THE BACTERIAL SURFACE

III. EFFECT OF PENICILLIN ON THE ELECTROPHORETIC MOBILITY OF *STAPHYLOCOCCUS AUREUS*

by

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A drug must first reach its appropriate site of action before it can affect an organism. This site may be at the surface of the cell or it may be deep within the cell. In the latter case, the toxic agent must penetrate whatever cell envelopes are present—capsule, wall, cytoplasmic membrane—before reaching the susceptible locus. The interaction which subsequently occurs leads to a succession of changes in the organism. Since the outer surface is the first point of contact and since many antibacterial agents cause obvious changes in size and shape or behaviour of the surface of sensitive cells, a study of the surface properties of bacteria after treatment with such substances is of interest. Of these properties, the electrokinetic behaviour is one which is amenable to quantitative investigation. Moreover, the method of micro-electrophoresis has the unique advantage of giving information on individual, living, unstained organisms with the least possible manipulation. Previous communications (McQUILLEN[1, 2]) dealt with the interaction of a cationic detergent and a heavy metal complex with various organisms. In this and the succeeding paper[2a] the effects of penicillin and streptomycin are reported.

The exact mechanism of action of penicillin is not known but a number of experimental facts have been discovered regarding changes which occur to susceptible bacteria some time after contact with the antibiotic. Almost the only washed suspension experiments giving positive results are uptake studies using isotopically labelled penicillin. ROWLEY, COOPER, ROBERTS AND LESTER SMITH[3] suspended sensitive staphylococci in saline solutions of penicillin (0.05 unit/ml = 0.01 μg/mg cells) and found an uptake of 1–2 μg penicillin per g of bacteria within 3 min at 0° C. The uptake was not much increased either at 37° C or during longer periods of incubation but amounts between 3–25 μg/g were taken up by sensitive cells growing rapidly in the presence of the drug for times up to 2½ h. MAAS AND JOHNSON[4] reported similar findings. They incubated a thick paste of *Staphylococcus aureus* with equal volumes of penicillin solutions of various concentrations and determined the distribution of the drug. Two types of uptake were found:

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a. specific uptake of 0.5 μg/ml cell paste, independent of the penicillin concentration

b. diffusion of penicillin into the bacterial cells so that the intracellular water had the same penicillin concentration as the surrounding medium.

The specific uptake was independent of time and extensive washing did not remove the specifically bound ^{35}S of radio-active penicillin nor did this exchange with ^{32}S of unlabelled penicillin even at concentrations of 10,000 unit/ml. MAAS AND JOHNSON[4] calculated that about 750 molecules of penicillin were bound per bacterial cell.

The location of the specifically attached antibiotic—if indeed it is penicillin as such—is not known but there is some evidence that it is not combined at the surface of the organisms. ROWLEY *et al.*[3] found that the bound ^{35}S was not removed by 30 min incubation with 0.1% cetyl-trimethyl-ammonium bromide (a cationic detergent which SALTON[5] has shown to "strip off" the outer envelope of *Staph. aureus*); 5% phenol (a solvent used by MITCHELL and the writer to prepare the material of the outer layers of *Staph. aureus*, see MCQUILLEN[6]); or by incubation with a number of other substances. Also COOPER, ROWLEY AND DAWSON[7] disintegrated *Staph. aureus* by shaking with "ballotini" (minute glass beads) and found the insoluble residue to consist of material which in electron micrographs appeared as empty sacs which were assumed to be the cell-walls of the organisms. When the bacteria were incubated with ^{35}S-labelled penicillin prior to disintegration, all the radio-active isotope was found in the supernatant and none attached to the "cell-wall" fraction.

It is generally accepted that active growth of an organism in the presence of penicillin is necessary for the drug to exert its antibacterial action. Such growth is accompanied by progressive cytological and metabolic changes (PRATT AND DUFRENOY[8], CHAIN AND DUTHIE[9]). The glutamic acid accumulating mechanism of *Staph. aureus* and other Gram-positive organisms is impaired after 30 min growth in the presence of 10 unit/ml penicillin or after longer periods in lower concentrations (GALE AND TAYLOR[10], GALE[11]). A relationship between the sensitivity to penicillin and the efficiency of the glutamic acid assimilatory mechanism was shown by GALE AND RODWELL[12]. In the Gram-positive bacteria an early effect of penicillin is interference with the active transport of glutamic acid across the cell-wall but whether this is the cause of death or whether it is a symptom of the general disorganisation of cellular function is not yet clear.

Experiments by a number of workers (KRAMPITZ AND WERKMAN[13], MACHEBOEUF[14], GROS AND MACHEBOEUF[15]) implicate the dissimilation of nucleotides as an important reaction which is inhibited by penicillin. MITCHELL[16] studying the nucleotide/nucleic acid balance of *Staph. aureus* found a displacement after 10 min growth in the presence of 1 μg/ml penicillin (5 μg/mg cells). This is the earliest effect of penicillin on growing cultures which has been reported.

One other very early change caused by the antibiotic has been observed. After short periods of growth in the presence of the drug, cultures of *Staph. aureus* become "sticky"—when spun down and washed, the cells are difficult to centrifuge, tend to adhere to the walls of the centrifuge tube, and swirl into suspension on slight agitation. This behaviour suggests a change in the surface properties of the organisms. The effects of penicillin on the electric charge of *Staph. aureus* have been studied by the method of micro-electrophoresis and whereas in no case has a change in charge been found in washed suspension experiments, significant alterations can be detected within 10 min of adding penicillin to bacteria growing at 25° C.

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METHODS

Bacteria

Escherichia coli H and *Staphylococcus aureus* Duncan (*Micrococcus pyogenes* var. *aureus* Duncan) were grown in 250 ml conical flasks containing 100 ml casein digest/glucose/marmite broth. A growth temperature of 25° C was chosen so that the time course of growth was extended. The washing procedure where used consisted of 1 × *M*/10 NaCl and 3 × glass distilled water on the centrifuge.

Antibiotic

Sodium penicillin G was used (1 unit = 0.6 μg penicillin).

Micro-electrophoresis

All experiments were carried out at 25° C in the horizontal, cylindrical cell used in previous studies (McQUILLEN[1, 2]). Observations were made at the stationary level determined after the manner of HENRY[17]. Platinum electrodes were used and the potential gradient calculated from the specific resistance of the bacterial suspension measured in the electrophoresis cell (McQUILLEN[6]) and the current as recommended by MOYER[18].

RESULTS

Experiments with Washed Suspensions of Staphylococcus aureus

In a number of preliminary experiments, washed suspensions of *Staph. aureus* were incubated with penicillin (1–100 unit/ml) but no change in electrophoretic mobility was detectable either after washing or in the presence of the drug, in buffer of ionic strength 0.01. Nor did determinations in distilled water after washing the bacteria, reveal differences in charge on treatment with penicillin. In view of the work of ROWLEY *et al.*[3] and MAAS AND JOHNSON[4] mentioned above (of the order of 500 molecules of penicillin specifically bound per cell) and the finding of COOPER *et al.*[7] that probably no penicillin is attached to the cell-wall, it is not surprising that there is no change in charge of washed bacteria on treatment with this antibiotic. The electrophoresis studies were made some time before the work with radio-active penicillin was published but conflict with the findings of DORFMAN, MOLDAVSKAYA, KASTORSKAYA AND ZASYPKINA[19]. These workers reported an increase in the zeta potential of sensitive cells within one minute of exposure to penicillin. Unfortunately, only abstracts of their paper have been available to the author.

Experiments with Growing Cultures of Staphylococcus aureus

Cultures of *Staph. aureus* were grown to a density of *c.* 200 μg/ml, diluted with an equal volume of fresh broth and divided into two parts. Each was incubated at 25° C in a 250 ml conical flask, penicillin being added to one to give a final concentration of 1 unit/ml. 5 ml samples were removed from each flask at times 0, etc. After centrifuging and washing these samples the organisms were resuspended in sodium phosphate buffer, p_H = 5.6, ionic strength = 0.01, for mobility determinations. All operations—growth, centrifuging and mobility measurements—were carried out in a 25° C room and since spinning down from the growth medium took 5 min, growth probably continued for a short time after the samples were taken. In general 20 bacterial cells were timed over a distance of 2 × 100 microns but on occasion the mobilities of 100 cells were determined. Typical results obtained on different days are shown in Table I.

The coefficient of variation for the control cells in the absence of penicillin is 8% (*i.e.* standard error *c.* 0.03 μ/sec/v/cm) and it can be seen than the mobility changes little during the 48 h growth period studied. The penicillin-treated cultures show early

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TABLE I

MOBILITY OF *Staph. aureus* GROWN IN 1 UNIT/ml PENICILLIN

Time	Mobilities in μ/sec per volt/cm					
	a Penicillin		b Penicillin		c Penicillin	
	—	+	—	+	—	+
0 min	1.50	1.48	1.70	1.68	1.61	—
10	—	—	1.68	1.65	—	1.53
30	—	—	1.69	1.57	1.64	1.55
60	1.53	1.25	1.67	1.47	—	1.47
90	—	—	—	—	1.67	1.40
3 h	—	—	—	—	1.65	1.30
5	1.53	0.80	1.61	1.11	1.67	1.05
12	—	—	1.65	1.23	—	—
24	—	—	1.69	1.46	—	—
48	—	—	1.65	—	—	—

Broth cultures of *Staph. aureus* grown in presence and absence of 1 unit/ml penicillin. Samples taken, spun down, washed and resuspended in phosphate buffer, $p_H = 5.6$, ionic strength 0.01, for determination of the electrophoretic mobility.

changes in the mean mobility. Also the distribution of mobilities alters. There is a progressive increase in the scatter of mobilities with time of growth in penicillin. For example, column (b) of Table I shows that the mobility of the penicillin-treated culture fell from 1.68 to 1.11 μ/sec/v/cm after 5 h but the range of mobilities of individual cells in the 5 h sample extended from 1.6 to 0.7 μ/sec/v/cm. Even after 12 h some cells had a mobility of 1.6 μ/sec/v/cm. Apparently the cells are by no means all affected to the same extent and after long periods of growth in the presence of this antibiotic a certain proportion of the bacteria has unchanged characteristics. The change in distribution of mobilities is illustrated by Fig. 1 where histograms are plotted of the time for 100 cells from the 5 h control and 5 h penicillin-treated cultures (Table I, column b) to travel 2 × 100 microns under a constant potential gradient.

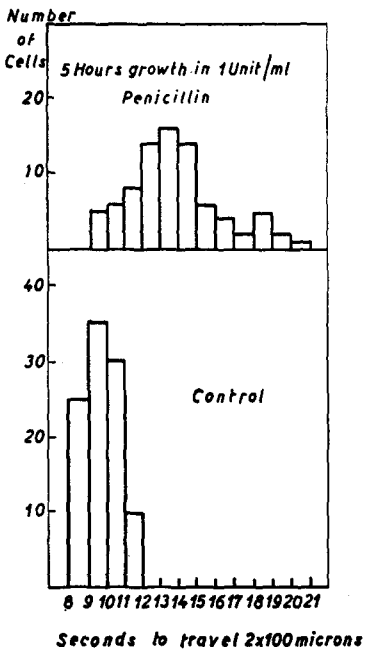

Fig. 1. Distribution of mobilities of *Staphylococcus aureus* growing 5 h in presence and absence of 1 unit/ml penicillin

The changes occurring during the first 30 min of growth were studied in greater detail by repeating the above experiment and timing 100 cells from samples taken at times 10, 20 and 30 min from both control and penicillin-treated cultures. Samples from the two cultures were also taken at times 0 and 60 min and the turbidities estimated on the Hilger Spekker photo-electric turbidimeter. Table II gives the results for the mean mobilities and histograms of the distribution of velocities are shown in Fig. 2.

The standard error of the control samples is less than 0.02 μ/sec/v/cm. In 10 min there is a significant decrease in the mean mobility of the penicillin-treated cells and

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this effect progresses with time as does the increase in scatter of individual mobilities. Some cells retain properties characteristic of the untreated organisms. The histograms of Fig. 2 should be compared with those in Fig. 1 for the 5 h cultures.

TABLE II

MOBILITY OF *Staph. aureus* GROWN IN 1 UNIT/ml PENICILLIN

Time min	Control		1 unit/ml Penicillin	
	Dry Weight μg/ml	Mobility μ/sec/v/cm	Dry Weight μg/ml	Mobility μ/sec/v/cm
0	89	—	94	—
10	—	1.44	—	1.35
20	—	1.41	—	1.30
30	—	1.39	—	1.26
60	146	—	146*	—

* See below in text.

Broth cultures of *Staph. aureus* grown in presence and absence of 1 unit/ml penicillin. Samples taken, spun down, washed and resuspended in phosphate buffer, $p_H = 5.6$, ionic strength = 0.01, for determination of electrophoretic mobilities of 100 cells. See Fig. 2 for histograms of the distribution of mobilities.

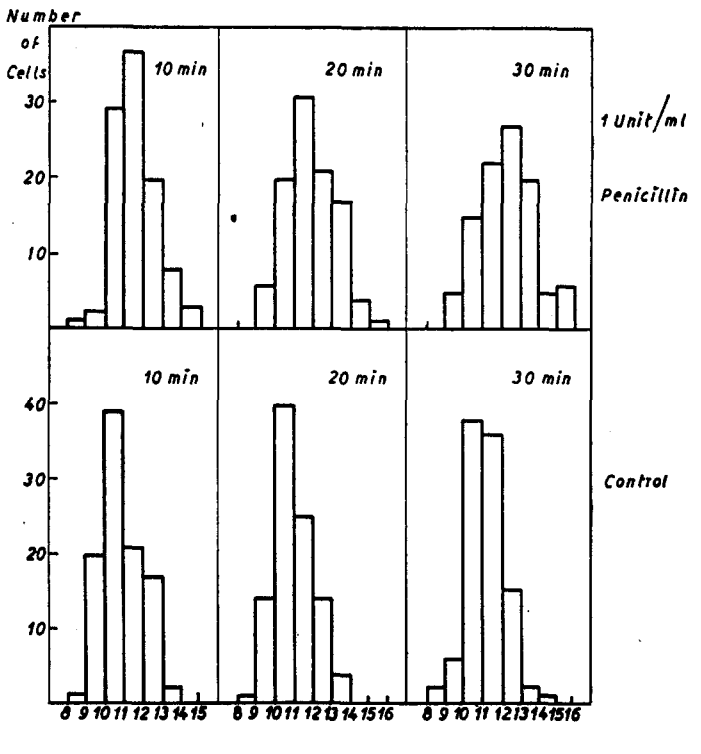

Fig. 2. Distribution of mobilities of *Staphylococcus aureus* growing for 10, 20 and 30 min in presence and absence of 1 unit/ml penicillin

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The mean generation time of the control culture is *c.* 90 min as calculated from the dry weight determinations. The values given for the dry weights are based on the observed values for log I_0/I obtained in the turbidimeter. The dry weight value for the 60 min penicillin-treated sample is almost certainly too high since it is based on the calibration curve for normal organisms. MITCHELL[16] showed that the scattering index of the same organisms growing in the same concentration of penicillin (*c.* 5 μg/mg cells) increases by about 25% during the first hour. This means that dry weights of penicillin-treated cultures based on calibration curves plotted from data on normal organisms are erroneously high. A value of about 110 μg/ml instead of 146 μg/ml for the 60 min penicillin-treated culture is, therefore, more probable.

Experiments with Growing Cultures of Escherichia coli

A few similar studies were carried out with the Rough *Esch. coli* H but in the case of this organism penicillin concentrations of the order of 100 unit/ml were necessary to obtain comparable results. Table III shows some of the findings. The "stickiness" and lysis were so great in many of the samples that it was not possible to centrifuge quantitatively and accurate turbidity measurements could not be made.

TABLE III

MOBILITY OF *Esch. coli* GROWN IN PENICILLIN

Time min	Penicillin units/ml					
	0		100		500	1000
	Dry weight μg/ml	Mobility μ/sec/v/cm	Dry weight μg/ml	Mobility μ/sec/v/cm	Mobility μ/sec/v/cm	Mobility μ/sec/v/cm
0	140	—	140	—	—	—
15	—	4.35	—	4.28	4.19	4.21
30	—	4.42	—	4.25	4.10	4.16
60	225	—	155	—	—	—
300	475	4.36	—	2.90	2.44	2.66

Coefficient of variation of control samples less than 4%

Broth cultures of *Esch. coli* grown in the presence and absence of penicillin. Samples taken, spun down, washed and resuspended in phosphate buffer, pH 5.6, ionic strength = 0.01, for determination of electrophoretic mobility.

The mean generation time of the control culture was *c.* 90 min. As with *Staph. aureus*, growth in the presence of the antibiotic resulted in a decrease in the mean mobility and an increase in the scatter of mobilities, some cells being much more affected than others. It is possible that the relatively smaller changes observed with this organism are due to rapid lysis of affected cells.

Mobility Determinations in Diluted Broth

In order to follow changes in the electric charge of *Staph. aureus* without centrifuging and washing the cells, cultures were grown to a density of *c.* 400 μg/ml, diluted tenfold with glass distilled water, and measurements of the mobility in this diluted broth made with and without addition of penicillin. The suspensions had specific resistances which probably correspond to ionic strengths of the order of 0.01. Mobilities

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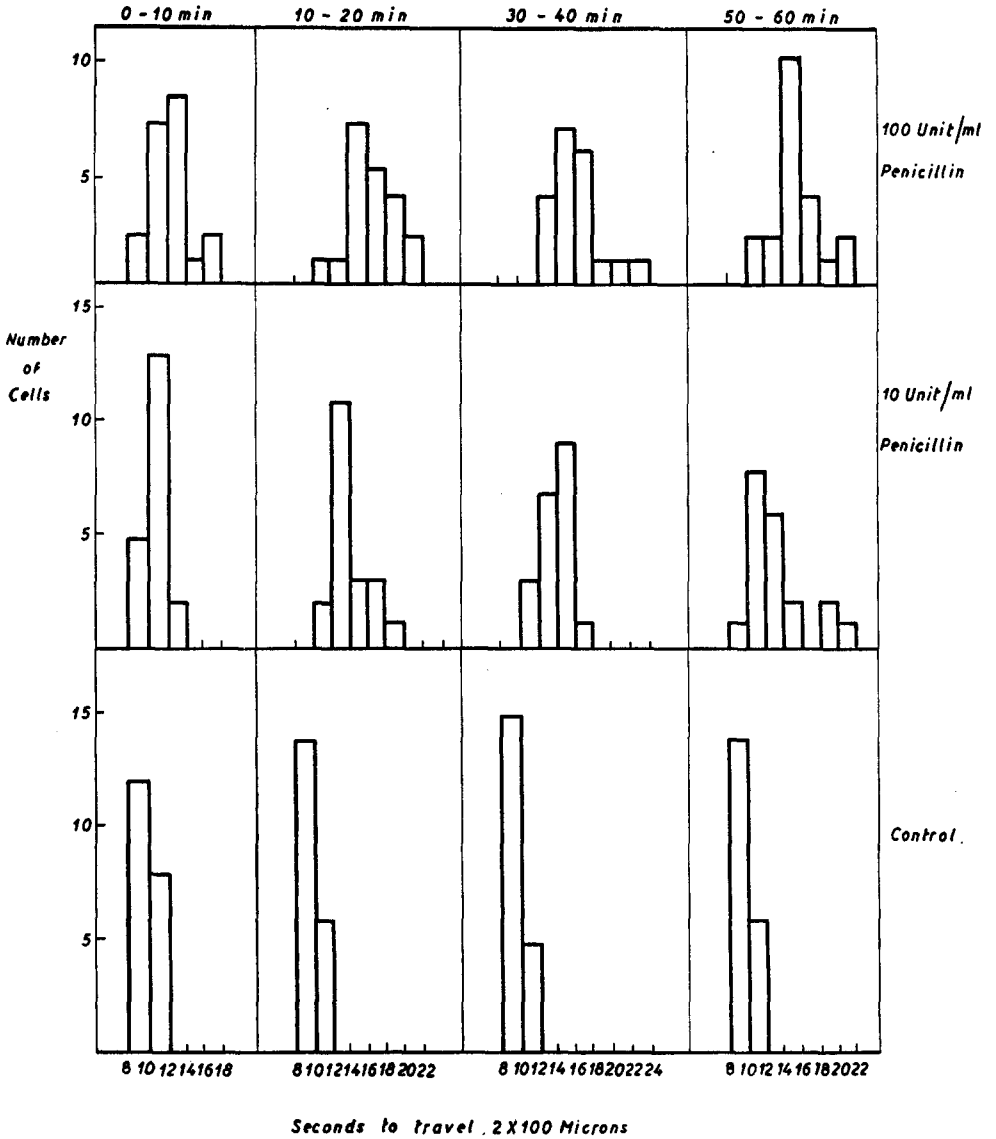

Fig. 3. Distribution of mobilities of *Staphylococcus aureus* growing in presence of 0, 10 and 100 unit/ml penicillin

in this medium were surprisingly consistent and the presence of 100 unit/ml penicillin altered the ionic strength by not more than 5%. It was possible, therefore, to follow mobilities of cultures growing in the micro-electrophoresis cell.

In the first of these experiments the organisms were grown to a density of 420 μg/ml and 2 ml samples of this culture were diluted to 20 ml with glass distilled water or with a solution of penicillin (final concentration of penicillin 10 or 100 unit/ml). The diluted broth cultures were immediately run into the micro-electrophoresis cell and measurements of the mobility begun within 2 min and continued for about 1 h. The results are

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given in Table IV while histograms of the behaviour of these cells appear in Fig. 3. 20 cells were timed for each sample and this took less than 10 min to complete.

TABLE IV

MOBILITY IN BROTH OF *Staph. aureus* GROWING IN PENICILLIN

Time min	Mobilities in μ/sec/v/cm in presence of penicillin concentration unit/ml		
	0	10	100
0–10	1.29	1.17	1.07
10–20	1.29	1.03	0.89
30–40	1.31	0.90	0.82
50–60	1.30	0.92	0.84
60–70	—	—	0.89

Standard error of controls 0.03 μ/sec/v/cm

Broth culture of *Staph. aureus* (420 μg/ml) diluted tenfold with water or penicillin solutions and samples run into electrophoresis cell. Determinations of mobility begun within 2 min and continued for about 1 h. See Fig. 3 for histograms of the distribution of mobilities.

The effects of 10 unit/ml penicillin in this growth medium are almost instantaneous and progress with time. 100 unit/ml is even more effective. The increasing scatter of mobilities can be seen particularly well in Fig. 3 for the 10 unit/ml culture.

Using a much lower concentration of drug (0.1 unit/ml), very similar results were obtained. The culture was grown to 375 μg/ml and then diluted tenfold with water or penicillin solution. Table V and Fig. 4 show the results obtained.

TABLE V

MOBILITY IN BROTH OF *Staph. aureus* GROWING IN 0.1 UNIT/ml PENICILLIN

Time min	Mobilities in μ/sec/v/cm	
	Control	0.1 unit/ml penicillin
0–10	1.28	1.23
10–20	—	1.15
30–40	—	1.04
70–80	—	0.87

Standard error of control 0.03 μ/sec/v/cm

Broth culture of *Staph. aureus* (375 μg/ml) diluted tenfold with water or penicillin solution (final concentration 0.1 unit/ml). Samples run into electrophoresis cell and mobility determinations begun within 2 min and continued for about 1 h. See Fig. 4 for histograms of the distribution of mobilities.

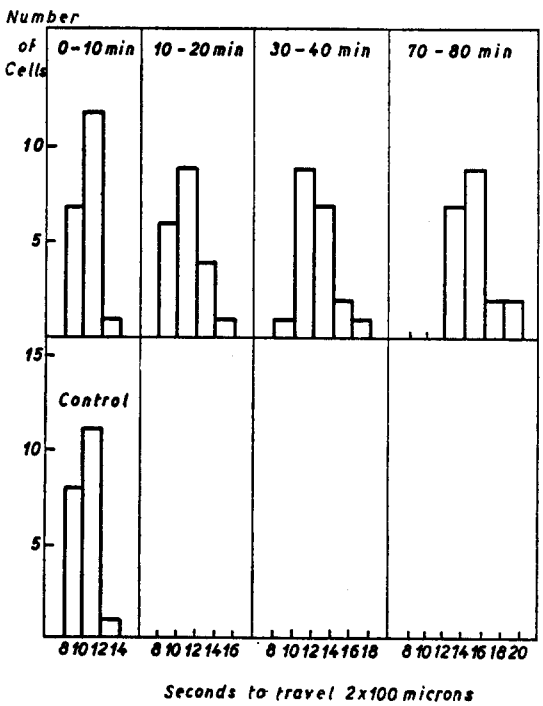

Fig. 4. Distribution of mobilities of *Staphylococcus aureus* growing in presence and absence of 0.1 unit/ml penicillin

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Again an almost immediate change in mean mobility is evident in the presence of the antibiotic. The progress of the effects is slower than with the higher concentrations but essentially the same overall picture emerges. A comparison of the time courses of the reactions with 0.1, 10 and 100 unit/ml penicillin is given in Fig. 5.

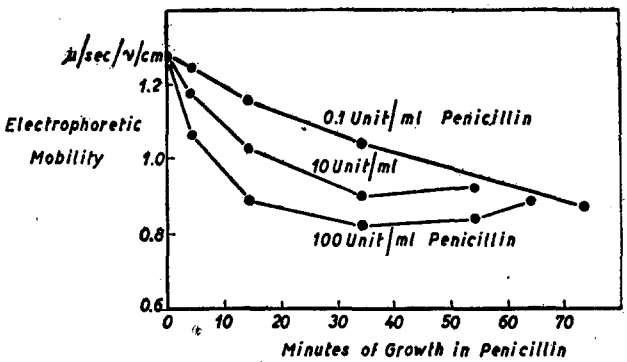

Fig. 5. Time course of interaction of penicillin with *Staphylococcus aureus*—electrophoretic mobilities measured in dilute broth

Attempt to Reverse Penicillin Effect on Staphylococcus aureus

Two 2 ml samples of a growing culture of *Staph. aureus* (dry weight 375 μg/ml) were diluted to 20 ml with water and 10 unit/ml penicillin solution respectively. The cells were at once centrifuged down (*c.* 15 min) and the supernatants interchanged. Mobilities of each sample were then determined in these media (Table VI).

TABLE VI

ATTEMPT TO REVERSE PENICILLIN EFFECT ON *Staph. aureus*

	Mobilities in μ/sec/v/cm
a. Control cells	1.28
b. 10 unit/ml cells + control supernatant	0.87
c. Control cells + 10 unit/ml supernatant	1.05 falling to 0.95 after 10 min

Broth culture of *Staph. aureus* (375 μg/ml). Samples diluted tenfold with water or penicillin solution (final concentration 10 unit/ml). Two samples spun down and supernatants interchanged. All mobilities determined in this diluted broth.

Cells (b) which had been put up in 10 unit/ml penicillin and after spinning down, resuspended in penicillin-free medium, had a mobility of 0.87 μ/sec/v/cm as would have been expected had the cells been left in the medium containing the drug. On the other hand, cells (c) which, after spinning out from penicillin-free medium, were resuspended in the supernatant from penicillin-treated cells, also had depressed mobilities (1.05 decreasing to 0.95 μ/sec/v/cm after 10 min as compared with the control value of 1.28). This argues that the change in mobility is not immediately reversible by replacing the medium, and secondly, that from this concentration of penicillin at any rate, no appreciable amount of antibiotic is removed by the bacteria.

Necessity for Presence of Growth Medium

The next studies were carried out on washed suspensions of *Staph. aureus* to which penicillin was added both in the presence and absence of very small amounts of growth medium. A broth culture (dry weight 350 μg/ml) was spun down, washed and four

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samples of the cells put up at a suspension density of 35 μg/ml in sodium phosphate buffer, p_H = 5.6, ionic strength = 0.01. The following additions were then made:

a. None
b. Penicillin to a final concentration of 10 unit/ml
c. 10 unit/ml penicillin + $^1/_{1000}$ fresh broth
d. 10 unit/ml penicillin + $^1/_{100}$ fresh broth.

Determinations of the mobility were made immediately after the additions and again at 10 min intervals. The results are shown in Table VII.

TABLE VII

NECESSITY FOR PRESENCE OF GROWTH MEDIUM FOR PENICILLIN EFFECT ON *Staph. aureus*

Time min	Mobilities in μ/sec per volt/cm			
	a Control	b 10 unit/ml	c 10 unit/ml + $^1/_{1000}$ broth	d 10 unit/ml + $^1/_{100}$ broth
0–10	1.30	1.35	1.35	1.25
10–20	—	1.35	1.32	0.99
30–40	—	1.33	1.30	—

Washed *Staph. aureus* suspended in phosphate buffer, p_H = 5.6, ionic strength 0.01, at a suspension density of 35 μg/ml with additions of penicillin and broth.

In the absence of broth, (b), no change in the mobility occurred but $^1/_{100}$ broth, (d), caused a rapid fall in the mean value and the characteristic increase in scatter of individual mobilities. The fall in mean mobility of the organisms in $^1/_{1000}$ broth, (c), may be just significant since the standard error of these readings is less than 0.03 μ/sec/v/cm.

Time Course of Penicillin Effect on Staphylococcus aureus

The changes occurring under these conditions were followed in greater detail. A broth culture of *Staph. aureus* (dry weight 400 μg/ml) was washed and two flasks set up as follows:

	Control	Penicillin 10 unit/ml
Staph. aureus (washed)	90 μg	92 μg
Fresh growth medium	1 ml	1 ml
Penicillin	—	1000 units
Sodium Phosphate buffer, p_H = 5.6, μ = 0.01	99 ml	99 ml

The flasks were plugged with cotton wool and incubated at 25° C. 10 ml samples were removed at times 0, 2, 4, 6 and 18 h. These samples were used for turbidity determinations and mobility measurements. Since both cultures were grown side-by-side in a 25° C room, it was necessary to work to a strict time-table in order to be able to specify appropriate ages. For any pair of samples the procedure was as follows, taking time 0 as the time at which the samples were removed from the flasks:

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0–5 min Turbidity readings
5–15 Mobility determinations on control cells
15–25 Mobility determinations on penicillin-treated cells.

A set of 20 mobility readings took about 10 min and the mean mobility values are referred to the middle of this 10 min period. The results are given in Table VIII

TABLE VIII

TIME COURSE OF PENICILLIN EFFECT ON *Staph. aureus*

Time min	Control		10 unit/ml Penicillin	
	Dry weight μg/ml	Mobility μ/sec/v/cm	Dry weight μg/ml	Mobility μ/sec/v/cm
0	90	—	92	—
10	—	1.23	—	—
20	—	—	—	0.99
120	101	—	92	—
130	—	1.27	—	—
140	—	—	—	0.93
240	106	—	84	—
250	—	1.27	—	—
260	—	—	—	1.02
300	109	—	86	—
310	—	1.25	—	—
320	—	—	—	1.07
360	112	—	83	—
370	—	1.25	—	—
380	—	—	—	1.08
18 h	126.5	1.24	70	1.07

Washed *Staph. aureus* suspended in phosphate buffer, $p_H = 5.6$, ionic strength = 0.01, containing $^1/_{100}$ broth with and without 10 unit/ml penicillin. Mobilities determined in this medium. See Fig. 6 for histograms of distribution of mobilities and Fig. 7 for progress curves.

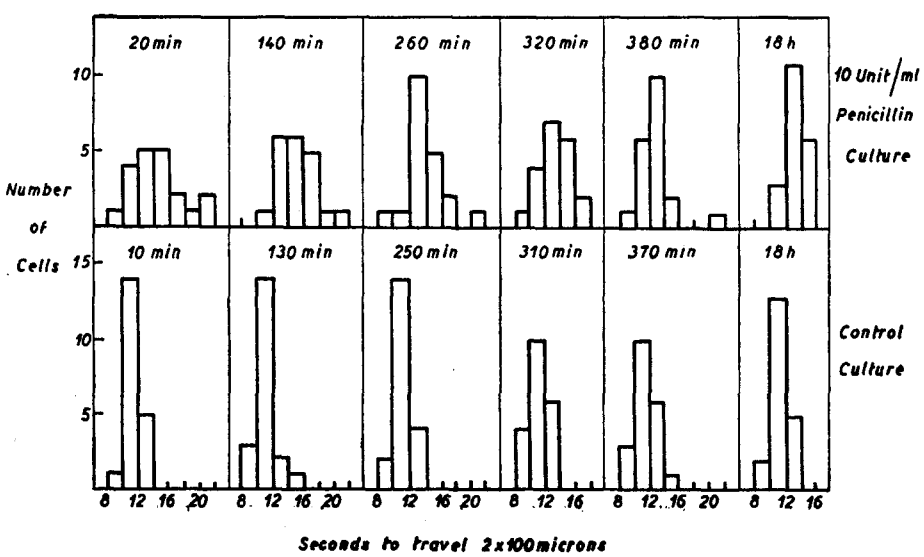

Fig. 6. Distribution of mobilities of *Staphylococcus aureus* growing in presence and absence of 10 unit/ml penicillin

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where the turbidity readings have been converted to dry weights on the basis of the control organisms' calibration curve. Histograms of the mobilities appear in Fig. 6. Progress curves for growth and mobility of both cultures are shown in Fig. 7.

The behaviour of the control culture was more or less constant up to 18 h which is about the duration of the mean generation time under these conditions. The penicillin-treated cells are already much changed in 20 min. The mean mobility continues to fall for about 2 h during which time there is no increase in the turbidity of the culture. Later the mean mobility rises somewhat, coincidently both with a decrease in the scatter of individual mobilities (*i.e.* a tendency towards a more homogeneous population) and with lysis of some of the cells. It is tempting to suppose that those cells whose mobility is most affected are the first to lyse, since this would account for the upward trend of the mean mobility noticeable in a number of experiments after the organisms have grown for some time in the presence of the antibiotic (cf. Table I, column (b) and Table IV).

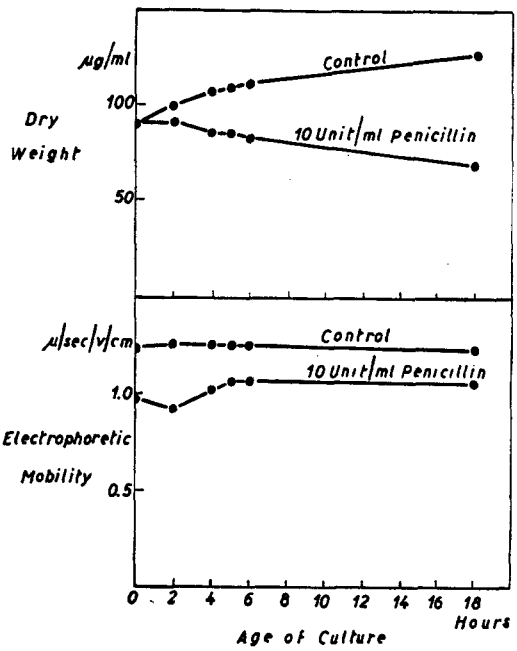

Fig. 7. Progress curves of dry weight (based on turbidity) and electrophoretic mobility of *Staphylococcus aureus* growing in presence and absence of 10 unit/ml penicillin

In many metabolic studies on the mode of action of penicillin it has been found that some growth and reproduction of bacteria takes place before irreversible changes result in the death of the organisms. If the penicillin concentration is not too great and if the affected cells are removed in time, growth in a drug-free medium may be resumed after a lag period. The length of this lag is related to the concentration of penicillin used (PARKER AND MARSH[20], PARKER AND LUSE[21]). The sequence of electrophoretic changes now reported integrates into the general picture which is gradually being built up. The surface changes, if not the first results of the action of penicillin, are certainly the earliest which have so far been reported.

ACKNOWLEDGEMENTS

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SUMMARY

1. No change has been found in the electrophoretic mobility of washed *Staphylococcus aureus* on incubation with penicillin (up to 100 unit/ml) in the absence of growth medium.

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2. Organisms grown in the presence of penicillin (0.1 unit/ml and upwards) show a progressive decrease in electric charge. This decrease is apparent within 10 min even when cultures are growing at 25° C (mean generation time *c.* 90 min).

3. The decrease in mean mobility is accompanied by a progressive increase in the scatter of the mobilities of individual cells. Even after 12 h growth in 1 unit/ml penicillin some of the cells have unchanged electrokinetic properties.

4. Washing does not reverse this effect of penicillin. Essentially the same results are obtained when organisms growing in a full medium containing penicillin are washed and resuspended in buffer, as when mobilities are determined in diluted broth containing the antibiotic.

5. The rate of progress of the penicillin effect is increased by increasing the concentration of drug.

6. The effect can be observed within 20 min in broth diluted 100 times when the mean generation time in the absence of penicillin is *c.* 20 h.

7. A late, partial recovery of the mean mobility may be due to lysis of those bacterial cells most affected.

8. Similar effects have been observed with *Escherichia coli* growing in 100 unit/ml penicillin.

RÉSUMÉ

1. Nous n'avons observé aucun changement de la mobilité électrophorétique de *Staphylococcus aureus* lavé, incubé avec de la pénicilline (jusqu'à 100 unités ml) en absence de milieu de croissance.

2. Des organismes cultivés en présence de pénicilline (0.1 unité/ml et davantage) montrent une décroissance progressive de la charge électrique. Cette décroissance est apparante en 10 minutes, même lorsque les cultures croissent à 25° (le temps de génération moyen étant à peu près 90 minutes).

3. A mesure que la mobilité moyenne décroit les mobilités des cellules individuelles diffèrent de plus en plus les unes des autres. Même après 12 heures de croissance dans un milieu contenant 1 unité de pénicilline par ml, quelquesunes des cellules conservent leur propriétés électrocinétiques inchangées.

4. Cet effet de la pénicilline n'est pas inversé par lavage. Si les organismes croissant dans un milieu complet, contenant de la pénicilline, sont lavés et suspendus à nouveau dans un tampon, l'on obtient essentiellement les mêmes résultats que si l'on détermine les mobilités dans du bouillon dilué contenant l'antibiotique.

5. L'effet de la pénicilline progresse plus rapidement lorsque la concentration de la drogue augmente.

6. Cet effet peut être observé après 20 minutes dans du bouillon dilué 100 fois, lorsque le temps moyen de génération en absence de pénicilline est de 20 heures.

7. Un retour tardif à la valeur initiale de la mobilité moyenne peut être dû à la lyse des cellules bactériennes qui ont été le plus gravement atteintes.

8. Nous avons observé des effets semblables avec *Escherichia coli* croissant dans 100 unités/ml de pénicilline.

ZUSAMMENFASSUNG

1. In der elektrophoretischen Mobilität von gewaschenem *Staphylococcus aureus* wurde bei Inkubation mit Penicillin (bis 100 Einheiten/ml) in Abwesenheit eines Wachtumsmittels keine Veränderung wahrgenommen.

2. In Gegenwart von Penicillin (0.1 Einheit/ml und mehr) gezüchtete Organismen zeigen eine fortschreitende Abnahme der elektischen Ladung. Diese Abnahme ist innerhalb 10 Minuten sichtbar, selbst wenn die Kulturen bei 25° wachsen (durchschnittliche "generation time" ca 90 Minuten).

3. Während der Durchschnittswert der Mobilität abnimmt, nehmen die Unterschiede zwischen den Mobilitäten der einzelnen Zellen allmählich zu. Selbst nach 12stündigem Wachstum in Penicillin (1 Einheit/ml) zeigen einige Zellen noch unveränderte elektrokinetische Eigenschaften.

4. Durch Waschen wird diese Wirkung des Penicillins nicht umgekehrt. Man erhält wesentlich dieselben Ergebnisse, wenn man in einem vollständigen, Penicillin enthaltenden Mittel wachsende Organismen wäscht und aufs neue in einem Puffer suspendiert, als wenn man die Mobilitäten in verdünnter, das Antibioticum enthaltender Bouillon bestimmt.

5. Wird die Penicillinkonzentration erhöht, so schreitet auch die Wirkung der Droge rascher fort.

6. Diese Wirkung kann innerhalb 20 Minuten in 100 mal verdünnter Bouillon beobachtet werden, wenn die durchschnittliche "generation time", in Abwesenheit von Penicillin, 20 Stunden ist.

7. Ein erneutes schwaches Ansteigen der durchschnittlichen Mobilität in späterem Stadium kann vielleicht auf die Lyse der am stärksten angegriffenen Bakterienzellen zurückgeführt werden.

8. Ähnliche Effekte wurden bei *Escherichia coli* in Penicillin (100 Einheiten/ml) beobachtet.

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