THE BACTERIAL SURFACE

IV. EFFECT OF STREPTOMYCIN ON THE ELECTROPHORETIC MOBILITY OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

by

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Further understanding of the mechanism of interaction of drugs of proven usefulness with susceptible organisms is necessary if hopes of a rational chemotherapy are to be sustained. FERGUSON[1] enumerated four kinds of drugs based on properties such as ionisation, surface-activity and lipoid solubility while HOTCHKISS[2], discussing the investigation of antibacterial action, mentions first "the physical-chemical process by which the agent attains a toxic concentration upon or within the affected cell". The method of micro-electrophoresis can sometimes be used to study the interaction between bacteria and physiologically "active" substances (*e.g.* sulphonamides, BRADBURY AND JORDAN[3]; detergents, DYAR AND ORDAL[4] and McQUILLEN[5]; uranyl salts, McQUILLEN[6]; penicillin, McQUILLEN[7]). This technique has now been applied to the antibiotic streptomycin acting upon *Escherichia coli* and *Staphylococcus aureus*.

Although the site of action of streptomycin has not been decided unequivocally, there is reason to believe that it interferes with an essential enzyme reaction—possibly the pyruvic acid/oxalacetic acid condensation (OGINSKY, SMITH AND UMBREIT[8]; SMITH, OGINSKY AND UMBREIT[9]; UMBREIT AND TONHAZY[10]). Several workers have shown that streptomycin combines with nucleic acids. COHEN[11] found that it reacts as a multivalent base precipitating with nucleic acids in certain proportions. MASSART, PEETERS AND VAN HOUCKE[12] demonstrated the displacement by streptomycin of acridine dyes from combination with the nucleic acid of living or dead yeast cells. Both groups of workers attributed biological significance to their findings. The streptomycin/nucleic acid complexes are solubilised by $M/10$ sodium salts and even in a concentration of $M/50$ these salts inhibit the formation of the complexes. GROS AND MACHEBOEUF[13] reported that streptomycin agglutinated a large number of strains of bacteria but that those organisms possessing the somatic *o* antigen (Smooth strains of the coli/salmonella group) were not agglutinated. These Smooth strains were also notably more resistant to the action of the antibiotic than the corresponding Rough strains.

METHODS

The two organisms *Escherichia coli* H and *Staphylococcus aureus* Duncan were those used in the previous study (McQUILLEN[7]) and the electrophoretic technique was as described in that paper.

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The antibiotic preparation was the Merck calcium chloride complex of streptomycin, 2 streptomycin: 1 $CaCl_2$ (1 unit $\equiv$ 1 μg free base).

Casein digest/glucose/marmite broth ten times diluted with distilled water had a specific conductivity which probably corresponded to an ionic strength of about 0.01.

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 electrophoretic mobility (ABRAMSON[14]). For a uni-univalent electrolyte:

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

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

To a first approximation it is assumed that the diluted broth behaves as a uni-univalent electrolyte of ionic strength 0.01. Then at 25° C:

$\sigma = 73{,}800\sqrt{\mu}$ sinh $v/4$ electronic charges per square micron when v is expressed in microns/second per volt/cm.

RESULTS

Growth Experiments with Staph. aureus—Mobility in Buffer

Staph. aureus was grown to *c.* 200 μg/ml, an equal volume of fresh growth medium added, and the culture divided into two parts. 100 unit/ml streptomycin was added to one part and both were further incubated at 25° C. Samples were taken at intervals from each flask and, after washing the organisms, mobilities were determined in sodium phosphate buffer, $p_H = 5.6$, ionic strength = 0.01. Turbidity measurements were also made. Table I gives typical results of such an experiment.

TABLE I

MOBILITY OF *Staph. aureus* GROWN IN 100 UNIT/ml STREPTOMYCIN

Time min	Control		100 unit/ml Streptomycin	
	Dry weight μg/ml	Mobility μ/sec/v/cm	Dry weight μg/ml	Mobility μ/sec/v/cm
0	90	1.54	90	1.51
15	—	1.53	—	1.53
30	—	1.56	—	1.51
90	133	—	102	—
270	375	1.53	168	1.52

Staph. aureus in broth culture grown with and without 100 unit/ml streptomycin. Samples taken, centrifuged, washed, and resuspended in phosphate buffer, $p_H = 5.6$, ionic strength = 0.01, for mobility determinations.

The mean generation time of the control culture was *c.* 120 min and 100 unit/ml streptomycin caused considerable inhibition of growth. There was, however, no significant change in the electrophoretic mobility even after 5 h growth in the presence of this antibiotic, nor were any visual differences (agglutination, etc.) apparent.

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Growth Experiments with Esch. coli—Mobility in Buffer

A parallel experiment on *Esch. coli* gave results shown in Table II. Although this concentration of streptomycin (100 unit/ml) was less effective in inhibiting the growth of *Esch. coli* than of *Staph. aureus*, it caused immediate agglutination of the cells of *Esch. coli*. Washing reversed this effect and the change in mobility was only 5%.

TABLE II

MOBILITY OF *Esch. coli* GROWN IN 100 UNIT/ml STREPTOMYCIN

Time min	Control		100 unit/ml Streptomycin	
	Dry weight μg/ml	Mobility μ/sec/v/cm	Dry weight μg/ml	Mobility μ/sec/v/cm
0	110	4.40	110	4.34
15	—	4.35	—	4.12
60	180	—	180	—
240	410	—	335	—

Esch. coli in broth culture grown with and without 100 unit/ml streptomycin. Samples taken, centrifuged, washed, and resuspended in phosphate buffer, $p_H = 5.6$, ionic strength 0.01, for mobility determinations.

Mobility of Staph. aureus in Broth Containing Streptomycin

In order to avoid this reversal by washing, all subsequent experiments were carried out by determining the mobility of organisms in broth diluted 10 times with glass distilled water (*cf.* penicillin experiments, MCQUILLEN[7]). *Staph. aureus* was grown to *c.* 400 μg/ml and two samples of the broth culture taken and diluted tenfold with distilled water. 100 unit/ml streptomycin was added to one sample and the mobilities of each determined immediately in the diluted broth. Table III records the results obtained.

TABLE III

MOBILITY OF *Staph. aureus* MEASURED IN BROTH CONTAINING STREPTOMYCIN

Time min	Mobility in μ/sec per volt/cm	
	Control	100 unit/ml Streptomycin
0	1.06	0.89
20	—	0.90

Staph. aureus 400 μg/ml in broth culture diluted tenfold with distilled water or 100 unit/ml streptomycin. Mobilities determined in this diluted broth.

The mobility in the presence of the antibiotic was immediately (less than 5 min) reduced by 15% and had not changed further after 20 min (contrast effects of penicillin which causes a progressive change in charge, MCQUILLEN[7]). Evidently washing in the previous streptomycin experiments had reversed any effects the drug might have had.

The specific conductivity of the diluted broth suspension probably corresponds to

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an ionic strength, μ, of the order of 0.01 and for the purposes of calculating the surface density of charge (which involves the function $\sqrt{\mu}$) the value $\mu = 0.01$ is accurate enough to give an indication of what is happening. The decrease in mobility from 1.06 to 0.89 μ/sec/v/cm (Table III) corresponds to a reduction in negative charge of about 3000 electrons per square micron. The surface area of a bacterial cell is of the order of a few square microns and these figures give some idea of the magnitude of the changes which occur.

Mobility of Esch. coli in Broth Containing Streptomycin

The effects on *Esch. coli* are more marked. Table IV shows the immediate changes in mobility and surface density of charge caused by 10, 100 and 1000 unit/ml streptomycin when the measurements are made in diluted broth.

TABLE IV

MOBILITY OF *Esch. coli* MEASURED IN BROTH CONTAINING STREPTOMYCIN

Streptomycin unit/ml	Mobility μ/sec/v/cm	Charge Density electrons/μ^2	Charge Reduction electrons/μ^2
0	2.31	45,000	—
10	2.12	41,000	4,000
100	1.04	19,500	25,500
1000	0.44	8,100	36,900

Esch. coli 400 μg/ml in broth culture diluted tenfold with distilled water or streptomycin solutions. Mobilities determined immediately in this diluted broth.

In another experiment, *Esch. coli* was grown to 500 μg/ml and samples diluted tenfold with streptomycin solutions to give final concentrations of 0, 10, 25, 50, 75 and 100 unit/ml. Mobility determinations were begun within 2 min of the addition of the drug. The results are given in Table V and a combination of these results with those of Table IV has been used to plot Fig. 1 where the reduction in surface density of charge is shown as a function of the streptomycin concentration. The smooth curve in Fig. 1 was drawn from a theoretical Langmuir adsorption isotherm of the form:

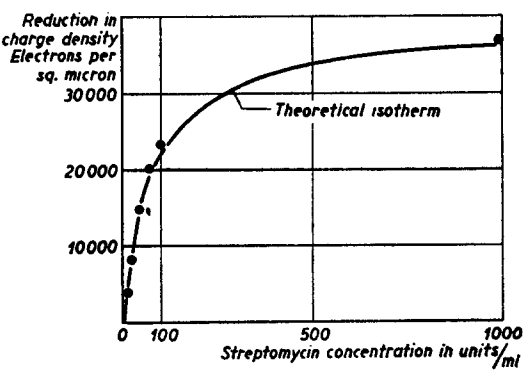

Fig. 1. Effect of streptomycin on the surface density of charge of *Escherichia coli*

$$\Delta\sigma = \Delta\sigma_2 \frac{\beta c}{1 + \beta c}$$

where $\Delta\sigma_2$ is the limiting value of $\Delta\sigma$
$\beta\,\Delta\sigma_2$ is the initial slope
c is the concentration of drug.

The excellence of the fit of the experimentally determined points to the theoretical curve suggests that the reduction in charge is due to adsorption of streptomycin on the bacterial surface.

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TABLE V

MOBILITY OF *Esch. coli* MEASURED IN BROTH CONTAINING STREPTOMYCIN

Streptomycin unit/ml	Mobility μ/sec/v/cm	Charge Density electrons/μ^2	Charge Reduction electrons/μ^2
0	2.39	47,000	—
10	2.19	42,700	4,300
25	2.01	38,500	8,500
50	1.68	32,000	15,000
75	1.42	26,800	20,200
100	1.25	23,400	23,600

Compare Table IV.

Reversibility of Streptomycin Effect on Esch. coli

In order to see whether the attachment of streptomycin to the surface of *Esch. coli* was reversible, samples of a broth culture 10 times diluted were put up as follows:

A_1 Control—no streptomycin (similarly A_2 and A_3)
B 25 unit/ml streptomycin
C_1 50 unit/ml streptomycin (similarly C_2 and C_3).

The mobilities of samples A_1, B and C_1 were determined immediately after preparation. Samples A_2 and C_2 were immediately mixed and the mobility determined. Samples A_3 and C_3 were centrifuged and the supernatants interchanged. The results are given in Table VI.

TABLE VI

REVERSIBILITY OF STREPTOMYCIN EFFECT ON *Esch. coli*

	Mobility in μ/sec/v/cm
A_1 Control	2.45
B 25 unit/ml streptomycin	1.99
C_1 50 unit/ml streptomycin	1.70
$A_2 + C_2$ (Control + 50 unit/ml)	1.95
A_3 cells + C_3 supernatant	1.85
C_3 cells + A_3 supernatant	2.45

Final suspension density of *Esch. coli* = 60 μg/ml.

All samples in 10 times diluted broth (see text).

The coefficient of variation of all samples was 4% and it is evident that mixing the control suspension with a suspension in 50 unit/ml streptomycin results in all the cells having a mobility equal to that in 25 unit/ml drug. Also, treatment of the bacteria with 50 unit/ml streptomycin and then suspension in diluted broth containing no antibiotic, results in release of the streptomycin, the charge on the organisms reverting to the value for the control. Apparently, then, a very small proportion of the total streptomycin present is attached to the cells under these conditions.

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The comparatively small differences in mobility on changing the suspension density in a given concentration of streptomycin are confirmatory evidence that a small proportion only of the total streptomycin is bound by the bacterial cells. For instance, in 50 unit/ml streptomycin the mobilities of 30, 60 and 120 μg/ml suspensions of *Esch. coli* were respectively 1.56, 1.63 and 1.67 μ/sec/v/cm. This is in marked contrast to the effects of altering the antibiotic concentration—see Table V.

In conclusion, streptomycin adsorbs on to the surface of *Esch. coli* resulting in a reduction of the net negative charge by about 20,000 electrons per bacterial cell in the presence of 10 unit/ml and by more than 100,000 electrons per cell in the presence of 100 unit/ml streptomycin. This high affinity of the bacteria for the antibiotic is probably of importance in their biological interaction. In view of the findings of COHEN[11], MASSART *et al.*[12] and GROS AND MACHEBOEUF[13] concerning the interaction of streptomycin and nucleic acids, it is possible that the combination of the drug at the surface of *Esch. coli* takes place *via* nucleic acid radicals.

ACKNOWLEDGEMENTS

The author's thanks are due to Dr E. F. GALE for his interest in this work and to the Medical Research Council for a personal grant.

SUMMARY

1. Streptomycin combines with surface groups of *Escherichia coli* decreasing the net negative charge of the organisms.

2. This also results in agglutination of the Rough form of *Esch. coli*.

3. Combination (and agglutination) is readily reversed by washing with *M*/10 NaCl or by resuspension in fresh broth.

4. The combination of streptomycin with surface groups of *Esch. coli* can be represented as an adsorption isotherm.

5. Mixing streptomycin-treated and untreated cells results in an equal distribution of the drug amongst all the cells.

6. Growth of *Esch. coli* in 100 unit/ml streptomycin yields cells which, after washing, have normal mobilities although considerable inhibition of growth has occurred.

7. It is suggested that streptomycin may combine with nucleic acid residues in the surface of *Esch. coli*.

8. Streptomycin has similar but less intense effects on *Staphylococcus aureus*.

RÉSUMÉ

1. La streptomycine se combine à des groupes de la surface de *Escherichia coli* diminuant ainsi la charge négative nette de ces organismes.

2. Il en résulte aussi l'agglutination de la forme dite "Rough" de *Esch. coli*.

3. Cette combinaison est rapidement scindée et l'agglutination disparaît par lavage avec du NaCl *M*/10 et par suspension renouvelée dans du bouillon frais.

4. La combinaison de la streptomycine avec des groupes de la surface de *Esch. coli* peut être représentée par une isotherme d'adsorption.

5. En mélangeant des cellules traitées à la streptomycine avec des cellules non traitées on obtient une distribution égale de la drogue entre toutes les cellules.

6. En cultivant *Esch. coli* dans 100 unités/ml de streptomycine on obtient des cellules qui montrent, après lavage, des mobilités normales bien qu'une inhibition considérable de leur croissance ait eu lieu.

7. Nous avons suggéré l'idée que la streptomycine pourrait se combiner à des restes d'acides nucléiques de la surface de *Esch. coli*.

8. La streptomycine a des semblables mais moins intenses sur le *Staphylococcus aureus*.

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ZUSAMMENFASSUNG

1. Streptomycin verbindet sich mit gewissen Gruppen der Oberfläche von *Escherichia coli* wodurch die netto negative Ladung der Organismen herabgesetzt wird.

2. Hierdurch wird ebenfalls Agglutination der "Rough"-Form von *Esch. coli* verursacht.

3. Diese Bindung (und die Agglutination) wird durch Waschen mit *M*/10 NaCl und suspendieren in frischer Bouillon rasch wieder aufgehoben.

4. Die Verbindung von Streptomycin mit gewissen Gruppen der Oberfläche von *Esch. coli* kann durch eine Adsorptionsisotherme dargestellt werden.

5. Durch Mischen von mit Streptomycin behandelten und nicht behandelten Zellen erreicht man eine regelmässige Verteilung der Droge über alle Zellen.

6. Wachstum von *Esch. coli* in Streptomycin (100 Einheiten/ml) ergibt Zellen, welche nach dem Waschen normale Mobilitäten zeigen, obwohl das Wachstum bedeutend verlangsamt ist.

7. Es ist möglich, dass das Streptomycin sich mit Nucleinsäureresten der Oberfläche von *Esch. coli* verbindet.

8. Streptomycin wirkt ähnlich aber weniger stark auf *Staphylococcus aureus*.

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Received September 18th, 1950