Effect of phenylethanol on *Pseudomonas aeruginosa*

Richards, Suwanprakorn & others (1969) and Richards & McBride (1971) reported that phenylethanol enhanced the activity of some ophthalmic preservatives against *Pseudomonas aeruginosa* by an effect on permeability. We have now measured the release of intracellular constituents absorbing at 260 nm from *P. aeruginosa* in the presence of phenylethanol.

The method was a modification of that described by Brown & Winsley (1969). Stationary phase cells of *P. aeruginosa* NCTC 6750 were obtained by centrifuging organisms grown overnight at 37°, at 2500 *g* for 20 min. The cells were washed twice in 0·5M sodium chloride and then resuspended at a concentration of 10^9 cells ml^{-1} (determined spectrophotometrically) in four 100 ml aliquots. After samples had been taken from each flask for viable counts and extinction measurements, sufficient phenylethanol was added to give concentrations of 0·05, 0·1 and 0·2% v/v in three flasks, an appropriate volume of water was added to the fourth flask as control. At 45, 90, 150, 210 and 270 min after the additions, 10 ml samples from each flask were removed for viable counts and extinction measurements. The viable counts were estimated as described by Richards & others (1969). The extinction of the supernatant liquid was measured at 260 nm after centrifugation and suitable dilution. Corrections for the extinction due to the phenylethanol and 0·5M sodium chloride were estimated by measuring the extinction of corresponding dilutions containing no bacteria. The results are shown in Figs 1 and 2.

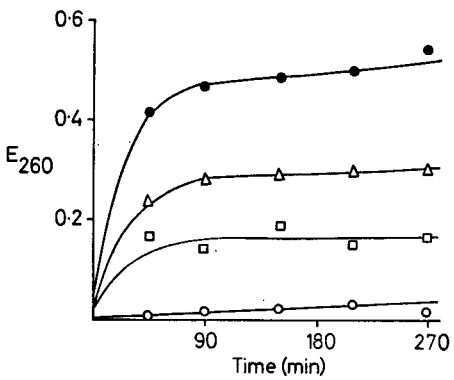

FIG. 1. Release of 260 nm absorbing material from washed cells of *P. aeruginosa* suspended in 0·5M sodium chloride containing 0 (○), 0·05 (□), 0·1 (△) and 0·2 (●) % v/v phenylethanol.

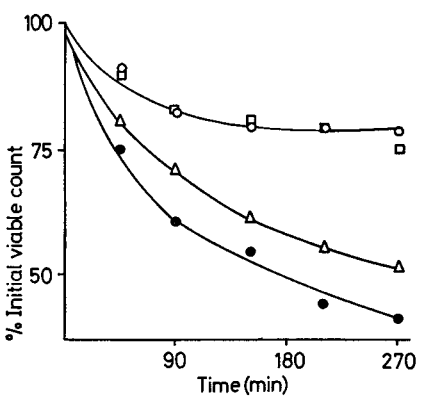

FIG. 2. Viability of washed *P. aeruginosa* suspended in 0·5M sodium chloride containing 0 (○), 0·05 (□), 0·1 (△) and 0·2 (●) % v/v phenylethanol.

The constituents absorbing at 260 nm were released quickly, and after 45 min further release was observed only when the higher concentrations of phenylethanol were present. At the lowest concentration, phenylethanol had no effect on viability, though there was an increase in leakage. Higher concentrations did have an effect of viability (Fig. 2), but the viable count was still about 10^8 cells ml^{-1} after 4 h.

Best, Best & others (1968) described how vancomycin can be used to detect changes in the integrity of the cell walls of Gram-negative organisms and showed that *P. fluorescens* adsorbs much less vancomycin than *B. subtilis*, but after treatment with sodium edetate uptake of vancomycin by *P. fluorescens* was greatly enhanced. We found that *P. aeruginosa* NCTC 6750, which is normally resistant to vancomycin, after exposure to phenylethanol 0·05 to 0·15% v/v showed increased uptake of vancomycin. Above the higher concentration of phenylethanol, uptake was only slightly increased. With an organism susceptible to vancomycin such as *B. subtilis*, 80% of the available vancomycin is taken up; *P. aeruginosa* in the absence of phenylethanol shows no uptake, and a maximum uptake of 26% in the presence of phenylethanol under the same conditions.

These results indicate that phenylethanol has an effect on the cell envelope of *P. aeruginosa* and supports the hypothesis of Richards & others (1969) that a combination of phenylethanol and a bactericide may enable higher concentrations of the bactericide to enter the cell than would enter in the absence of phenylethanol.

Department of Pharmacy,
Heriot-Watt University,
79 Grassmarket,
Edinburgh, EH1 2HJ.

R. M. E. RICHARDS
R. J. MCBRIDE

June 5, 1973

REFERENCES

BEST, G. K., BEST, W. H., FERGUSON, D. V. & DURHAM, N. W. (1968). *Biochem. biophys. Acta*, **165**, 558–560.

BROWN, M. R. W. & WINSLEY, B. E. (1969). *J. gen. Microbiol.*, **56**, 99–107.

RICHARDS, R. M. E., SUWANPRAKORN, P., NEAWBANIJ, S. & SURASDIKUL, N. (1969). *J. Pharm. Pharmac.*, **21**, 681–686.

RICHARDS, R. M. E. & MCBRIDE, R. J. (1971). *Ibid.*, **23**, 1415–1465.

Effects of dopamine receptor stimulation and blockade on Ro 4-1284-induced enhancement of electroshock seizure

Recent investigations have indicated that dopamine or noradrenaline, or both, are functionally important in convulsive seizure mechanisms. For example, Jobe, Picchioni & Chin (1973) reported that noradrenaline is a modulator of audiogenic convulsions, and Azzaro, Wenger & others (1972) suggested that the reserpine-induced reduction of electroshock seizure threshold is related to a reduction of brain catecholamines and 5-hydroxytryptamine (5-HT). Furthermore, De Shaepdryver, Piette & Delaunois (1962) and Billiet, Bernard & others (1970a, b) have presented data indicating that electroshock seizure threshold in rabbits is regulated by the level of brain dopamine. The present investigation provides additional evidence that dopamine is an important modulator in electroshock seizures.

The electroshock seizure pattern (either clonic or tonic) in Sprague-Dawley female (120–180 g) rats was used to test the effects of selected drugs. Since the tonic pattern is more intense than the clonic pattern (Woodbury & Esplin, 1959; Swinyard, 1963), drugs which increase the incidence of tonus obviously enhance seizure intensity, whereas drugs which decrease the incidence of tonus diminish seizure intensity.