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The Absorption of Polymyxin E by Bacteria and Bacterial Cell Walls and its Bactericidal Action

BY A. V. FEW AND J. H. SCHULMAN

Department of Colloid Science, University of Cambridge

SUMMARY: Isotherms of the absorption of polymyxin E by *Bacillus subtilis*, *Pseudomonas denitrificans* and *Streptococcus faecalis* after 20 min. contact with the antibiotic were determined. Tested on seven different bacteria, the maximum amounts of polymyxin E absorbed showed that the polymyxin-sensitive bacteria were capable of absorbing much greater quantities of the antibiotic than resistant organisms. When the bacteria were suspended in buffer solutions, the ultraviolet spectra of their supernatant fluids showed a maximum absorption at 260 m μ ; purines and pyrimidines contribute to this maximum. Addition of polymyxin E to suspensions of sensitive bacteria caused an immediate increase in the height of this maximum. Similar treatment of resistant organisms caused very little immediate increase in the amount of 260 m μ . absorbing material released. On prolonged incubation of untreated and polymyxin E treated cells there was a steady increase in the amount of 260 m μ . absorbing cell solutes released into the suspension media. Within the pH range 4.4-7.6 no optimum pH value was found for the polymyxin E absorption process or for the cellular release mechanism. The absorption isotherms of polymyxin E obtained with cell-wall preparations derived from sensitive and resistant bacteria showed that cell walls derived from sensitive organisms bound more polymyxin E than cell-wall preparations from resistant organisms. On treatment of sensitive bacteria with concentrations of polymyxin E less than those required for 99 % killing in 20 min. at 25°, there was a linear relationship between the amounts of 260 m μ . absorbing material released and the percentage of cells killed. Complete saturation of sensitive organisms with the antibiotic was not necessary to kill the cells. The amounts of polymyxin E required for 99.5 % killing corresponded closely to those sufficient for the formation of a closely packed monolayer within the bacterial cell wall. It is suggested that, at these bactericidal concentrations, polymyxin E combines with and thereby disorganizes the structures responsible for the maintenance of osmotic equilibrium within the cell wall.

The surface-active cationic polypeptide antibiotics of the polymyxin group form stable unimolecular films at the air/water interface when spread upon concentrated ammonium sulphate solution (Few & Schulman, 1953*a*). This observation provides a simple technique for the assay of these antibiotics and makes possible a detailed investigation of the absorption of these agents by susceptible and resistant bacteria and bacterial components.

The five members of this group of antibiotics (polymyxin A, B, C, D and E) were shown by Brownlee, Bushby & Short (1952) to possess similar antibacterial spectra and potency, whilst considerable evidence has accumulated showing that they are rapidly bactericidal and not bacteriostatic (Brownlee & Bushby, 1948; White, Alverson, Baker & Jackson, 1949; Bliss, Chandler & Schoenbach, 1949). White *et al.* (1949) demonstrated the bactericidal and bacteriolytic action of polymyxins A and D, and also found that

electron micrographs of susceptible bacteria exposed to the antibiotics were much less electron dense than those of untreated cells. In microbiological assay procedures, the dependence of the minimum lethal concentration of polymyxin upon the size of the inoculum suggests that bacterial absorption of the antibiotic is high. In particular, the treatment of polymyxin with washed resting suspensions of a susceptible organism resulted in a loss of antibacterial activity, whereas similar treatment of solutions with a resistant organism caused no loss of activity (Bliss *et al.* 1949). This was interpreted as the result of absorption of the antibiotic upon the bacterial bodies of the susceptible organisms.

Phosphatides of the lecithin type interfere with the antibacterial action of polymyxin (Bliss *et al.* 1949), and in this respect polymyxin exhibits properties similar to those of the cationic detergents (Baker, Harrison & Miller, 1941). Rybak (1950) showed that insoluble complex formation takes place between polymyxin and serum albumin; a similar action also occurs with yeast ribonucleic acid (Latterade & Macheboeuf, 1950).

Although there is a similarity in the charge of the cationic detergents, the basic polypeptide tyrocidin and polymyxin, the cationic detergents and tyrocidin are more active against the Gram-positive bacteria, whilst polymyxin is, with some exceptions, only active towards the Gram-negative bacteria. By chemical analysis of a range of bacterial cell walls, Salton (1953) has shown that the cell-wall preparations from the Gram-positive bacteria contain very much less lipid than those derived from the Gram-negative organisms. From studies on the absorption of cetyltrimethylammonium bromide (CTAB) by bacteria and its action in releasing soluble cellular components, Salton (1951) supported the view expressed by Baker *et al.* (1941) that the primary action of surface active agents is that of irreversible damage to the cellular membrane, resulting in the release of cell constituents and dilution of these into the surrounding fluid. Treatment of susceptible bacteria with tyrocidin also causes the release of cellular constituents (Gale & Taylor, 1947).

The present communication describes experiments on the absorption of polymyxin E by sensitive and resistant bacteria and bacterial cell walls, and on its action in releasing cellular constituents possessing a maximum absorption in their ultraviolet spectra at 260 m μ .

EXPERIMENTAL

Polymyxin E. A crystalline sample of polymyxin E sulphate, kindly given by Dr J. W. Trevan, F.R.S., Wellcome Research Laboratories, Langley Court, Beckenham, Kent, was used.

Organisms. Strains of *Pseudomonas denitrificans*, *Bacillus subtilis*, *Bacterium coli*, *Micrococcus lysodeikticus*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Proteus vulgaris*, kindly given by Dr E. F. Gale, were used in this investigation. Determination of the sensitivities of these organisms to polymyxin E, by streaking 24 hr. broth cultures upon nutrient agar containing increasing quantities of the antibiotic, showed that the first four organisms were inhibited by 5 μ g./ml., whilst the others were not inhibited by 100 μ g./ml.

Preparation of bacterial suspensions. The organisms were grown in surface culture on a beef tryptic digest agar medium contained in 20 oz. bottles. The bottles were inoculated with c. 3 ml. of a 24 hr. broth culture, and the organisms were harvested after 16 hr. growth at 37°. The bacteria were washed 4 times by centrifugation in 0.01 M-phosphate buffer (pH 6.3), unless otherwise stated, and finally re-suspended in this medium at a concentration equivalent to 3 mg. dry wt. bacteria/ml. The dry weights of bacteria and of bacterial cell walls were determined directly by drying 1 ml. samples to constant weight in an air-oven at 105°.

Bacterial cell walls. Bacterial cell walls were prepared by disintegration of cells by shaking with no. 12 glass 'ballotini' beads (English Glass Co. Ltd., Empire Road, Leicester) in a Mickle disintegrator. By shaking 9 ml. of a 10 mg. dry wt./ml. bacterial suspension in 0.01 M-phosphate buffer (pH 6.3) with an equal volume of beads more than 95 % of the cells were disrupted after 5 min. The progress of disruption could be followed qualitatively by the examination of Gram-stained smears. The glass beads were removed on a sintered glass filter and the suspensions were then centrifuged at low speed to remove debris and intact bacteria. Centrifugation of the supernatant at 8000 g caused the deposition of the cell-wall fraction, which was washed thoroughly by centrifugation with the phosphate buffer. The cell walls were finally resuspended in this medium and the dry weight determined. With the assistance of Mr H. Pearson of the Cavendish Laboratory, these preparations were examined in the electron microscope; this showed that less than 1 % of intact cells were present. Provided the bacteria were disrupted in 0.01 M-phosphate buffer and subsequently thoroughly washed with this medium no electron-dense cytoplasmic material was found adhering to the cell walls.

Langmuir-Adam surface-balance method for the assay of polymyxin E solutions. Polymyxin E forms stable unimolecular films at the air/water interface when 70 % (w/w) ammonium sulphate solution is used as the aqueous phase in the trough (Few & Schulman, 1958a). At a surface pressure of 16 dynes/cm., where compressibility of the film is at a minimum, 1 mg. polymyxin E occupies an area of 0.80 sq.m. To determine the concentration of polymyxin E in aqueous solution, a sample is spread on the $(\text{NH}_4)_2\text{SO}_4$ trough solution, and the area occupied by the unimolecular film at a surface pressure of 16 dynes/cm. is noted. By comparison of this area with that occupied by 1 mg. of antibiotic the concentration of the polymyxin solution can be calculated. This method is rapid, requires less than 0.5 ml. of solution, and can estimate the polymyxin at a concentration of 5 $\mu\text{g.}/\text{ml.}$ to $\pm 10\%$ and at 100 $\mu\text{g.}/\text{ml.}$ to $\pm 1\%$ (Few & Schulman, 1958b).

Examination of soluble cellular constituents in the supernatant fluid of bacterial suspensions. The ultraviolet spectra of the supernatant fluids obtained from buffered suspensions of untreated and polymyxin E treated cells were investigated by means of a Unicam quartz spectrophotometer. Where necessary, corrections were applied for the ultraviolet absorption of polymyxin E.

Absorption of polymyxin E by bacteria and bacterial cell walls. Unless otherwise stated, bacteria and bacterial cell walls were re-suspended to *c.* 1 mg. dry wt./ml. in 0.01 M-phosphate buffer (pH 6.8) alone and in buffered solutions of polymyxin E. Samples (8 ml.) were incubated in a water-bath at 25°. The organisms, or cell walls, were then removed by centrifugation and the supernatant fluids cleared by recentrifuging. Samples of these solutions were then assayed for unabsorbed polymyxin E by the surface balance method, and for the presence of released cellular constituents by means of the Unicam spectrophotometer. Control experiments in the absence of polymyxin E showed that in general the amount of cellular material released during the incubation period of 20 min. and which formed an insoluble monolayer on the $(\text{NH}_4)_2\text{SO}_4$ trough solution was small, but where necessary a correction for this was applied in the calculation of the amount of polymyxin E absorbed. Furthermore, with certain organisms almost complete bacterial absorption of the polymyxin E occurred, and the trough areas were no greater than those of the controls. This indicated that the ultraviolet absorbing cellular material released by susceptible bacteria in the presence of the antibiotic did not form monolayers upon the trough solution and hence did not interfere with the assay method.

Plate count estimation of surviving cells. Samples (1 ml.) of untreated and polymyxin E treated bacterial suspensions were withdrawn after 20 min. incubation at 25° and added to 9 ml. sterile broth. Further dilutions were then made in broth diluted with 3 vol. of sterile distilled water, and plated out in duplicate on agar medium. The colonies were counted after 24 hr. incubation at 37°.

Surface tensions of polymyxin E solutions. The surface tensions of buffered solutions of polymyxin E were determined at the air/water and *n*-hexane/water interfaces using the micrometer syringe drop volume method. The corrections discussed by Harkins & Brown (1919) were applied in the calculation of the interfacial tensions. Measurements were carried out at 20°.

RESULTS

Absorption of polymyxin E by bacteria

In Fig. 1 the absorption of polymyxin E by *Ps. denitrificans*, *B. subtilis* and *Strep. faecalis* is expressed as a function of the polymyxin concentration in the supernatant. The results show that marked differences existed, both in the shape of the absorption isotherms and in the amounts absorbed by the polymyxin-sensitive organisms, *Ps. denitrificans* and *B. subtilis*, when compared with the isotherm obtained with the polymyxin-resistant *Strep. faecalis*. The initial absorption process was complete at a supernatant concentration of *c.* 50 µg. polymyxin E/ml.; thereafter further absorption was slight. Table 1 shows the maximum amount of polymyxin E absorbed in 20 min. at 25° for a number of sensitive and resistant bacteria. In general the sensitive bacteria absorbed approximately four times as much polymyxin as the resistant organisms. Of four strains of *Bact. coli* investigated, three were agglutinated in the presence of the antibiotic, and complete saturation of the organisms with

polymyxin E was not obtained even at concentrations of 800 μg . polymyxin/ml. of supernatant.

Fig. 1. Absorption of polymyxin E by bacteria. Organisms were incubated at 25° for 20 min. in 0.01 M-phosphate buffer pH 6.8 containing polymyxin E. —●—, *Strep. faecalis*; —○—, *B. subtilis*; —○—, *Ps. denitrificans*.

Table 1. Maximum amounts of polymyxin E absorbed by bacteria in 20 min. at 25°, its relation to their Gram reaction and polymyxin sensitivity

Suspensions in 0.01 M-phosphate buffer pH 6.8.

Organism	Gram reaction	Polymyxin sensitivity	Maximum amount of polymyxin E absorbed ($\mu\text{g./mg. dry wt. bacteria}$)
<i>Ps. denitrificans</i>	—	Sensitive	350
<i>M. lysodeikticus</i>	+	Sensitive	375
<i>Bact. coli</i>	—	Sensitive	> 220
<i>B. subtilis</i>	+	Sensitive	310
<i>Strep. faecalis</i>	+	Resistant	71
<i>Staph. aureus</i>	+	Resistant	84
<i>Pr. vulgaris</i>	—	Resistant	82

Release of ultraviolet absorbing material by bacteria

Examination of the ultraviolet spectra of the supernatant fluids from buffered suspensions of *Ps. denitrificans* alone, and in the presence of polymyxin E, revealed a maximum in their absorption spectra at 260 $m\mu$. The presence of free purines and pyrimidines in the supernatant fluids contribute to this maximum (Dehér, 1906; Salton, 1951). Fig. 2 shows the ultraviolet spectra of these fluids; a marked increase in the 260 $m\mu$. absorption maximum occurred with the cells treated with polymyxin E. Similar results were obtained with other polymyxin-sensitive bacteria, whereas with suspensions of resistant organisms the addition of polymyxin caused only a very small increase in this absorption maximum. In Fig. 3 the release of 260 $m\mu$. absorbing cellular material is plotted as a function of the amount of polymyxin E added to

Fig. 2. Ultraviolet spectra of supernatant fluids from *Ps. denitrificans* suspensions (1 mg. dry wt. bacteria/ml.) incubated at 25° for 20 min. in 0.01 M-phosphate buffer (pH 6.8). —●—, untreated cells; —○—, cells treated with 30 μg. polymyxin E.

Fig. 3. Effect of the addition of polymyxin E on the release of 260 mμ. absorbing material by bacterial suspensions. Organisms were incubated at 25° for 20 min. in 0.01 M-phosphate buffer pH 6.8 containing polymyxin E. —●—, *Strep. faecalis*; —○—, *B. subtilis*; —○—, *Ps. denitrificans*.

suspensions of *Ps. denitrificans*, *B. subtilis* and *Strep. faecalis*. The release of 260 m μ . absorbing cellular material by suspensions of seven organisms treated with sufficient of the antibiotic to give complete saturation of the absorbing sites are recorded in Table 2. These results illustrate the considerable difference in behaviour exhibited by sensitive and resistant organisms.

Table 2. *Maximum amounts of 260 m μ . absorbing material released by bacteria in 20 min. at 25° in the presence of polymyxin E*

Suspensions in 0.01 M-phosphate buffer pH. 6.3.

Organism	260 m μ . absorbing material released (log I_0/I /mg. dry wt. bacteria/ml.)
<i>Ps. denitrificans</i>	0.46
<i>M. lysodeikticus</i>	0.92
<i>Bact. coli</i>	0.24
<i>B. subtilis</i>	0.40
<i>Strep. faecalis</i>	0.06
<i>Staph. aureus</i>	0.02
<i>Pr. vulgaris</i>	0.05

Effect of incubation time upon the absorption of polymyxin E and on the release of 260 m μ . absorbing material

In the preceding sections the absorption of polymyxin E by bacteria and its action in releasing cellular material were studied after an incubation time of 20 min. The effect of incubation time upon these two processes with *B. subtilis*, *Bact. coli* and *Strep. faecalis* are recorded in Table 3. Polymyxin E was added to suspensions of these organisms, and samples were withdrawn at intervals for the determination of polymyxin E absorbed and cellular material released. The bacterial suspensions were shaken by hand for c. 10 sec. at 10 min. intervals. With *B. subtilis* the absorption process was rapid and unaffected by incubation times from 10 to 250 min., whereas with *Bact. coli* and *Strep. faecalis* absorption of polymyxin increased progressively with time of incubation. However, with

Table 3. *Effect of time of contact upon the bacterial absorption of polymyxin E, and the release of 260 m μ . absorbing material*

Suspensions in 0.01 M-phosphate buffer (pH 6.3). A=260 m μ . absorbing material (log I_0/I /mg. dry wt. bacteria/ml.); B=polymyxin E absorbed (μ g./mg. dry wt. bacteria).

Organism		Time (min.)				
		10	30	70	170	250
<i>B. subtilis</i> , untreated	A	0.13	0.14	0.15	0.18	0.31
Polymyxin E treated (400 μ g./ml.)	A	0.50	0.52	0.54	0.62	0.96
	B	310	310	310	303	303
<i>Bact. coli</i> , untreated	A	0.09	0.10	0.11	0.15	0.18
Polymyxin E treated (350 μ g./ml.)	A	0.30	0.33	0.36	0.46	0.53
	B	142	147	174	186	218
<i>Strep. faecalis</i> , untreated	A	0.07	0.07	0.08	0.13	0.19
Polymyxin E treated (250 μ g./ml.)	A	0.11	0.15	0.27	0.39	0.53
	B	62	76	99	130	142

the *Bact. coli* suspensions agglutination of the cells occurred on addition of the polymyxin E, and the dependence upon time of contact may in this case be due to the slow diffusion of the antibiotic into the agglutinated bacteria. Untreated suspensions of the three bacteria showed a steady release of 260 m μ . absorbing material with time of incubation. Addition of polymyxin E to suspensions of *B. subtilis* and *Bact. coli* caused an immediate increase in the amount of 260 m μ . absorbing material released, further material being released as incubation proceeded. Treatment of *Strep. faecalis* suspensions with polymyxin E caused very little immediate release of cellular material, but as incubation continued 260 m μ . absorbing constituents were released, as with the other organisms.

Fig. 4. Effect of pH value upon the absorption by *B. subtilis* of polymyxin E and the release of 260 m μ . absorbing material. Cells incubated at 25° for 20 min. in 0.01 M-acetate buffers (pH 4.4 and 5.3) and 0.01 M-phosphate buffers (pH 6.5 and 7.6) alone, and in the presence of 500 μ g. polymyxin E/ml. —○—, absorption of polymyxin E; —●—, release of 260 m μ . absorbing material from untreated and polymyxin-treated cells respectively.

Effect of pH value upon the absorption of polymyxin E and on the release of 260 mμ. absorbing material

Variation of the pH value of bacterial suspensions medium between pH 4.4 and 7.6 revealed that within this range no optimum pH occurred for the polymyxin E absorption process or for the cellular release mechanism. With *B. subtilis*, *Ps. denitrificans*, *Bact. coli* and *Strep. faecalis* a progressive increase occurred in the amount of polymyxin E absorbed, and 260 m μ . absorbing material released by both untreated and polymyxin E treated organisms when the pH of the suspension medium was between pH 4.4 and 7.6. The results obtained for *B. subtilis* are recorded in Fig. 4.

Relationship between the bactericidal action of polymyxin E and release of 260 m μ . absorbing material

The bactericidal action of polymyxin E and the release of 260 m μ . absorbing material was followed with suspensions of *Ps. denitrificans*, *Bact. coli* and *Strep. faecalis*. Samples were taken after 20 min. incubation at 25° for the estimation of surviving cells and release of cellular material; the results are shown in Table 4.

Table 4. *Bactericidal action of polymyxin E and release of 260 m μ . absorbing material*

Bacterial suspensions at 1 mg./ml. dry wt. in phosphate buffer (pH 6.8); samples taken after 20 min. incubation at 25°. A=log no. viable cells/ml. B=260 m μ . absorbing material released (log I_0/I /ml.).

Polymyxin E concentration (μ g./ml.)	<i>Ps. denitrificans</i>		<i>Bact. coli</i>		<i>Strep. faecalis</i>	
	A	B	A	B	A	B
.	9.48	.	9.20	.	9.72	.
5	9.18	0.08	8.85	0.04		
10	8.85	0.11	8.40	0.06	9.69	0.008
15	8.48	0.15	7.60	0.08		
20	7.40	0.19	6.78	0.10	9.69	0.01
25	4.80	0.24	4.48	0.18		
30	3.10	0.28	.	0.16	9.70	0.03

Absorption of polymyxin E by bacterial cell walls

The absorption isotherms of polymyxin E obtained with buffered suspensions of cell walls derived from *Ps. denitrificans*, *Bact. coli* and *Strep. faecalis* are shown in Fig. 5. As with the absorption isotherms obtained for the intact bacteria, a marked difference in behaviour was shown by cell-wall preparations derived from the polymyxin-sensitive *Ps. denitrificans* and *Bact. coli* when

Fig. 5. Absorption of polymyxin E by bacterial cell walls. Cell walls were incubated at 25° for 20 min. in 0.01 M-phosphate buffer (pH 6.8) containing polymyxin E. —●—, cell walls from *Strep. faecalis*; —○—, from *Bact. coli*; and —○—, from *Ps. denitrificans*.

compared with cell walls prepared from the resistant *Strep. faecalis*. Similar results were obtained with cell-wall preparations of the sensitive *B. subtilis* and the resistant *Staph. aureus*. No 260 m μ . absorbing cellular material was released by the cell-wall preparations on addition of polymyxin E.

Interfacial tensions of polymyxin E solutions

The interfacial tensions of buffered solutions of polymyxin E were investigated at the air/water and *n*-hexane/water interfaces. In Fig. 6 the depression of interfacial tension is plotted against the concentration of polymyxin E. At low polymyxin concentrations, the presence of the non-polar phase greatly facilitates orientation at the interface, whilst at concentrations greater than 20 μ g./ml. further interfacial tension lowering occurs approximately at the same rate at both interfaces.

Fig. 6. Interfacial tensions of polymyxin E in 0.01 M-phosphate buffer (pH 6.8) at 20°. —●—, air/water interface (interfacial tension 72.4 dynes/cm.); —○—, *n*-hexane/water interface (interfacial tension 51.0 dynes/cm.).

DISCUSSION

Polymyxin-sensitive bacteria have a high affinity for polymyxin. Addition of low concentrations of the antibiotic to suspensions of the four sensitive organisms studied resulted in almost complete absorption of the antibiotic upon the bacteria. At higher concentrations saturation of the organisms occurred and, with the exception of *Bact. coli*, uptake of the antibiotic was independent of the concentration of polymyxin E in the supernatant fluid. It appears that as a result of the agglutination of *Bact. coli* suspensions in the presence of the antibiotic the absorption process is impeded and complete saturation of the cells

is not obtained even at high concentrations of polymyxin E. With this exception, the general absence of agglutination when cells are suspended in polymyxin E solutions even at saturation levels suggests that there is little absorption of the antibiotic upon the bacterial cell surface. This is in contrast to the agglutination of bacteria which occurs on treatment with CTAB observed by Salton (1951), although similar quantities of both compounds are absorbed by the bacteria. The shape of the absorption isotherms of polymyxin E with resistant bacteria shows that absorption is less complete at low concentrations of antibiotic, and that the saturation levels obtained after 20 min. contact with the antibiotic are much lower than those observed with the sensitive organisms. However, the amount of polymyxin absorbed at saturation levels by resistant and by sensitive organisms corresponds to more than that required for a closely packed monolayer of the antibiotic upon the bacteria. With the assumption that 1 mg. polymyxin E occupies an area of 0.80 sq.m., i.e. 160 A.²/mol. (Few & Schulman, 1953a) the amount taken up at the saturation level by *Ps. denitrificans* would occupy an area of c. 12 × the cell surface area, assuming the cell dimensions to be 0.8 μ. width and 2.5 μ. length. The corresponding ratios for *Bact. coli* and *Strep. faecalis* are c. 10 × and c. 4 × the cell surface areas, assuming cell dimensions of 1 μ. width, 2 μ. length, and a sphere of 1 μ. diameter, respectively.

The absorption isotherms obtained with cell walls derived from sensitive and resistant bacteria are similar to those obtained for the intact bacteria. Complete absorption of the antibiotic takes place upon cell walls prepared from sensitive bacteria on addition of low concentrations of polymyxin E, whilst absorption is incomplete upon cell walls derived from resistant bacteria. Assuming that the cell wall of the bacteria accounts for c. 15 % of the intact cell on a dry-weight basis (Few, Cooper & Rowley, unpublished observations; Mitchell & Moyle, 1951) the amount of polymyxin E absorbed by the cell walls at saturation levels corresponds to c. 1.8 × c. 2.3 × the cell surface area in the case of *Ps. denitrificans* and *Bact. coli*, respectively, and c. 0.4 × the cell surface area of *Strep. faecalis*. Thus, with the intact organisms, it is evident that at saturation levels polymyxin E absorbs at sites both upon the cell wall and inside the cell.

Addition of polymyxin E to suspensions of susceptible bacteria causes an immediate release of 260 mμ. absorbing material; more than 75 % of the material capable of release in 20 min. at 25° appears when the organisms are less than one-third saturated with polymyxin E. The amount of 260 mμ. absorbing material appearing in the supernatant fluid corresponds closely to that released on treatment of cells with sufficient CTAB to cause 99.99 % mortality (Salton, 1951). Salton also analysed chemically certain of the cellular constituents released by CTAB, and found that the amounts of inorganic and total phosphorus, pentose, glutamic acid, adenine and uracil were comparable to the quantities released when the cell suspensions were pipetted into boiling water. On treatment of sensitive bacteria with polymyxin E at concentrations less than those required for 99 % killing the amount of 260 mμ. absorbing material released in 20 min. is proportional to the percentage of cells killed. A similar

relationship was observed by Gale & Taylor (1947) between the proportion of cells killed by different amounts of tyrocidin and the release of amino-acids, and by Salton (1951) between the proportion of cells killed by CTAB and the release of 260 m μ . absorbing material.

Complete saturation of susceptible organisms with polymyxin E is not necessary to render the cells non-viable. More than 99.5 % mortality is obtained in 20 min. when *Bact. coli* and *Ps. denitrificans* (at 1 mg. dry wt. bacteria/ml.) are treated with 20 and 25 μ g. polymyxin E/ml. At these low polymyxin E concentrations the shape of the absorption isotherms of both intact bacteria and cell-wall preparations shows that complete absorption of the antibiotic takes place. Since the amounts of the antibiotic required for > 99.5 % killing are much below the levels required for complete saturation of the bacterial cell wall it would be expected from the shape of these curves that the distribution of the antibiotic would be such as to favour absorption at sites within the bacterial cell wall, with but little penetration inside the cell. With this assumption, the amounts of polymyxin E required for c. 99.5 % killing approximate closely to those sufficient for the formation of a closely packed monolayer within the bacterial cell wall. Since, as a result of this absorption, there is a rapid release of soluble cellular constituents it is suggested that polymyxin combines with and also disorganizes, by a detergent-type action, the structures within the bacterial cell wall which are responsible for the maintenance of osmotic equilibrium. Once this has taken place there is both a diffusion of cell solutes into the external medium and, provided polymyxin E is present above the bactericidal concentration, a penetration of the antibiotic into the cell and reaction with intracellular components. The oil/water interfacial tension curve for polymyxin E shows that the antibiotic exhibits a strong tendency to absorb at hydrophobic interfaces. It has been pointed out by Hotchkiss (1946) for the case of synthetic surface-active agents that penetration of these substances into the cell would be expected to be appreciable only if accompanied by damage to the cell membrane. The results obtained here with polymyxin E support this general picture, the low and time-dependent absorption of the antibiotic by resistant bacteria being due to its poor penetration of the cell membrane.

It must be emphasized that the reactions of the surface-active antibiotics, and probably the synthetic detergents with the bacterial cell wall, would be expected to be specific interactions dependent not only upon the hydrophobic/hydrophilic balance and stereochemical arrangement of the polar groups present in the antibacterial agent, but also upon the nature of the structures within the bacterial cell wall. A similarity with these bacterial interactions is shown by the study of the mechanism of haemolysis by ionic detergents which possess identical non-polar groups (Pethica & Schulman, 1958). Haemolytic reactivity appears to depend upon detergency, or upon the lowering of interfacial tension, by complex formation with components present in the erythrocyte membrane, and is analogous to the ease of absorption and penetration by these agents of cholesterol monolayers at the air/water interface.

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