimation of ionic detergents was adapted for determination of the amount of CTAB taken up by *Staph. aureus*. Suspensions containing 0.1 mg/ml *Staph. aureus* were prepared in buffered solutions of CTAB as for the electrophoresis experiments. After 5 mins the cells were centrifuged down and the residual CTAB in the supernatant estimated by titration with sodium cetyl sulphate in the presence of pinacyanol bromide as indicator. The uptake of CTAB by the bacteria was obtained by difference.

RESULTS

Escherichia coli

Fig. 1 shows the changes in the electrophoretic mobility of *Esch. coli*, a typical

References p. 471.

Gram-negative organism, on treatment with various concentrations of CTAB. In the absence of the detergent the organisms bear a negative charge which is gradually reduced as the CTAB concentration is increased until at about 200 μM it is neutralized and the bacteria consequently have little or no movement in an electric field. At higher concentrations the charge is reversed.

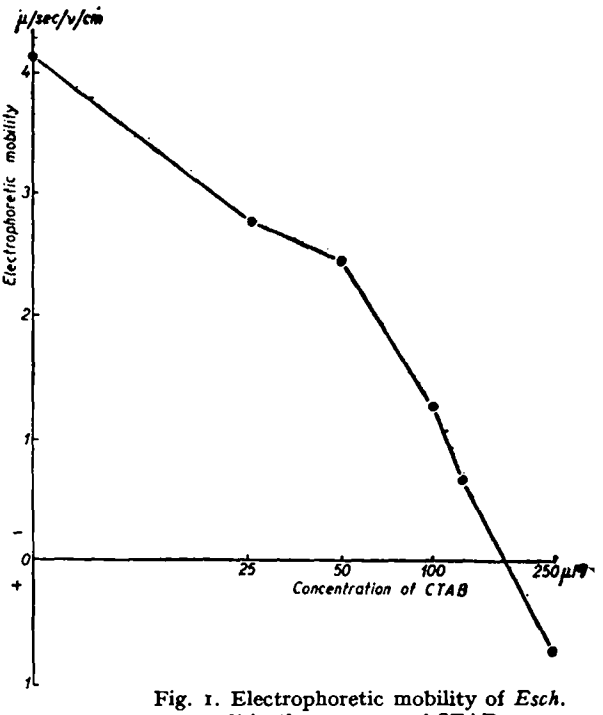


Fig. 1. Electrophoretic mobility of *Esch. coli* in the presence of CTAB

Staphylococcus aureus

The behaviour of *Staph. aureus* was found to be quite different from that of *Esch. coli*. Fig. 2 illustrates typical curves for strains of this organism. With increasing concentrations of CTAB, after a small initial fall in mobility there is a sharp discontinuity beginning at about 50 μM with a rise to a maximum negative mobility in the presence of 100 μM CTAB. Thereafter the mobility decreases until in 250 μM detergent it is almost zero.

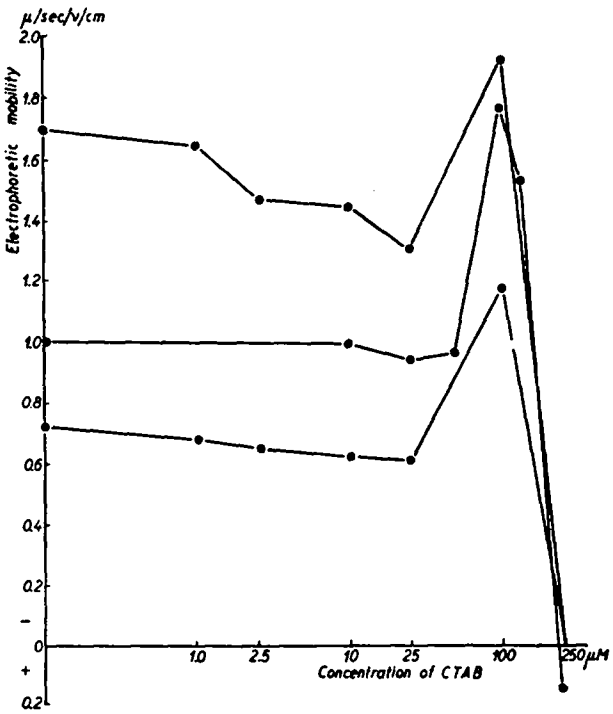


Fig. 2. Electrophoretic mobility of strains of *Staph. aureus* in the presence of CTAB

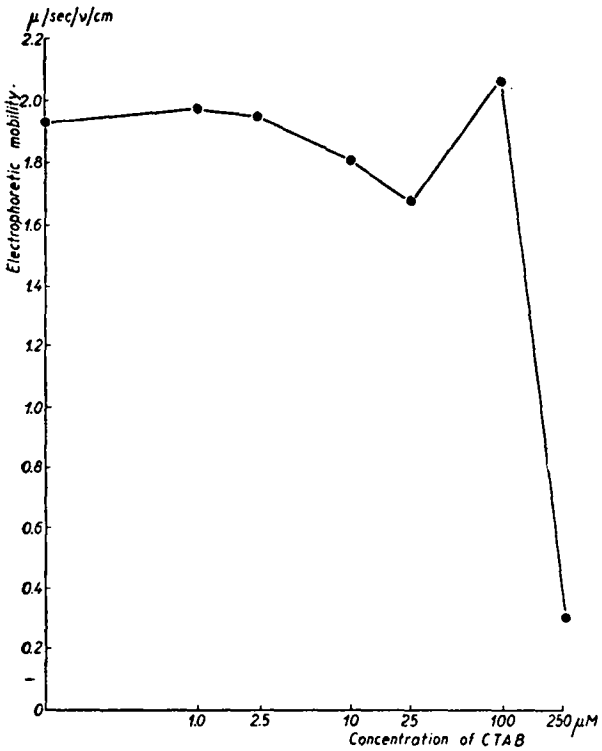
Streptococcus faecalis

This behaviour of *Staph. aureus* differs from that reported by DYAR AND ORDAL[9], and it consequently became of importance to determine whether another typical member of the Gram-positive cocci would behave similarly. Results obtained with *Strep. faecalis* are shown in Fig. 3; the pattern of behaviour is substantially the same as that of *Staph. aureus* even to the occurrence of the maximum mobility in the presence of 100 μM CTAB.

Distribution of Mobilities

The micro-electrophoresis cell used in these studies was checked

References p. 471.



using human red blood cells which in $M/15$ phosphate buffer p_H 7.35 have a reproducible mobility of 1.31 microns/sec per volt/cm (ABRAMSON[14]). The mean of 78 determinations with various field strengths was 1.33 microns/sec per volt/cm with a standard deviation of 0.049 microns/sec per volt/cm (3.7%). The bacteria so far studied have a scatter of mobilities of about the same magnitude as red blood cells, and single observations on 20 organisms, each travelling first in one and then in the reverse direction, give a mean value with a standard error of *c.* 1%. It has been found however, in agreement with STEARNS AND ROEPKE[15], that dissociating strains of bacteria may show a much greater scatter of mobilities.

Fig. 3. Electrophoretic mobility of *Strep. faecalis* in the presence of CTAB

In the course of the experiments with CTAB-treated bacteria it was noticed that in certain concentrations of the surface-active substance the scatter was greater than in the controls in buffer alone. By plotting histograms of the distribution of mobilities in different concentrations of CTAB (Fig. 4) it was found that *Staph. aureus* showed a gradually increasing scatter of mobilities as the CTAB concentration increased from 0–25 μM; this was concurrent with a small decrease in mean mobility. In 100 μM CTAB, however, the cell population was again more or less homogeneous as regards charge, which had at this stage increased to the maximum

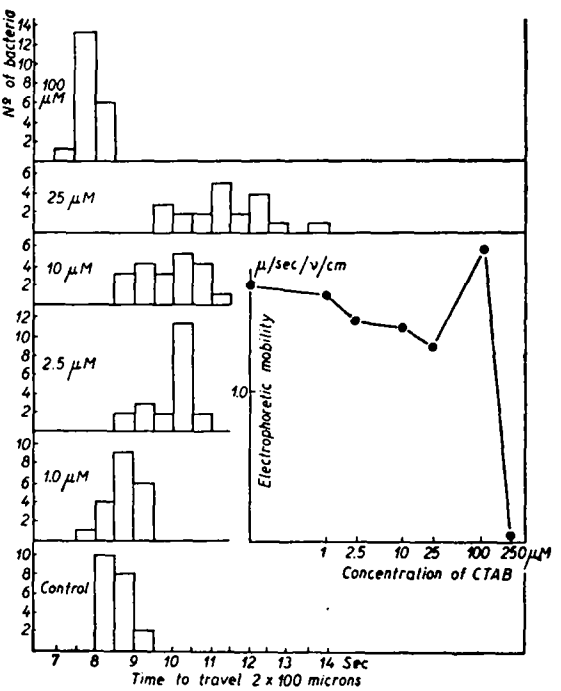


Fig. 4. Distribution of mobilities of *Staph. aureus* in various concentrations of CTAB
Inset: Electrophoretic mobility of *Staph. aureus* in CTAB

References p. 471.

mentioned above. These findings indicate that in the more dilute solutions of detergent the individual organisms are not all affected to the same extent.

Effect of Suspension Density and Reaction Time

DYAR AND ORDAL[9] found that the suspension density of bacteria (within limits reasonable for observation) and the time of contact with the detergent had no effect on the charge acquired in a given concentration of surface-active agent. The present findings differ in both respects.

The technique has been to standardise the suspension density at 0.1 mg/ml in all experiments, since alteration of this caused changes in the electrophoretic mobility observed in a given concentration of detergent. For example, *Strep. faecalis* 0.1 mg/ml in 250 μM CTAB had approximately zero mobility, but at a suspension density of 0.25 mg/ml the mobility was 1.38 microns/sec per volt/cm. This correlates with the finding of GALE AND TAYLOR[5] that there is an almost stoichiometric relation between the number of cells lysed and the amounts of CTAB present. The results of PUTNAM AND NEURATH[16] on electrophoretic analysis of protein-detergent mixtures which indicate the "all-or-none" character of complex formation are also of interest in this connection. Data presented below show that uptake of CTAB by bacteria can markedly alter the resulting bulk concentration of detergent; at saturation $^2/_3$ of the added CTAB is taken up by the cells, while at lower concentrations a proportion less than $^1/_3$ is left in the supernatant. From these considerations the number of organisms present would be expected to affect the amount of CTAB adsorbed by each cell and hence influence the mobility of the bacteria.

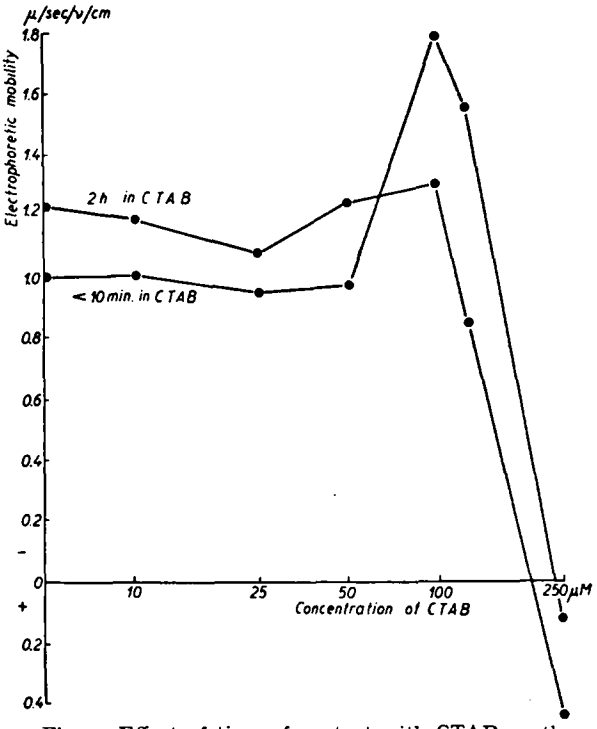


Fig. 5. Effect of time of contact with CTAB on the electrophoretic mobility of *Staph. aureus*

The change of charge brought about by adding CTAB to either staphylococci or streptococci is affected by the time of contact with the surface-active substance. In the case of streptococci a drift in the mobility values is apparent after 10 mins in the presence of detergent while Fig. 5 shows the changes which occur after *Staph. aureus* has been in contact with CTAB for 2 h at 25° C. SALTON[17] has studied the release of phosphates, free amino-acids and purines and pyrimidines from *Staph. aureus* 21.5 treated with CTAB and has found the rapid release occurring during the first 20 mins to be followed by a slower process which is still incomplete after some hours.

Adsorption of CTAB by Staphylococcus aureus

The extension of SALTON AND ALEXANDER'S[13] method to the estimation of such

References p. 471.

small quantities of CTAB as a few μg/ml appears to be satisfactory. The results for the uptake of CTAB by *Staph. aureus* obtained by this method are plotted in Fig. 6. The curve has the form expected for an adsorption isotherm and at saturation the bacteria have taken up an amount of CTAB equal to about 30% of their dry-weight. If both uptake of CTAB and resulting electrophoretic mobility are plotted against concentration of CTAB added (Fig. 7), it can be seen that the peak in the mobility curve corresponds approximately with saturation of the organisms with detergent.

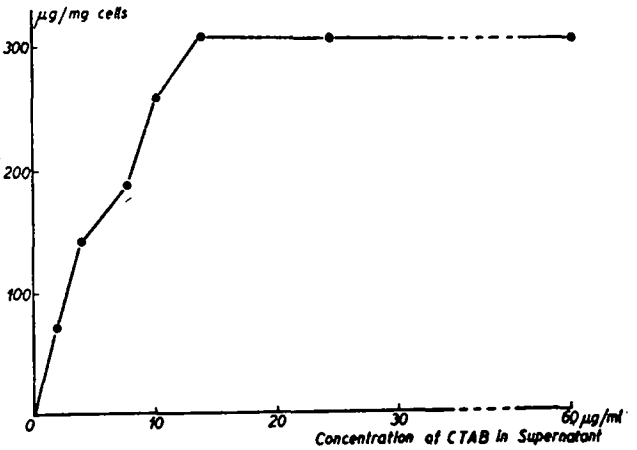


Fig. 6. Adsorption isotherm of uptake of CTAB by *Staph. aureus*

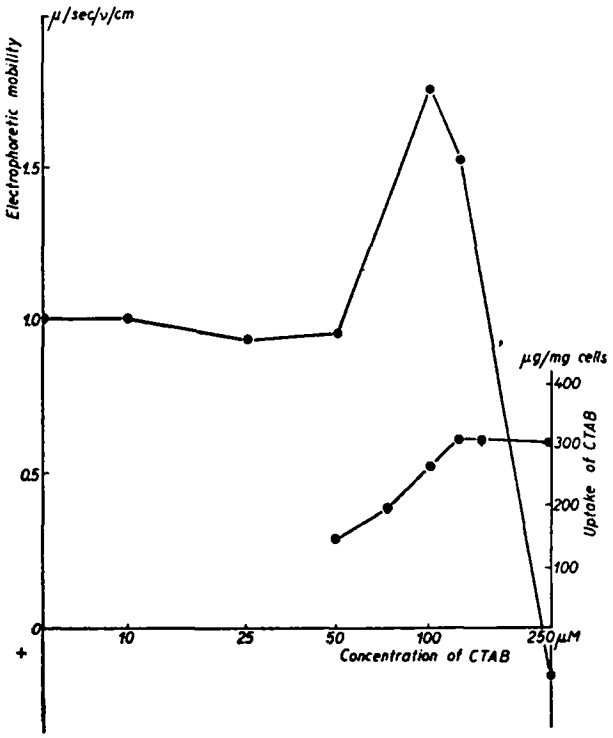


Fig. 7. Uptake of CTAB by *Staph. aureus* and resulting electrophoretic mobility

DISCUSSION

Esch. coli after treatment with CTAB is found to behave in a manner comparable to that described by DYAR AND ORDAL[9] using another cationic detergent. It seems likely that an adequate explanation of the results is to be found in the supposition that increasing quantities of the detergent cation combine with the negatively-charged organism resulting in reduction and eventual reversal of the net negative charge. The behaviour of the Gram-positive *Staph. aureus* and *Strep. faecalis* is quite different and allows of no such simple interpretation. The peak in the mobility curves of these organisms is coincident with saturation of the bacteria by CTAB (Fig. 7) and with a return to a relatively homogeneous distribution of mobilities among the individual cells (Fig. 4). There are several possible explanations of this maximum. It may be that this concentration of detergent strips off some external material from the bacterial cell, exposing underlying material which is inherently more negatively charged. Alternatively, a re-orientation of the surface layers and/or of the complexes between detergent

References p. 471.

and cell-surface constituents may reveal more negatively-charged groups and thus increase the negative mobility. A third possibility is that material released from the bacterial cells by the action of the surface-active agent may adsorb back on to the surface of the organisms and increase their net negative charge. In this connection it is interesting that SALTON[17, 18] has found that CTAB causes a release of 260 mμ-absorbing material from *Staph. aureus* and *Strep. faecalis*. The amount of this material appearing in the supernatant increases, falls to a minimum and rises again as the CTAB concentration is increased. Significantly, the minimum occurs in all cases at a CTAB/bacterial dry-weight ratio of about the same value as that for the peak in the electrophoresis curves obtained in the present study. If 260 mμ-absorbing material is released from bacteria by certain concentrations of CTAB and then taken up at higher concentrations only to be again released when the concentration is still further raised, then this would argue strongly in favour of the third possibility mentioned above, that the peak in the mobility curves is due to adsorption back on to the surface. It should be noted that the secondary release of the adsorbed material takes place without further uptake of CTAB by the bacteria which are already saturated.

The data compiled as Fig. 4 showing the distribution of mobilities make it clear that in the lower concentrations of detergent there is a lack of uniformity of change of charge among the individual bacteria comprising the suspension. This suggests that attachment of a certain amount of detergent to a given bacterial cell may facilitate further uptake. This may well be the case since the strong VAN DER WAALS' forces between adjacent non-polar parts of the detergent molecules (16-carbon chains in the case of CTAB) will tend to anchor further molecules once some are attached to the bacteria.

There are not yet enough data to decide at which sites in the bacterial cell the surface-active substances are adsorbed. Some is almost certainly attached to the surface but at saturation the amount of CTAB bound by the cell would, if spread as a closely-packed monolayer, occupy an area greater than that of the surface of the cell. It is conceivable that a series of concentric shells of detergent is built up round the bacterial cell with polar heads facing alternately inwards and outwards, but the electrophoresis data do not afford any confirmation of this picture. The uptake of CTAB by *Staph. aureus* at saturation amounts to about $^1/_3$ of its own dry-weight, and it is known that proteins form stable complexes with about the same proportion of detergent. CTAB may, therefore, be adsorbed at sites within the bacterial cytoplasm as well as at the surface (c.f. MCMULLEN AND ALEXANDER[19]). Such sites might be expected to be about as frequent in bacterial substance as in protein. VALKO AND DIBBLEE[20], for example, found that towards dimethyl-dodecyl-benzyl-ammonium chloride, *Esch. coli* had about the same equivalent weight as many animal proteins.

ACKNOWLEDGEMENT

The author is indebted to Dr E. F. GALE for his encouragement and help and to the Medical Research Council for a personal grant. He is also grateful to Mr P. D. MITCHELL for advice and to Mr M. R. J. SALTON for samples of CTAB, sodium cetyl sulphate and pinacyanol bromide as well as for the strain 21.5 of *Staph. aureus*.

References p. 471.

SUMMARY

1. A study has been made of the electrophoretic mobility of *Esch. coli*, *Staph. aureus*, and *Strep. faecalis* treated with the cationic detergent, cetyl-trimethyl-ammonium bromide (CTAB), at concentrations 1–250 μM in phosphate buffer p_H 7.0 and ionic strength 0.01.

2. *Esch. coli* shows a progressive decrease in negative mobility as the CTAB concentration is increased, the charge being neutralized in *c.* 200 μM detergent and reversed at higher concentrations.

3. The two Gram-positive organisms behave differently; after a small initial decrease in mobility there is an abrupt rise at concentrations above 50 μM, to a maximum in 100 μM CTAB, followed by a decrease such that these organisms bear only a small negative or positive charge in 250 μM detergent.

4. The maximum is coincident with saturation of the cells with CTAB and at this concentration the bacteria again all bear approximately the same charge, although in more dilute CTAB solutions there is a considerable scatter of mobilities among the individual bacterial cells.

5. Both the suspension density of bacteria and the time of contact influence the charge acquired by organisms in a given concentration of detergent.

6. Data concerning the adsorption of CTAB by *Staph. aureus* are also presented.

RÉSUMÉ

1. Nous avons étudié la mobilité électrophorétique de *Esch. coli*, de *Staph. aureus* et de *Strep. faecalis* sous l'influence d'un détergent cationique, le bromure de cétyl-triméthyl-ammonium (CTAB) à des degrés de concentrations de 1 à 250 μM en tampon phosphate de p_H 7.0 et de force ionique 0.01.

2. *Esch. coli* révèle une diminution progressive de mobilité négative à mesure que la concentration de CTAB est augmentée, la charge étant neutralisée par un détergent d'environ 200 μM et inversée par des concentrations plus fortes.

3. Les deux organismes Gram-positifs réagissent différemment; la mobilité commence par diminuer légèrement, mais au-dessus de 50 μM elle augmente brusquement et finit par atteindre un maximum dans 100 μM de CTAB, maximum qui est suivit par une diminution telle que ces organismes ne portent qu'une petite charge négative ou positive dans le détergent 250 μM.

4. Le maximum coïncide avec la saturation en CTAB des cellules et à cette concentration les bactéries portent de nouveau à peu près la même charge, bien que dans des solutions plus diluées les mobilités se dispersent considérablement entre les cellules bactériennes individuelles.

5. La charge acquise par les organismes varie selon la densité de suspension des bactéries et aussi selon la durée de leur présence dans une concentration donnée du détergent.

6. On trouvera aussi des données sur l'adsorption de CTAB par *Staph. aureus*.

ZUSAMMENFASSUNG

1. Die electrophoretische Mobilität von *Esch. coli*, *Staph. aureus* und *Strep. faecalis* wurde eingehend untersucht wenn dieselben mit dem kationischen Dispersionsmittel, Cetyl-trimethyl-ammonium-bromid (CTAB), bei Konzentrationen von 1 bis 250 μM in Phosphat-pufferlösungen von p_H 7.0 und Ionenkonzentration 0.01 behandelt wurden.

2. Die negative Mobilität von *Esch. coli* nimmt allmählich mit zunehmender CTAB Konzentration ab, und die Ladung wird in *ca.* 200 μM Dispersionsmittel neutralisiert, und bei stärkeren Konzentrationen sogar umgekehrt.

3. Die beiden Gram-positiven Organismen reagieren auf andere Weise; nach einer anfänglichen geringen Abnahme der Mobilität zeigt sich ein plötzlicher Anstieg bei einer Konzentration von mehr als 50 μM bis zu einem Maximum bei 100 μM CTAB, dem ein Abstieg bis zu einer geringen negativen oder positiven Ladung der Organismen bei 250 μM Dispersionsmittel folgt.

4. Das Maximum fällt mit der Sättigung der Zellen an CTAB zusammen, und bei dieser Konzentration haben die Bakterien wieder alle ungefähr dieselbe Ladung, obwohl in verdünnteren CTAB-Lösungen eine beträchtliche Streuung der Mobilitäten der einzelnen Bakterienzellen vorkommt.

5. Die Suspensionsdichte der Bakterien sowohl als auch die Berührungsdauer beeinflussen die Ladung, die von den Organismen in einer gegebenen Konzentration des Dispersionsmittel erlangt wird.

6. Die Resultate von Untersuchungen der Adsorption des CTAB durch *Staph. aureus* werden ebenfalls beschrieben.

References p. 471.

REFERENCES

[1] M. T. DYAR, *J. Bact.*, 56 (1948) 821.
[2] F. R. BRADBURY AND D. O. JORDAN, *Biochem. J.*, 36 (1942) 287.
[3] Z. BAKER, R. W. HARRISON, AND B. F. MILLER, *J. Exptl Med.*, 73 (1941) 249; 74 (1941) 611, 621.
[4] R. D. HOTCHKISS, *Ann. N.Y. Acad. Sci.*, 46 (6) (1946) 479.
[5] E. F. GALE AND E. S. TAYLOR, *J. Gen. Microbiol.*, 1 (1947) 77.
[6] P. D. MITCHELL AND G. R. CROWE, *J. Gen. Microbiol.*, 1 (1947) 85.
[7] P. D. MITCHELL AND K. MCQUILLEN, unpublished observations (1948).
[8] M. R. J. SALTON, unpublished observations (1949).
[9] M. T. DYAR AND E. J. ORDAL, *J. Bact.*, 51 (1946) 149.
[10] D. C. HENRY, *J. Chem. Soc.*, (1938) p. 997.
[11] L. S. MOYER, *J. Bact.*, 31 (1936) 531.
[12] P. D. MITCHELL AND K. MCQUILLEN, in preparation (1950).
[13] M. R. J. SALTON AND A. E. ALEXANDER, *Research*, 2 (1949) 247.
[14] H. A. ABRAMSON, *J. Gen. Physiol.*, 12 (1929) 711.
[15] T. W. STEARNS AND M. H. ROEPKE, *J. Bact.*, 42 (1941) 745.
[16] F. W. PUTNAM AND H. NEURATH, *J. Biol. Chem.*, 159 (1945) 195.
[17] M. R. J. SALTON, *J. Gen. Microbiol.*, in press.
[18] M. R. J. SALTON, personal communication (1949).
[19] A. I. MCMULLEN AND A. E. ALEXANDER, *Surface Chemistry*, (1949) p. 309. Joint Faraday Society and Société de Chémie Physique Discussion.
[20] E. I. VALKO AND D. DIBBLEE, (1944) mentioned in E. I. VALKO, *Ann. N.Y. Acad. Sci.*, 46(6) (1946) 476.

Received December 2nd, 1949