creased wetting and dispersion of the powder such that at the highest concentrations used the powder was dispersed to a semi-colloidal state. Short & others (1972), studying similar systems found a pronounced maximum dissolution rate near to the cmc while we did not observe a maximum.

Solubilities of hydrocortisone are shown in Fig. 2. Solulan 16 and 25 are ethoxylated lacohol fractions of lanolin containing respectively 16 and 25 moles of ethylene oxide. Mulley (1964) has shown that altering the ethylene oxide chain number does not alter the general shape of solubility curves hence the solubility of hydrocortisone was determined in Solulan 25 only. Similarly there are only two data points for Solulan 16 dissolution (Fig. 1). As these fit the Solulan 25 line, any further points could be expected to follow the trend. The rate of increase of solubility relative to solubility in 0·1 M HCl is much less than for relative rate of increase of initial dissolution rate. This indicates that changing solubility is unlikely to be a major factor in increased dissolution rates in these systems. The main factor thus appears to be increased dispersion and wetting of the powder. That wetting and dispersion occur appreciably only above the cmc is unexpected. However, on the basis of cmc values, assumptions about the molecular weight of surfactants and of the particle size of hydrocortisone, it is possible to calculate the approximate surface area of the particles and the approximate surface area of surfactant molecules at the cmc. Comparison shows that even if all surfactant molecules were adsorbed they would cover less than 1% of the surface of the hydrocortisone particles. Thus concentrations of surfactant well above the cmc are required to permit sufficient to be adsorbed onto the hydrocortisone particles to give adequate wetting. Solulans, which have a ring structure similar to hydrocortisone, might be expected to be adsorbed more strongly on hydrocortisone than polysorbate. The observed measurements do not indicate any significant difference.

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Electron-microscope studies of the effect of subinhibitory concentrations of phenylethanol and polysorbate 80 on *Pseudomonas aeruginosa* 'sensitive' and 'resilient' to benzalkonium chloride

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The effect of benzalkonium chloride and disodium edetate on the morphology of the cell envelope of *Pseudomonas aeruginosa* has been examined by Richards & Cavill (1976). Evidence was obtained, about the mode of action of the two chemicals used singly and in combination, which further explained their effectiveness in combination against *P. aeruginosa*.

Phenylethanol and polysorbate 80, when used in combination with benzalkonium chloride, also show enhanced activity against *P. aeruginosa* (Richards & McBride, 1972; Brown & Richards, 1964a; Richards, 1975). The current investigation was to further elucidate the action of phenylethanol and polysorbate 80 on the cell envelope of *P. aeruginosa* using similar techniques to those used with benzalkonium and edetate disodium.

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The test organism was *P. aeruginosa* NCTC 6750 and the liquid and solid culture media were nutrient broth No. 2 and Oxoid nutrient agar (Oxoid) respectively. The 2-phenylethanol was a BDH laboratory reagent, the polysorbate 80 was from Hopkin and Williams and benzalkonium chloride (alkyl dimethyl benzylammonium chloride C_{14} 50%, C_{12} 40%, C_{16} 10%) from Rhom and Haas.

Cultures were maintained as described by Brown & Richards (1964b) and incubation was at 37°.

Benzalkonium 'sensitive' cells were prepared from the agar stab stock cultures by inoculating into broth and incubating for 16 h before using as an inoculum.

Benzalkonium 'resistant' cells were prepared using a 16 h broth culture of 'sensitive' cells to inoculate nutrient agar containing benzalkonium chloride. After incubation, a sample was removed from the surface of the agar and used to inoculate nutrient agar plus a higher concentration of benzalkonium until cells of a suitable resistance were obtained. Cells resistant to 200 μg ml^{-1} benzalkonium were used for the experiments with phenylethanol and cells resistant to 800 μg ml^{-1} benzalkonium were used for the experiments using polysorbate 80. In each case a clone of resistant cells was mixed with 5 ml sterile broth and 0·2 ml quantities of this suspension used as an inoculum.

'Sensitive' and 'resistant' cultures were used as sources of inocula for 100 ml quantities of broth or broth plus chemical agent. These cultures were incubated in 250 ml conical flasks shaken at 100 throws min^{-1}, and then examined for cell damage by electron microscopy (Richards & Cavill, 1976).

Phenylethanol. The effect of growing benzalkonium 'sensitive' *P. aeruginosa* for 24 h in the presence of 0·2% v/v phenylethanol is seen in Fig. 1A. When compared with similar cells grown in broth alone (Fig. 1B) it would appear that the outer layers of the cell envelope are not damaged, but the ghosted cell in Fig. 1A may indicate damage to the cytoplasmic membrane. If this is so then cells grown in the presence of benzalkonium and therefore having the outer layers damaged/or stripped off or both (Richards & Cavill, 1976) may on subsequent culture in the presence of phenylethanol produce cells with abnormal outer layers. Benzalkonium 'resistant' cells are known to recover to a normal morphological appearance when grown 16 h in nutrient broth (Richards & Cavill, 1976). This is confirmed.

Fig. 2A shows the effect of growing *P. aeruginosa* on agar plus 200 μg ml^{-1} benzalkonium chloride. An inoculum of these 'resistant' cells grown for 24 h in broth with phenylethanol 0·2% v/v produced cells

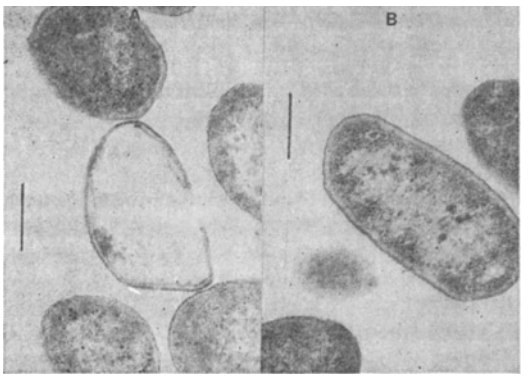

FIG. 1A. *P. aeurginosa* grown in broth plus phenylethanol 0·2%. The bar represents 0·25 μm.

1B. *P. aerunginosa* grown in broth. The bar represents 0·25 μm.

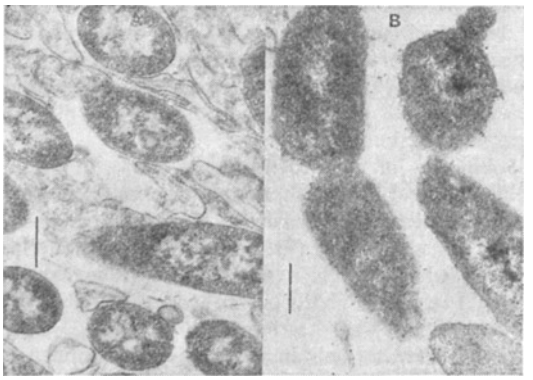

FIG. 2A. *P. aeruginosa* grown on agar containing benzalkonium chloride 200 μg ml^{-1}. The bar represents 0·25 μm.

2B. *P. aeruginosa* resulting from growing cells on agar plus benzalkonium 200 μg ml^{-1} then growing in broth plus phenylethanol 0·2%. The bar represents 0·25 μm.

having outer layers wavy in cross section (Fig. 2B). Phenylethanol 0·2 or 0·3% v/v did not produce this effect on benzalkonium 'sensitive' cells. But this effect was seen when 'sensitive' cells were grown in the presence of disodium edetate (Richards & Cavill, 1976).

These results indicate that the damage to the outer layers of the cell envelope seen in Fig. 2B results from an action of the phenylethanol on the cytoplasmic membrane. The membrane would then be unable to carry out fully its function of producing the outer layers of the cell. This provides an explanation of how phenylethanol exerts a much more potent effect on benzalkonium-treated cells than on cells which have had no contact with benzalkonium chloride. Such an action is likely to form a significant part in the enhanced action of the benzalkonium-phenylethanol combination against *P. aeruginosa*. This mode of action is consistent with the knowledge that phenylethanol is particularly effective against *P. aeruginosa* when in combination with benzalkonium chloride (Richards, 1971; Richards & McBride, 1972) and with the results of Richards & McBride (1973) showing that phenylethanol affects the permeability properties of *P. aeruginosa*.

Polysorbate 80. The benzalkonium 'sensitive' cells grown in 0·5% v/v polysorbate 80 appear to have normal external layers (cf. Fig. 3A with 1B).

Benzalkonium 'resistant' cells, grown for 16 h in nutrient broth, produced cells having a normal morphological appearance, as before, and so did 'resistant' cells grown in broth plus benzalkonium chloride 10 μg ml^{-1} (Fig. 3B). However, 'resistant' cells grown in the presence of ether polysorbate 80 0·02% v/v, or benzalkonium 10 μg ml^{-1} plus 0·2% v/v polysorbate 80, indicate damage to cytoplasmic membrane (Fig. 4A) and cytoplasmic membrane and external layers (Fig. 4B) respectively.

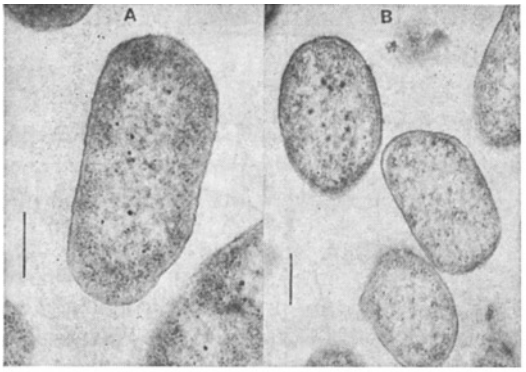

FIG. 3A. *P. aeruginosa* cultured in broth plus 0·5% polysorbate 80. The bar represents 0·25 μm.

3B. *P. aeruginosa* grown on nutrient agar plus 800 μg ml^{-1} benzalkonium chloride then in broth plus 10 μg ml^{-1} benzalkonium chloride. The bar represents 0·25 μm.

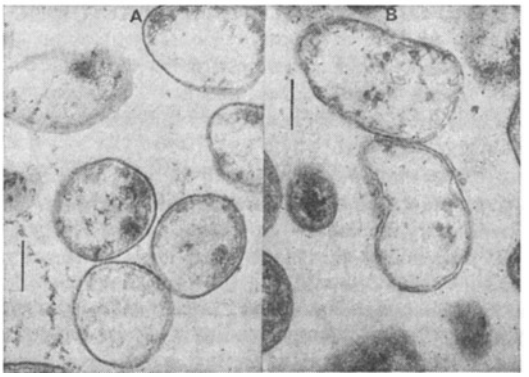

FIG. 4A. *P. aeruginosa* grown on nutrient agar plus 800 μg ml^{-1} benzalkonium chloride then in broth plus 0·02% polysorbate. The bar represents 0·25 μm.

4B. *P. aeruginosa* grown on nutrient agar plus 800 μg ml^{-1} benzalkonium chloride then in broth plus 0·02% polysorbate 80 and 10 μg ml^{-1} benzalkonium chloride. The bar represents 0·25 μm.

Brown (1973) reported that although polysorbate 80 up to 5% had no lytic effect on whole cells of *P. aeruginosa*, much lower concentrations had surprisingly large lytic effects on spheroplasts which were sensitive to 0·005% polysorbate 80.

Thus it may be postulated that polysorbate 80 is able to exert a lethal effect on *P. aeruginosa* cells having cytoplasmic membranes less than wholly protected by an incomplete outer cell envelope. This phenomenon could explain in part the results of Brown & Richards, (1964a) and it should be added to the original postulate that, polysorbate 80, by altering the permeability properties of the cell, enables the benzalkonium to reach its site of activity more effectively. Brown (1975) discusses various actual and possible effects of polysorbate 80 on the permeability of *P. aeruginosa*.

It would appear likely from the results presented here that phenylethanol and polysorbate 80 are both able to take advantage of the damage caused to *P. aeruginosa* by benzalkonium and each exert lethal effects on the cytoplasmic membrane of the cells. Two further points must also be noted; phenylethanol alone has antipseudomonal activity but polysorbate 80 alone has no antipseudomonal activity. In addition polysorbate 80 reacts physically with benzalkonium chloride so that at high concentration polysorbate 80 can totally inactivate the antipseudonomal activity of benzalkonium chloride.

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