Phenylethanol-antibacterial combinations killed the inoculum within 15 min except for one formulation with chlorhexidine and physostigmine salicylate (30 min), and all formulations with PMN (45–90 min). Nevertheless simple solutions of antibacterial alone in these formulations had much slower sterilization times than the phenylethanol combinations.

Benzalkonium with pilocarpine and with physostigmine sulphate had sterilization times within 15 min, but with atropine the time was 60 min. Benzalkonium is either less effective in atropine solutions than in solutions of the two other alkaloids or the inoculum into the atropine had higher intrinsic resistance. The phenylethanol-benzalkonium and EDTA-benzalkonium combinations, however, were both effective within 15 min.

Chlorbutol in simple solution has slower sterilization times with physostigmine salts (30 and 45 min) than with pilocarpine and atropine (15 min). This can be explained in terms of pH. The pH values of the autoclaved solutions are in the range 2·2–2·4 but the range for the steamed solutions is 3·1–3·6.

EDTA-PMN, EDTA-chlorbutol and EDTA-chlorocresol combinations show no clear advantage over the antibacterials in simple solution. The mode of action of the antibacterial agent and the state of resistance of the *P. aeruginosa* may determine whether EDTA enhances antibacterial activity or not.

Chlorhexidine had a slow sterilization time of 180 min with physostigmine salicylate. The sodium metabisulphite in the preparation may be reducing the effectiveness of the chlorhexidine. Phenylethanol-chlorocresol and phenylethanol-chlorbutol combinations sterilize physostigmine salicylate within 15 min.

These results, in conjunction with previous work (Richards, Suwanprakorn & others, 1969; Richards & McBride, 1971a,b; Richards, 1971) support the use of phenylethanol 0·4% in combination with other antibacterial agents in the preservation of ophthalmic solutions against contamination with *P. aeruginosa.*

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Effects of drying on polymyxin sensitivity of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa cultures dried over P_2O_5 during studies on cell wall composition were about 200-fold more resistant to polymyxin than before desiccation. Webb (1967) has reported possible mutagenic effects of desiccation on *Escherichia coli.*

P. aeruginosa strains NCTC 6750 and 1999, NCIB 8625 and several laboratory strains were cultured at 37° in nutrient broth either 100 ml in flasks in a shaking water bath or in 8 litre stirred magnetically. Cells were harvested by centrifugation, unwashed or washed three times in 0·9% NaCl, and the pellets stored in a vacuum desiccator over P_2O_5. The minimum inhibitory concentration (MIC) of polymyxin B sulphate (units/ml) in broth using inocula of 10^6 (total count) in final volume of 5 ml was measured before and after drying. The MIC increased from about 10–20 units/ml to over 2000 units/ml. These increases in resistance occurred with all strains on several occasions. With *P. aeruginosa* NCTC 6750 several consecutive attempts to increase resistance by vacuum drying were unsuccessful although previous and subsequent attempts using the same procedures were successful with this strain.

The resistance persisted through repeated subculture and was associated with colonial variants. These were small cream-yellow colonies similar in appearance to polymyxin resistant mutants obtained by selection. Colonies of both kinds occurred after drying and were picked off the surface of agar plates, diluted and standardized by optical density and the MIC measured. Typical green colonies were polymyxin sensitive and cream-yellow colonies were resistant. Colony plate counts and most probably number estimations in broth showed

between about 99 and 99·9% kill on vacuum drying. Differential colony counts and MIC determinations with varying sized inocula indicated that a high proportion of bacteria surviving vacuum drying were polymyxin resistant. Comparison was made with cultures freeze dried in Stamp's (1947) medium. Freeze drying reduced the count only to about 70% of the original and sensitivity was unaltered.

Vacuum dried whole cells were used as inocula for 8 litre nutrient broth cultures. Whole cell and cell wall preparations were analysed for readily extractable lipid (REL), calcium and magnesium (Brown & Watkins, 1970). The greatest difference was in wall phospholipid and wall Mg which were several-fold less in preparations from vacuum dried inocula.

These results support the hypothesis that drying had mutagenic effects.

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Effect of slime on the sensitivity of *Pseudomonas aeruginosa* to EDTA and polymyxin

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The slime of *Pseudomonas aeruginosa* has been implicated in its resistance to chemotherapy (Brown & Richards, 1964). We have been unable to find published work investigating the rôle of slime in the resistance of this organism. We report *in vitro* studies with ethylene-diaminetetra-acetic acid (EDTA) and polymyxin B sulphate using slime producing gluconate cultures and non-slime producing glucose cultures of *P. aeruginosa* in chemically defined media (Brown, Scott Foster & Clamp, 1969). Sensitivity was measured using methods described by Brown & Melling (1969).

Inocula from 6 day, slimy, stationary phase cultures were incubated in fresh gluconate media and challenged with EDTA and polymyxin immediately they entered the exponential phase. Comparison was made both with 6 day non slimy glucose cultures treated in this way, and also with cultures inoculated with cells in the exponential phase in both media.

Early exponential cultures derived from 6 day stationary phase inocula were more sensitive to both agents than were cultures derived from log phase inocula. Slime slightly enhanced resistance to both agents, especially to polymyxin.

Stationary phase glucose and gluconate cultures incubated for 2 and 7 days were tested for lysis by EDTA and polymyxin. Slime had little effect on polymyxin sensitivity; 2 day cultures were the most sensitive. Slime had a significant effect in reducing EDTA sensitivity.

In general, these *in vitro* results suggest that slime has only a minor rôle in sensitivity to the agents tested. A significant rôle *in vivo* is not excluded.

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