Interactions between amethocaine (tetracaine) and non-growing cultures of *Escherichia coli*

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That local anaesthetics possess antimicrobial activity has been well documented (Schmidt & Rosenkranz 1970) and concern has been expressed about their influence when they have been administered before sampling for microbiological analysis (Erlich 1961). Studies of their spectrum of activity (Kleinfeld & Ellis 1967; Weinstein et al 1975) and mode of action (Leung & Rawal 1977) have been performed, indicating a similarity with cationic surfactants.

Induced increases in turbidity in non-growing bacterial cultures may be used to provide supporting evidence of a mode of action similar to quaternary ammonium compounds (Salt 1976). This communication reports interactions between the local anaesthetic amethocaine (tetracaine) and *Escherichia coli* (NCTC 1093).

Cells were grown at 37 °C for 6 h in a Gallenkamp orbital incubator using cleated 1 litre flasks containing a medium (400 ml) consisting of (g litre^{-1}): NH_4Cl, 3; $MgCl_2.6H_2O$, 0·200; KH_2PO_4, 0·250; Na_2SO_4, 0·100; glucose, 2; tris-hydroxymethylaminomethane, 12; pH 7·7 (HCl). Exponentially growing cells (100 ml) were harvested by centrifuging the suspension at 5000 rev min^{-1} for 10 min, washing the pellet with, and then resuspending it in, approximately 80 ml fresh medium from which glucose had been omitted and the tris buffer had been replaced with citric acid/phosphate (pH 6·5). The absorbance of the suspensions was adjusted 0·60 (650 nm, 1 cm path) with more medium and the suspension used within 15 min of preparation.

Induced turbidity changes were measured after equal volumes of cell suspensions and solutions of amethocaine had been mixed for 10 min at 20 °C by reading the absorbance of samples (3 ml) of these mixtures at 650 nm using a 1 cm path. For comparison the changes induced by cetylpyridinium bromide (CPB) (BDH) were also assessed. Neither amethocaine nor CPB absorb significantly at 650 nm at the concentrations used.

The minimum inhibitory concentration (MIC) for amethocaine was determined by the tube dilution method using the medium and inoculum concentrations used in the turbidity experiments. Glucose (to 2 mg ml^{-1}) was added after 10 min contact between the non-growing cells and the amethocaine; incubation was for 16–24 h at 37° C.

A critical micelle concentration (cmc) for amethocaine in the glucose-free, citrate buffered medium was deduced from data obtained from the solubilization of Victoria Blue B at 20° C after 5 min agitation. The dye in solution was assayed (absorbance 625 nm) after centrifuging the medium at 5000 rev min^{-1} for 10

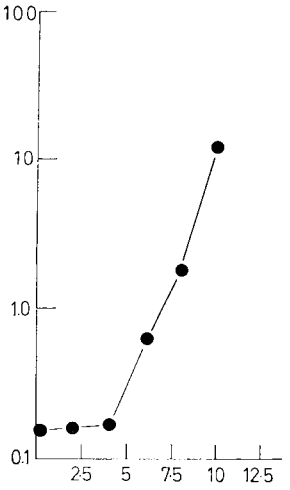

FIG. 1. Solubilization of Victoria Blue B by amethocaine HCl at 20° C in glucose-free medium[4]pH 6·5. Ordinate: Concentration Victoria Blue B (absorbance 625 nm). Abscissa: Amethocaine HCl (mg ml^{-1}).

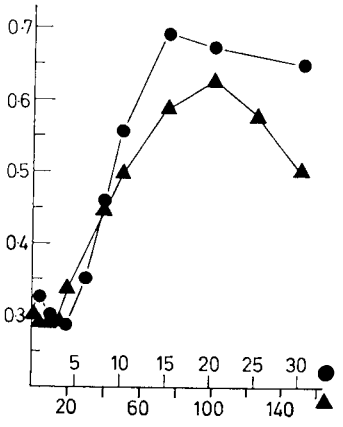

FIG. 2. Turbidity changes in cells of *E. coli* suspended in glucose-free medium pH 6·5 containing (A) CPB ▲—▲ and (B) amethocaine HCl ●—●. Cell concentration 3·2 × 10^8 cells ml.^{1}. Temp. 20° C. Contact time 10 min. Ordinate: Absorbance (650 nm). Abscissa: A CPB (μg ml^{-1}); B Amethocaine HCl (mg ml^{-1}).

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min to remove undissolved dye. The results (Fig. 1) show a distinctive change in slope at approximately 5 mg ml^{-1} amethocaine and this value was taken as the cmc for amethocaine in this medium.

The influence of CPB or amethocaine on culture turbidity is shown in Fig. 2; while both curves are similar, amethocaine is required at a higher concentration to produce the same increase in turbidity. Both compounds exhibit a diphasic pattern of turbidity increase similar to that described for *N*-alkyl trimethylammonium bromides (Salt 1976). Significant changes in turbidity occurred at concentrations in excess of 15 $\mu g\ ml^{-1}$ CPB and 5 mg ml^{-1} amethocaine, a maximum value being attained at approximately 100 $\mu g\ ml^{-1}$ CPB and 16 mg ml^{-1} amethocaine.

The MIC for amethocaine was 0·6 mg ml^{-1}, which was much less than the concentration that caused turbidity increases in non-growing cells. However, values for bactericidal concentrations of amethocaine and other local anaesthetics have been reported by Schmidt & Rosenkranz (1970) and Weinstein et al (1975) and which, allowing for medium and inoculum variation, approximate to concentrations that are active turbidimetrically.

There is a close correlation between the deduced cmc for amethocaine and that concentration in excess of which large increases in culture turbidity are detected. It is thus possible that the uptake of amethocaine by the cells occurs preferentially when the molecules are in the micellar state. However, it is equally possible that amethocaine is taken up by the cells in significant quantities only as the "non-micellar" molecules approach a maximum concentration at or near the cmc.

The results are consistent with the view that amethocaine has a similar mode of antibacterial action to that of the cationic surfactants.

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Mechanisms of dihydroergotoxine's effect on prolactin release

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Dihydroergotoxine (DHE), a mixture of dihydroergocryptine, dihydroergocrystine and dihydroergocornine, has been used for the treatment of mental function disturbances in the elderly (Hughes, Williams & Currier, 1976). Its mode of action has been attributed to various different mechanisms such as improvement of brain blood flow. Particular interest has been devoted to the possible interference of the drug with the central dopaminergic system since it has been hypothesized that during ageing dopaminergic function is impaired (Finch 1973; Carlsson & Winglad 1976; Samorajski 1977; Cotzias et al 1977). In this context, Govoni et al (1977, 1978) have demonstrated that the function of the dopaminergic receptor system is altered in various brain areas of old rats. In the same group of animals serum prolactin concentrations have been reported to be high (Trabucchi et al, in preparation) suggesting a possible decrease of the inhibitory control exerted by dopamine on prolactin release.

Other ergot derivatives such as bromocriptine have been previously studied with behavioural and biochemical methods (Corrodi et al 1973; Fuxe et al 1975; Johnson et al 1976) for their stimulatory effect on the dopaminergic system and for their capacity to reduce prolactin release (Müller et al 1977). In apparent contrast to these observations, it has been shown that bromocriptine and DHE behave as dopamine antagonists (Trabucchi et al 1976; Spano & Trabucchi 1978; Spano et al 1978), when dopamine-stimulated adenylate cyclase and [^{3}H]spiroperidol binding are used as experimental models. We now report the effect of DHE at very low doses on prolactin secretion and dopamine turnover in the striatum.

Mature male Sprague Dawley (Charles River, Italy) rats, 150–175 g, were kept at constant room temperature and humidity and received a standard diet and had free access to water. DHE was injected intraventricularly under ether anaesthesia according to the method of Noble et al (1967). All rats were decapitated at 3 p.m. and the brains quickly removed. The striata were immediately dissected and frozen on dry ice until dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations were assayed. The trunk blood was collected in centrifuge tubes, allowed to clot at 4 °C, and centrifuged at 3000 *g*

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