## Resistance of *Pseudomonas aeruginosa* to chemical inactivation

SIR,—The resistance of *Pseudomonas aeruginosa* to chemical antibacterial agents causes serious difficulties in ophthalmology (Brown, Foster, Norton & Richards, 1964) and in the control of cross infection (Rogers, 1960). We have found that the antibacterial activity of polymyxin B sulphate, benzalkonium chloride and chlorhexidine against this organism is substantially increased in the presence of the non-ionic surface-active agent polyoxyethylene sorbitan mono-oleate (Tween 80).

Cultures of *Ps. aeruginosa* NCTC 8203 in nutrient broth were measured in the log phase of growth spectrophotometrically at 420 mμ. Difficulties were met in establishing a satisfactory growth curve because of the short initial log phase, and it was also observed microscopically that the initial rate slowed at the same time as cell clumping increased substantially. *Ps. aeruginosa* produces much slime (Rhodes, 1959) and it seemed likely that this contributed to the clumping. Tween 80 added to the broth in an attempt to disperse the slime and prolong the initial phase. Concentrations of Tween 80 greater than 0·01% satisfactorily increased the length of the initial log phase in nutrient broth, and microscopic examination made concurrently showed that clumping was eliminated.

Antibacterial activity was measured by adding a small volume of a pre-warmed solution of the antibacterial agent to cultures of logarithmically dividing cells and measuring subsequent changes in growth rate (Brown & Garrett, 1964). There was no appreciable effect upon the growth rate in nutrient broth when benzalkonium chloride 35 μg/ml was added to log phase *Ps. aeruginosa* cells. The same concentration immediately reduced to zero the rate in broth

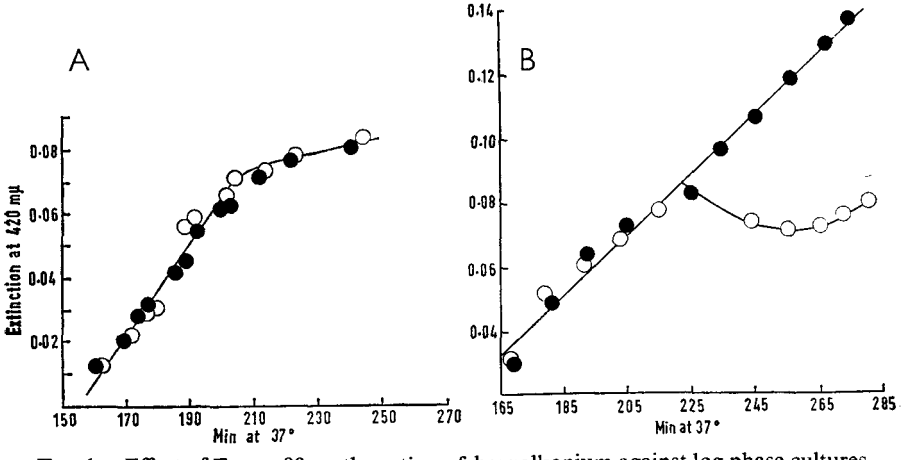


FIG. 1. Effect of Tween 80 on the action of benzalkonium against log phase cultures of *Ps. aeruginosa*. ● Control culture. ○ Benzalkonium added A, to nutrient broth after 18 min; B, to nutrient broth + 0·02% Tween 80 after 223 min.

containing 0·02% Tween 80 (Fig. 1). This effect is particularly remarkable in view of the fact that Tween 80 is an antagonist of benzalkonium (Kohn, Gershenfeld & Barr, 1963). We found that the growth rate in broth with 0·5% Tween 80 was unaffected by the addition of benzalkonium 35 μg/ml. A similar phenomenon was observed with chlorhexidine, when 0·02% Tween 80 significantly enhanced its activity but 0·5% Tween 80 eliminated any observable effect.

The inhibitory effect of polymyxin B sulphate on *Ps. aeruginosa* was enhanced in the presence of all concentrations of Tween 80 tested (0·004–0·5%); the effect increasing with increasing concentrations of Tween 80.

Preliminary experiments using end-point methods have confirmed these results and shown that the activity of our test agents is substantially increased by the presence of Tween 80.

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## Pharmacologically active constituents of *Girardinia heterophylla* (Dcne)

SIR,—*Girardinia heterophylla* (Dcne), a stinging nettle obtained from the Kumaoun hills (India) was tested for the active constituents.

An acetone extract (100% w/v) from the leaves of the plant was prepared. The acetone was immediately evaporated at room temperature and the volume of the extract was made up to the original by adding normal saline. The presence of 5-hydroxytryptamine (5-HT) in the acetone extracted material from *G. heterophylla* was indicated by a spasmogenic response on atropinised oestrus rat uterus, which was completely blocked by brom-lysergic acid diethylamide. Identification of histamine was done after boiling the acetone extracted material with strong hydrochloric acid and removing the acid by distillation (Gaddum, 1953). This acid-treated extract contracted the atropinised guinea-pig ileum and elicited a fall in the cat blood pressure, the two responses were blocked after mepyramine.

Chromatographically, 5-HT was detected by the method of Jepson & Stevens (1953) and histamine by that of Pratt & Auclair (1948). The Rf values obtained by using the solvents (*a*) n-butanol : acetic acid : water (4 : 1 : 5 v/v) were for 5-HT and the extract each 0·25; for histamine and the extract 0·19 and (*b*) n-butanol : ethanol : acetic acid : water (8 : 2 : 1 : 3 v/v) were for 5-HT and the extract each 0·60; for histamine and the extract 0·20.

Thus, the presence of 5-HT and histamine in *G. heterophylla* (Dcne) has been demonstrated both biologically and chromatographically.

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