THE BACTERIAL SURFACE

II. EFFECT OF URANYL CHLORIDE ON THE ELECTROPHORETIC MOBILITY OF BACTERIA

by

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INTRODUCTION

In the course of studies on agglutination of bacteria by various agents BECHOLD[1] remarked on the effect of uranyl salts and reported some approximate results of electrophoresis experiments. BOOY[2] found that uranium caused inhibition of yeast glucose metabolism and this has recently been investigated in detail by BARRON, MUNTZ AND GASVODA[3] and by ROTHSTEIN AND LARRABEE[4]. BARRON, MUNTZ AND GASVODA[3] found that the oxidation of glucose by yeast was 91% inhibited by 20 μM-uranyl nitrate and that the inhibition was reversed by addition of phosphate. Since cell-free enzyme preparations were not reactivated by phosphate to the same extent the authors suggested that uranium combines with the protein part of the cell membrane thus altering its permeability. The uptake of uranium at saturation of the cells amounted to 0.2 μg/mg dry weight of yeast and assuming 1 mg $= 7.2 \cdot 10^7$ cells, this corresponds to $c.\,7 \cdot 10^6$ atoms of uranium per yeast cell. Oxidations of other substrates by *Escherichia coli* and gonococci were inhibited by 1000 μM-uranyl nitrate and the inhibition completely or partially reversed by 10 mM-phosphate. ROTHSTEIN AND MEIER[5] using isotopic phosphorus (^{32}P) demonstrated the presence of a number of phosphatases at the surface of yeast cells. ROTHSTEIN AND LARRABEE[4] found that yeast cells placed in 46 μM-uranyl nitrate took up almost half of the uranium within two minutes. Evidence was presented that a highly undissociated complex of uranium is formed at the surface of the cells, and in a later paper (ROTHSTEIN, FRENKEL AND LARRABEE[6]) it was shown that the dissociation constant of the uranium-"active group" complex is about $2 \cdot 10^{-7}$ and that the total number of such groups per cell is about $4.5 \cdot 10^7$. The "active groups" are necessary for glucose metabolism, and complex formation results in inhibition which can be overcome by addition of phosphate.

In view of these findings on yeast and bacteria it was considered to be of interest to investigate the effects of a uranyl salt on the electrophoretic mobility of bacteria. Since the uranyl ion forms complexes with such groups as carboxyl, amino, hydroxyl and carbonyl it should alter the surface charge of bacteria if combination with such groups occurs near the surface of the organisms. The present communication reports results obtained with *Staphylococcus aureus*, *Streptococcus faecalis* and *Escherichia coli*.

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METHODS

Bacteria

The organisms used in these studies were *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) Duncan, *Streptococcus faecalis* ST (N.T.C.C. no. 6782) and two strains of *Escherichia coli*, H (a strain isolated in this Department) and 21.9 (kindly provided by Mr M. R. J. SALTON). These organisms were grown for 16 h at 37° C in a medium containing tryptic digest of casein, 1% glucose and 0.1% marmite.

Reagents

Sodium acetate buffers p_H 4.7 and ionic strengths ranging from 0.0001 to 0.01 were used for the electrophoresis experiments. Uranyl chloride solutions 1–1000 μM were made up in acetate buffers of ionic strengths 0.001 and 0.01.

Micro-electrophoresis

All experiments were carried out at 25° C in a horizontal, cylindrical cell 20 cm long and 2.55 mm diameter with platinum or silver/silver chloride electrodes. This micro-electrophoresis cell was designed and made by Mr P. D. MITCHELL. The standard procedure was to harvest the organisms from the growth medium, wash three times on the centrifuge with appropriate acetate buffer and resuspend in buffered uranyl chloride for a standard time. The suspension was then either run directly into the electrophoresis cell or the organisms were centrifuged down and washed before resuspension in buffer for determination of the mobility. 20 bacterial cells were timed over a distance of 100 microns in a field of about 10 volts/cm with the current passing in one direction and again with the current reversed. The specific resistance of the bacterial suspension was determined in the electrophoresis cell and observations of the velocity of migration were made at the stationary level (HENRY[7]).

The electrophoretic mobility is expressed as microns/second per volt/cm and is calculated from the mean velocity of the bacteria, the specific resistance of the suspension and the current. The standard error is about 1% of the mean value of the mobility.

Under the conditions employed in these studies the bacteria all bear a negative charge and the surface density of charge, σ, can be calculated from the mobility data (ABRAMSON[8]).

For a uni-univalent electrolyte (as sodium acetate):

$$\sigma = 2\sqrt{\frac{N D k T}{2000\,\pi} \cdot \mu} \cdot \sinh \frac{\zeta \varepsilon}{2kT}$$

where ζ, the zeta potential $= \frac{4\pi\eta}{D} \cdot v$

and N = Avogadro's number.
D = Dielectric constant.
k = Boltzmann's constant.
T = Absolute temperature.
η = Viscosity of suspension.
ε = Charge on electron.
v = Electrophoretic mobility.
μ = Ionic strength.

At 25° C assuming D and η have the values for water:

σ = 738,000 sinh $v/4\sqrt{\mu}$ electronic charges/sq. micron when v is expressed in microns/sec per volt/cm.

RESULTS

In a preliminary experiment a sample of washed *Esch. coli* H was divided into two parts, one of which was suspended in distilled water and the other in 1000 μM-uranyl chloride, the suspension density being 1 mg dry weight of bacteria per ml in each case. After 5 min the organisms were centrifuged down and resuspended in acetate buffer μ = 0.001. The mobility of the control was 4.25 μ/sec per volt/cm while that of the uranyl chloride-treated sample was 1.91 μ/sec per volt/cm. A similar experiment was carried out on six samples of *Esch. coli* H, 1–6 (see Table I). Samples 1 and 2 were

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suspended in acetate buffer $\mu = 0.001$, samples 3–6 in 100 μM-uranyl chloride in acetate buffer, the suspension density in all cases being 1 mg dry weight of cells/ml. After 5 min the samples were centrifuged down; sample 4 was washed once with buffer; sample 5 was washed twice with buffer; while sample 6 was washed first with sodium dihydrogen phosphate $\mu = 0.01$ and then twice with acetate buffer. All six samples were resuspended in acetate buffer for mobility determinations.

TABLE I

EFFECT OF 100 μM-URANYL CHLORIDE ON *Esch. coli* H (1 mg/ml). MOBILITIES DETERMINED IN ACETATE BUFFER $\mu = 0.001$, pH = 4.7

Sample	Uranyl Chloride 100 μM	Washing	Mobility μ/sec/volt/cm
1	—	—	5.37
2	—	—	5.41
3	+	—	3.60
4	+	Acetate × 1	3.77
5	+	Acetate × 2	3.84
6	+	Phosphate then acetate × 2	5.04

It is evident that low concentrations of uranyl chloride decrease the negative charge of *Esch. coli* and that washing with acetate buffer has only a small reversing effect. Washing with phosphate, however, restores the charge to almost its original value.

The effects of varying concentrations of uranyl chloride on different organisms was studied by treating washed bacteria with 1, 10, 50, 100, 500 and 1000 μM reagent in acetate buffer $\mu = 0.001$. After 5 min the cells were spun out, washed once with buffer and resuspended in buffer for mobility determinations. Fig. 1 shows the changes in mobility of *Esch. coli* and *Staph. aureus* as a result of such treatment. The higher concentrations of uranyl chloride cause an appreciable fall in the mobility although, in most cases, the lower concentrations cause a small increase in negative charge determined after the organisms are washed (but see below the results obtained in the presence of uranyl chloride *i.e.* without washing). The effect of altering the suspension density of *Esch. coli* H from 1 mg/ml to 0.35 mg/ml is also illustrated in Fig. 1. Apparently the relative amounts of uranyl chloride and bacteria are more important than the absolute concentration of reagent (*cf.* the effects of cationic detergents, McQUILLEN[9]).

The behaviour of *Strep. faecalis* ST after treatment with similar concentrations of uranyl chloride is quite different and the results of two independent experiments are shown in Fig. 2. Concentrations up to 1000 μM-uranyl chloride cause an increase in the negative charge of these organisms. Again the proportion of reagent to bacteria appears to be important rather than the absolute concentration. The data in Table II are further confirmation of this point. The mobility of a 10 mg/ml suspension after treatment with a certain concentration of uranyl chloride is the same as that of a 1 mg/ml suspension treated with $^1/_{10}$ the concentration of reagent.

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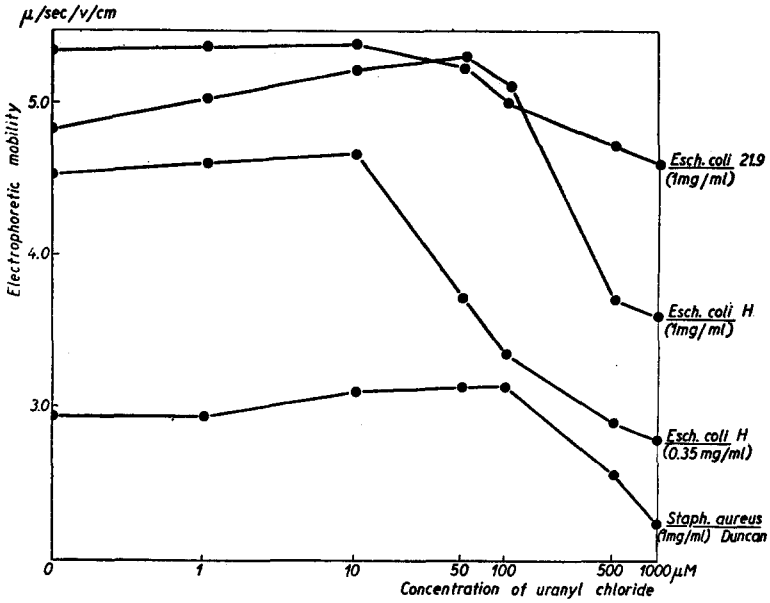

Fig. 1. Effect of Uranyl chloride on Electrophoretic Mobility of *Esch. coli* and *Staph. aureus*

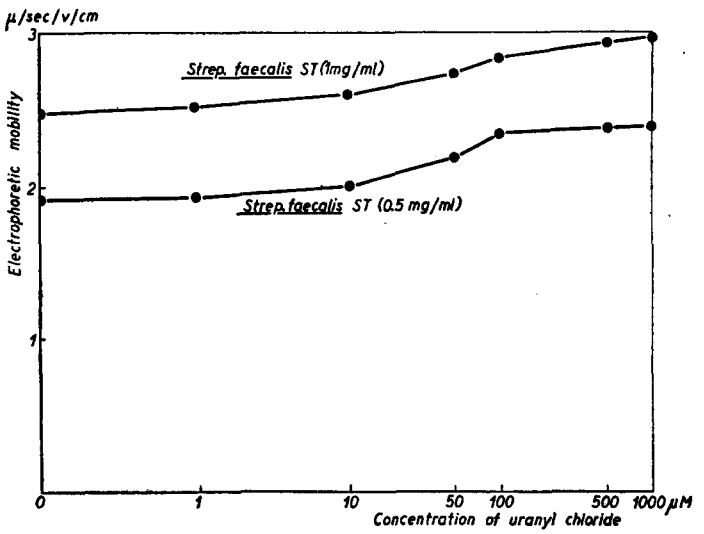

Fig. 2. Effect of Uranyl Chloride on Electrophoretic Mobility of *Strep. faecalis*

TABLE II

Strep. faecalis ST TREATED FOR 5 min WITH URANYL CHLORIDE IN ACETATE BUFFER μ = 0.001, SPUN DOWN, WASHED ONCE WITH BUFFER AND RESUSPENDED IN BUFFER

Suspension Density mg dry wt./ml	Uranyl Chloride μM	Mobility μ/sec/volt/cm
1	—	2.50
1	10	2.58
1	100	2.80
10	100	2.58

Table III shows that treatment of *Esch. coli* H for 5 min or 5 h with uranyl chloride results in approximately the same electrophoretic mobility.

TABLE III

Esch. coli H (1 mg/ml) TREATED WITH URANYL CHLORIDE IN ACETATE BUFFER μ = 0.001 FOR 5 min AND 5 h. CELLS SPUN DOWN, WASHED ONCE WITH BUFFER AND RESUSPENDED IN BUFFER

Uranyl Chloride μM	Time min	Mobility μ/sec/volt/cm
1	5	4.64
1	300	4.68
100	5	3.39
100	300	3.35
1000	5	2.81
1000	300	2.90
Control	—	4.53

The influence of phosphate in reversing the effect of uranyl chloride is indicated by the results in Table IV. Addition of phosphate 5 min after treatment with uranyl chloride and at 10 × the concentration causes almost complete nullification of the uranium effect on both *Esch. coli* where this is a decrease in negative charge and *Strep. faecalis* where the effect is an increase. Aggregation and flocculation of the bacteria were observed in concentrations greater than 50 μM-uranyl chloride, even in the case of *Strep. faecalis*, but the clumps dispersed on washing and resuspension in buffer so that electrophoresis measurements could be made on discrete bacterial cells. Addition of phosphate to uranyl chloride-treated *Staph. aureus*, however, resulted in clumps which were not always readily dispersed and the reversing effect of phosphate could not be demonstrated reliably in the case of this organism.

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TABLE IV

EFFECT OF PHOSPHATE IN REVERSING URANYL CHLORIDE ACTION. ORGANISMS TREATED FOR 5 min WITH URANYL CHLORIDE IN ACETATE BUFFER μ = 0.001, THEN LEFT FOR A FURTHER 5 min WITH OR WITHOUT ADDITION OF PHOSPHATE. CELLS SPUN DOWN, WASHED ONCE WITH ACETATE BUFFER AND RESUSPENDED IN ACETATE BUFFER

Organism	Uranyl Chloride μM	Sodium Dihydrogen Phosphate mM	Mobility μ/sec/volt/cm
Esch. coli H (1 mg/ml)	—	—	4.53
	1000	—	2.81
	1000	10	4.44
Strep. faecalis ST (0.5 mg/ml)	—	—	1.92
	1000	—	2.38
	1000	10	1.96
Strep. faecalis ST (1 mg/ml)	—	—	2.50
	100	—	2.80
	100	1	2.66
	1000	—	2.92
	1000	10	2.64

IONIC STRENGTH EFFECTS

In the work so far described all measurements were made in acetate buffer of ionic strength 0.001. When an experiment was carried out on *Strep. faecalis* using acetate buffer of ionic strength 0.01 it was found that the mobility was changed by less than 5% even after treatment with 10,000 μM-uranyl chloride (Table V).

TABLE V

Strep. faecalis ST (1 mg/ml) TREATED FOR 5 min WITH URANYL CHLORIDE IN ACETATE BUFFER μ = 0.01, SPUN DOWN, WASHED ONCE WITH BUFFER AND RESUSPENDED IN BUFFER

Uranyl Chloride μM	Mobility μ/sec/volt/cm
—	2.02
1	2.00
10	1.99
100	2.04
1000	2.05
10,000	2.08

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In order to determine whether or not the effect of treatment with the reagent in the more concentrated buffer was demonstrable by subsequent determination of the mobility in buffer of lower ionic strength, duplicate samples of *Strep. faecalis* ST, 1 mg/ml, were treated with uranyl chloride in acetate buffer μ = 0.01 and after 5 min, one set was spun down, washed and resuspended in this same buffer, while the other set was washed and resuspended in buffer of ionic strength 0.001. The results obtained are shown in Fig. 3 and it is clear that the effects of the treatment are much more apparent when the mobilities are determined in the buffer of lower ionic strength.

Similar investigations were extended to a wider range of ionic strengths and to the three bacterial species under consideration here. The suspension density was 1 mg/ml throughout and the washed organisms were treated with 1000 μM-uranyl chloride in acetate buffer μ = 0.001 for 5 min. After spinning down, samples of the cells were

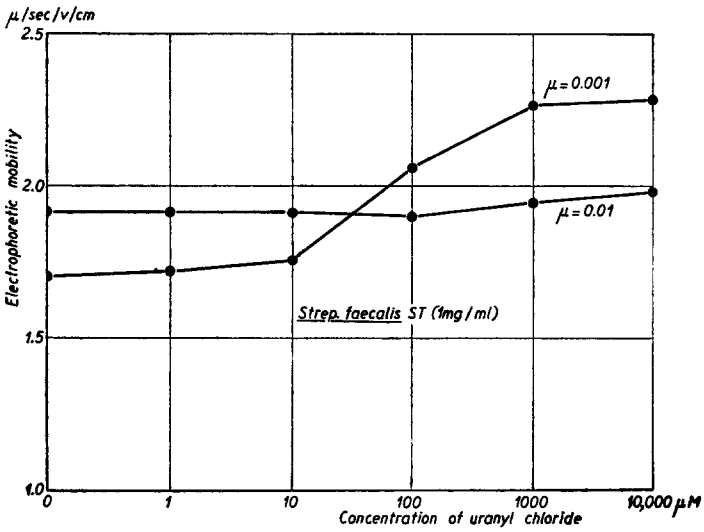

Fig. 3. Effect of Ionic Strength on Electrophoretic Mobility of *Strep. faecalis* treated with Uranyl Chloride

washed once with acetate buffers of ionic strengths 0.01, 0.002, 0.001, 0.0005, 0.0002, and 0.0001 and then resuspended in the same buffers. Control samples were treated in the same way with omission of the uranyl chloride treatment. The results are shown in Figs. 4–6. *Strep. faecalis* ST (Fig. 4) and *Staph. aureus* Duncan (Fig. 5) have the same mobility in buffer of ionic strength 0.01 whether or not they are treated with uranyl chloride but at lower ionic strengths an increasing difference in mobility is apparent. It will be noticed, however, that whereas the charge of *Staph. aureus* is decreased, that of *Strep. faecalis* is increased by treatment with uranyl chloride. The behaviour of *Esch. coli* is different (Fig. 6). The change in mobility caused by uranyl chloride is considerable in the concentrated buffers and decreases at lower ionic strengths.

The surface density of charge obtained from the equation:

σ = 738,000 sinh $v/4\sqrt{\mu}$ electronic charges/sq. micron was calculated from the mobility data in Figs. 4, 5 and 6 and the derived results are plotted in Figs. 7, 8 and 9.

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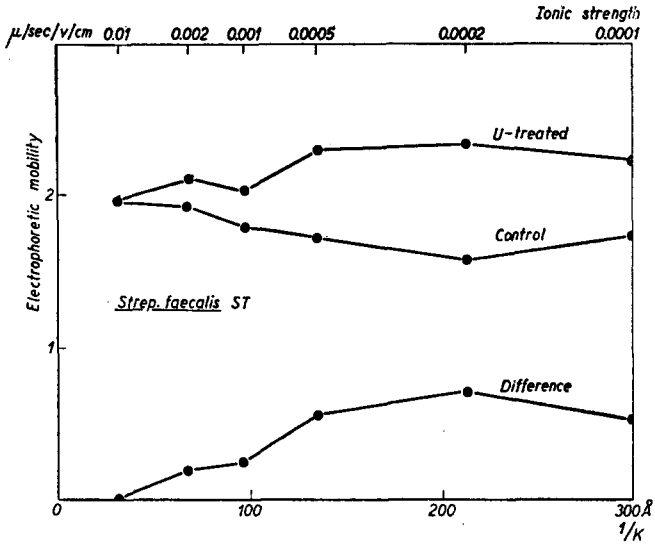

Fig. 4. Variation of Electrophoretic Mobility of *Strep. faecalis* with Ionic Strength before and after Treatment with Uranyl Chloride

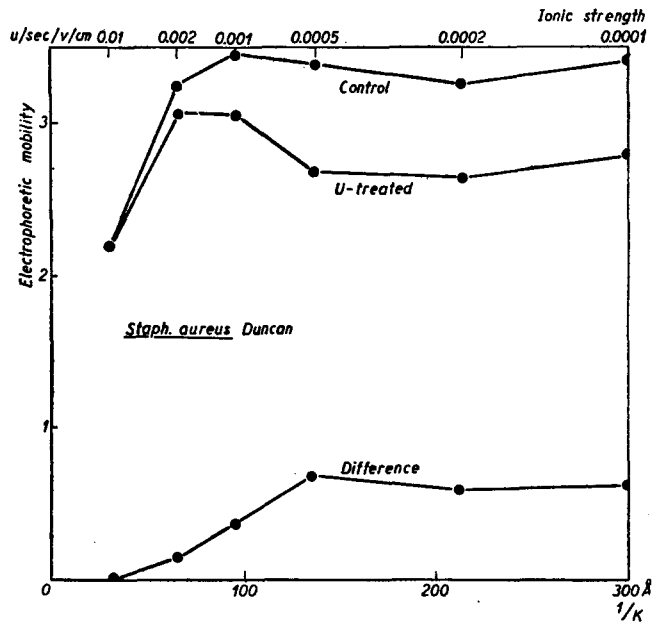

Fig. 5. Variation of Electrophoretic Mobility of *Staph. aureus* with Ionic Strength before and after Treatment with Uranyl Chloride

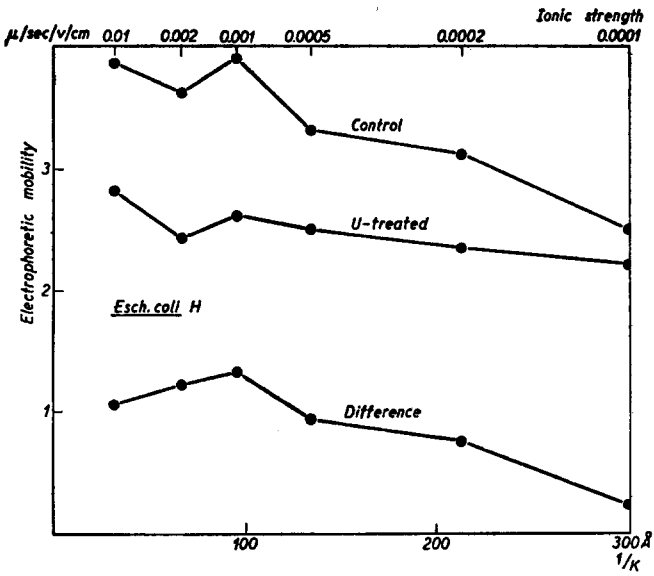

Fig. 6. Variation of Electrophoretic Mobility of *Esch. coli* H with Ionic Strength before and after Treatment with Uranyl Chloride

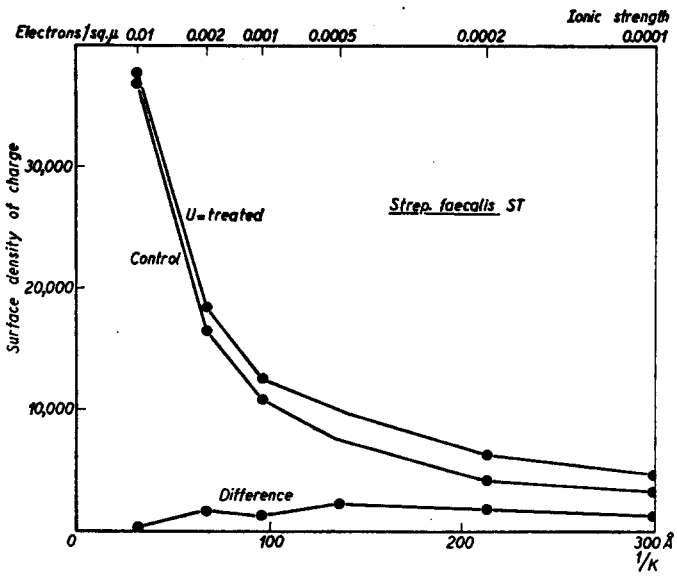

Fig. 7. Variation of Surface Density of Charge of *Strep. faecalis* with Ionic Strength before and after Treatment with Uranyl Chloride

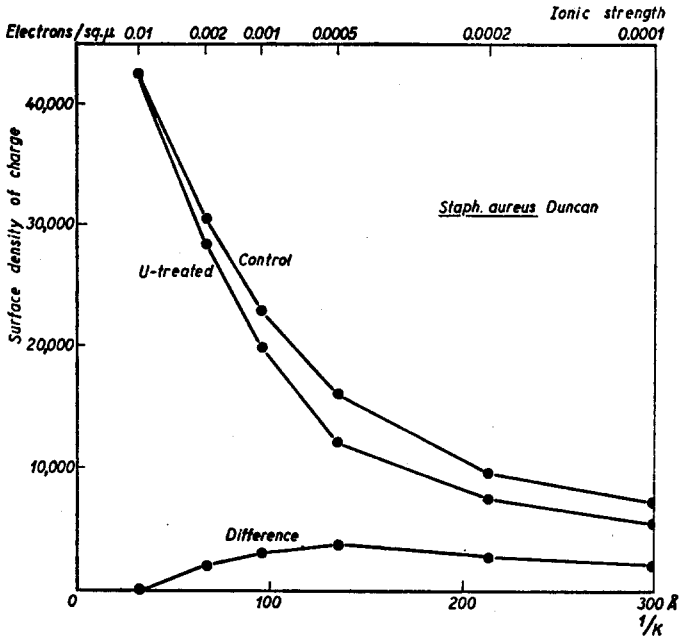

Fig. 8. Variation of Surface Density of Charge of *Staph. aureus* with Ionic Strength before and after Treatment with Uranyl Chloride

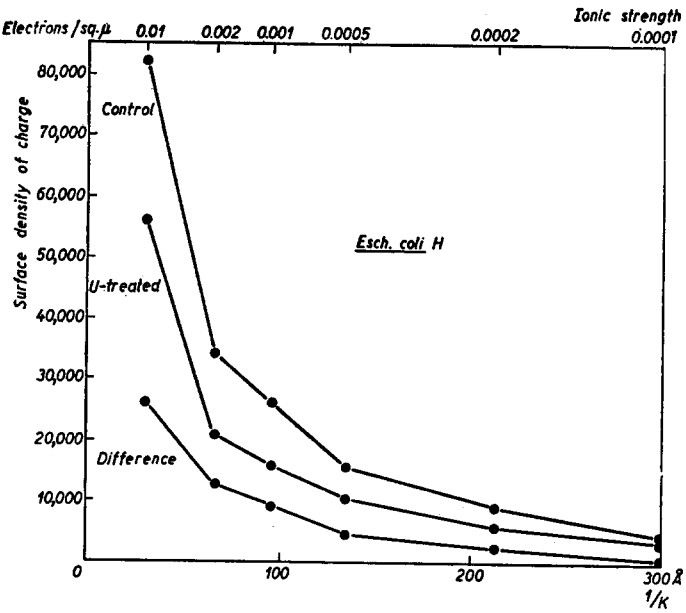

Fig. 9. Variation of Surface Density of Charge of *Esch. coli* H with Ionic Strength before and after Treatment with Uranyl Chloride

MOBILITY DETERMINATIONS IN THE PRESENCE OF URANYL CHLORIDE

A further group of experiments was carried out in which all the mobility determinations were made in the presence of uranyl chloride without centrifuging or washing the bacteria after addition of the reagent. The suspension density of bacteria was 0.1 mg/ml (10 times less than in most of the experiments reported above) since this is a suitable for use in the micro-electrophoresis cell. Samples of the washed bacteria were suspended in buffer or buffered uranyl chloride and the mobility determined. The range of concentrations 1–100 μM-uranyl chloride was studied in acetate buffers of ionic strengths 0.01 and 0.001. Fig. 10 includes the results for all three species of bacteria and shows patterns of behaviour essentially similar to those in Figs. 1 and 2 except that there is no evidence of a significant rise in the mobility of *Staph. aureus* and *Esch. coli* in the lowest concentrations of reagent.

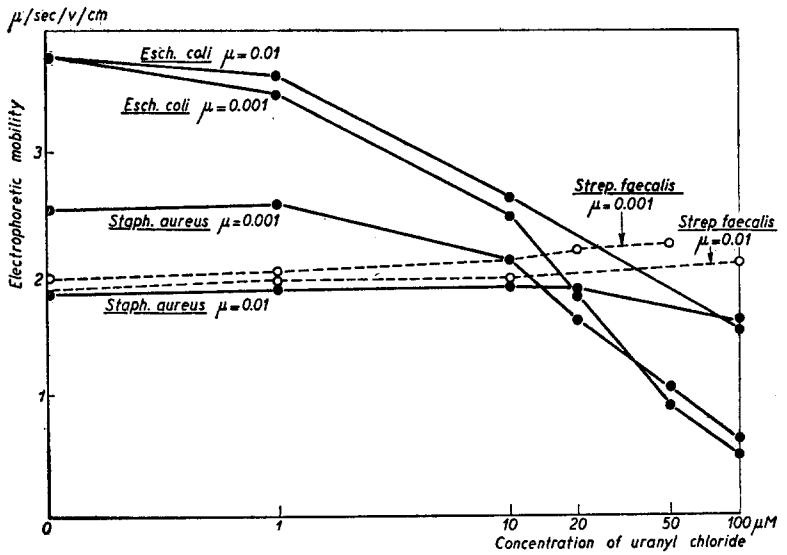

Fig. 10. Variation of Electrophoretic Mobility of *Esch. coli* H, *Staph. aureus* Duncan, and *Strep. faecalis* ST in the Presence of Various Concentrations of Uranyl Chloride

BARRON, MUNTZ AND GASVODA[3] using yeast cells of surface area 73–388 sq. microns found that the uptake of uranium at saturation amounted to about $7 \cdot 10^6$ atoms per yeast cell, *i.e.* say, $3.5 \cdot 10^4$ atoms/sq. micron assuming an average surface area of 200 sq. microns. The surface area of the bacteria used in the present study is of the order of 3 sq. microns. If the surface properties with respect to uranium are comparable with those of yeast, the saturation uptake might be expected to be *c.* 10^5 atoms per bacterial cell. From the data in Fig. 10 the surface densities of charge in terms of electrons/3 sq. microns have been calculated. They are shown in Table VI. For *Esch. coli* and *Staph. aureus* the change in charge in the presence of uranyl chloride reaches values of the order of 10^5 electrons per "cell". The results for *Strep. faecalis* again show an increase in negative charge on treatment with uranyl chloride.

It is noticeable both in Table VI and in Figs. 7–9 that the effect of the ionic strength of the suspending medium on the charge acquired by the organisms in media of constant

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TABLE VI

SURFACE DENSITY OF CHARGE OF *Esch. coli*, *Staph. aureus* AND *Strep. faecalis* (0.1 mg/ml) IN THE PRESENCE OF URANYL CHLORIDE. MOBILITIES DETERMINED IN ACETATE BUFFERS μ = 0.01 AND 0.001 (see Fig. 10)

Organism	Uranyl Chloride μM	Surface Density of Charge in Electrons/3 μ^2			
		Ionic strength 0.01		Ionic strength 0.001	
		Per cell*	Reduction	Per cell*	Reduction
Esch. coli H	0	379 000	—	120 800	—
	1	361 000	18 000	109 500	11 300
	10	250 000	129 000	74 000	46 800
	100	139 000	240 000	13 400	107 400
Staph. aureus Duncan	0	166 000	—	75 200	—
	1	167 000	— 1 000	75 400	— 200
	10	169 500	— 3 500	60 800	14 400
	100	145 000	21 000	15 900	59 300
Strep. faecalis ST	0	165 000	—	55 100	—
	1	166 000	— 1 000	58 000	— 2 900
	10	177 000	— 12 000	60 700	— 5 600
	100	191 500	— 26 500	—	—

* 3 sq. microns is the approx. surface area of the bacterial cells.

pH is considerable. The generally accepted explanation of this is that a large part of the charge is due to adsorption of ions from the medium on to the surface of the organism and that a plot of surface density of charge against ionic strength gives in fact an adsorption isotherm (MOYER[10]). HARTLEY AND ROE[11] have pointed out that the pH of the surface of a particle may differ by several units from that of the bulk of the suspending medium particularly at low salt concentrations. They derive the expression:

$$(pH)_s = (pH)_b + A \cdot v$$

where $(pH)_s$ and $(pH)_b$ are respectively the pH values for the surface and the bulk, and v is the electrophoretic mobility. A is a constant whose value at 25° C in water is 0.217 for particles of the dimensions of bacteria if v is expressed in microns/sec per volt/cm.

As the salt concentration is increased the surface pH tends towards that of the bulk and titration of ionisable surface groups may occur. MITCHELL[12] considers it likely that in certain specially defined systems at low ionic strenghts, ionisable material some distance below the surface of a particle can contribute to the net charge (in an electrokinetic sense) of the particle. It is for this reason that Figs. 4–9 are plotted with alternative abscissae of ionic strength and $1/\kappa$, the thickness of the electrical double layer.

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The relation between μ and κ (which has dimensions of reciprocal length) is approximately:

$$\frac{\mu}{\kappa^2} = 9 \text{ sq. Å.}$$

The data presented in this paper concerning the variation of electrophoretic mobility and surface density of charge with ionic strength may be of use in the development of electrokinetic theory quite apart from the considerations relating to the action of uranyl chloride.

ACKNOWLEDGEMENTS

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SUMMARY

1. A study has been made of the effect of uranyl chloride (1–1000 μM) on the electrophoretic mobility of *Esch. coli*, *Staph. aureus* and *Strep. faecalis* in acetate buffer, $p_H = 4.7$, $\mu = 0.0001$ to 0.01.

2. The negative charge of *Esch. coli* and *Staph. aureus* is decreased by treatment with uranyl chloride; that of *Strep. faecalis* is increased.

3. Phosphate reverses these effects.

4. The surface density of charge of the bacteria has been calculated for various systems.

RÉSUMÉ

1. Nous avons étudié l'effet du chlorure d'uranyle (1 à 1000 μM) sur la mobilité électrophoretique de *Esch. coli*, de *Staph. aureus* et de *Strep. faecalis* en tampon acétate, $p_H = 4.7$, $\mu = 0.0001$ à 0.01.

2. La charge négative de *Esch. coli* et de *Staph. aureus* est diminuée par traitement avec du chlorure d'uranyle; celle de *Strep. faecalis* est augmentée.

3. Le phosphate renverse ces effets.

4. Nous avons calculé la densité de la charge superficielle des bactéries en divers systèmes.

ZUSAMMENFASSUNG

1. Die elektrophoretische Mobilität von *Esch. coli*, *Staph. aureus* und *Strep. faecalis* wurde eingehend studiert, wenn dieselben mit Uranichlorid (1 bis 1000 μM) in Azetatpufferlösungen, $p_H = 4.7$, $\mu = 0.0001$ bis 0.01, behandelt wurden.

2. Die negative Ladung von *Esch. coli* und *Staph. aureus* nimmt durch die Behandlung mit Uranichlorid ab; die von *Strep. faecalis* nimmt zu.

3. Diese Wirkungen sind bei Phosphatlösungen umgekehrt.

4. Die Oberflächenladungsdichte der Bakterien wurde für verschiedene Systeme berechnet.

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