A STUDY OF FACTORS AFFECTING THE INACTIVATION OF QUATERNARY AMMONIUM COMPOUNDS ON AGAR

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PHARMACEUTICAL preparations for topical use containing antibacterial quaternary ammonium compounds have been reported to show reduced *in vitro* activity when tested by the seeded agar plate method. The "resistance" met by an alkyldimethylbenzylammonium chloride, reported by Heineman[1], was ascribed by Sherwood[2] to the presence of agar. Tobie and Ayres[3], and later Hoogerheide[4], attributed the reduced activity to a slow rate of diffusion owing to the high molecular weights of quaternary ammonium compounds. Phillips[5] amplified this, suggesting that "the ionic aggregates of quaternary ions may be physically too large to pass through the agar (gel) network." Quisno, Gibby and Foter[6] showed that granular agar and agar in sol were equally effective in adsorbing quaternary ammonium compounds and claimed that discrepancies between tests with solid and liquid media could not therefore be explained by differences in diffusion rates. Other factors affecting the development of inhibition zones by quaternary ammonium compounds on seeded agar plates have now been investigated.

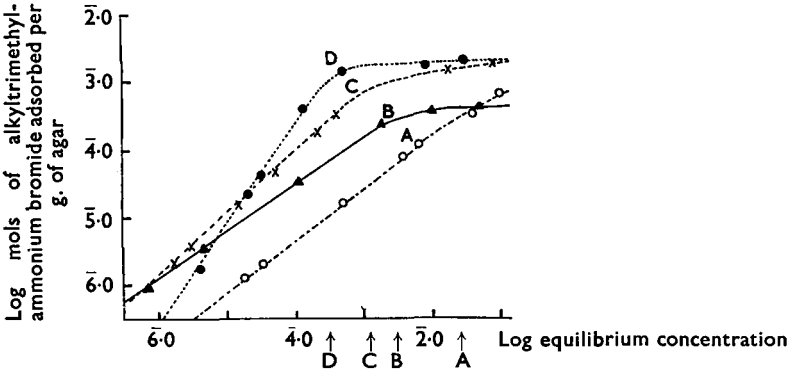

FIG. 1. The adsorption of alkyltrimethylammonium bromide homologues by New Zealand agar at 37°.

A Dodecyl-trimethylammonium bromide
B Tetradecyl- „ „ „
C Hexadecyl- „ „ „
D Octadecyl- „ „ „

The respective critical micelle concentrations[1,2] are indicated by the arrows.

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The adsorption of an homologous series of alkyltrimethylammonium bromides by granular agar was examined by a method adapted from that of Quisno and others[6]. Preliminary work showed that the particle size of the granules made no significant difference to the amount of quaternary ammonium compound adsorbed or to the time required to reach equilibrium. *Davis* New Zealand agar (44/60 mesh) was used throughout: 250 mg. of the agar was stirred for two hours at 37° with 10 ml. of quaternary ammonium compound solution, filtered and the filtrate assayed by the method of Few and Ottewill[7]. This was repeated for concentrations of each homologue from 10^{-1} to 10^{-5} molar and results are given in Figure 1 in the form of Freundlich adsorption isotherms.

TABLE I

THE ADSORPTION OF A SERIES OF ALKYLTRIMETHYLAMMONIUM BROMIDE HOMOLOGUES ON NEW ZEALAND AGAR

(Calculated from Fig. 1)

Homologue	Molecular weight	Freundlich adsorption isotherm for concentrations below the critical micelle concentration	Critical micelle concentration (from Harkins[12])	Approximate maximum weight adsorbed moles/g. of agar
Dodecyl-	308	$X/M = 0{\cdot}006C^{0{\cdot}75}$	0·015	0·001
Tetradecyl-	336	$X/M = 0{\cdot}018C^{0{\cdot}72}$	0·0034	0·00045
Hexadecyl-	364	$X/M = 0{\cdot}448C^{0{\cdot}95}$	0·001	0·0018
Octadecyl-	393	$X/M = 560{\cdot}0C^{1{\cdot}6}$	0·00025	0·002

For each of the four homologues there is a point on the isotherm above which the adsorption process ceases. This point appears to correspond to the critical micelle concentration for any one of the quaternary ammonium homologues and a similar type of phenomenon was observed by Weatherburn and Bayley[8] who investigated the adsorption of surface-active agents on cotton and wool. These authors attributed the saturation achieved to the adsorption of unassociated ions, the ionic aggregates, or micelles, taking little part in the process at or above the critical micelle concentration. The amount of any one homologue adsorbed per unit mass of agar increases with increase of molecular weight up to the critical micelle concentration (Table I) and the maximum amount adsorbed per gram of agar is about 10^{-3} mols.

Whilst this figure may not apply to quaternary ammonium compounds differing in structure from the alkyltrimethylammonium bromide series, its order of magnitude appeared sufficiently small to suggest that, if the interpretation of Quisno and others[6] is tenable and unequal diffusion rates are not responsible for the reduced activity, zones of inhibition should be obtainable on seeded agar plates. In a further search for suitable conditions a number of different quaternary ammonium compounds were investigated using a method based on the seeded agar well-plate method. The materials employed were chemically pure and a series of aqueous solutions from 10^{-2} to 10^{-5} molar were prepared. These were placed in wells prepared in nutrient broth solidified with 1 per cent New Zealand agar seeded with *Staphylococcus aureus*. After incubation the

zones of inhibition were measured and the results plotted as a function of the concentration.

Results obtained to date, in addition to those reported in Figure 2, indicate that if the dose-response curves are interpreted as suggested by Humphrey and Lightbown[9] the "critical" concentration of any quaternary ammonium compound on an agar plate is about the same as the bacteriostatic concentration in liquid media.

The addition of 10^{-4} molar Sky Blue FF to 1 per cent agar gel enables the detection of micelle aggregates within the gel, such a dye being a micelle indicator (Corrin and Harkins[10]), and this is evidence which seems

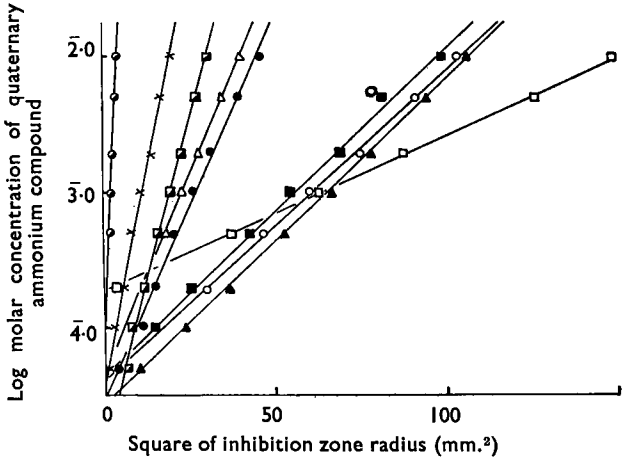

FIG. 2. Dose-response curves for a number of quaternary ammonium compounds when tested against *Staph. aureus* by means of the agar well-plate method.

○ = Dodecyldimethylbenzylammonium chloride. ● = Tetradecyldimethylbenzylammonium chloride. ◔ = Hexadecyldimethylbenzylammonium chloride. □ = Dodecyltrimethylammonium bromide. ■ = Tetradecyltrimethylammonium bromide. ◭ = Hexadecyltrimethylammonium bromide. × = Hexadecylpyridinium chloride. △ = Domiphen bromide. ▲ = Phenoctide chloride ("Octaphen").

to disprove the hypothesis of Phillips[5]. It is not known if the micelles diffuse from the solution in contact with the gel or form at any point within the gel where the concentration of diffusing ions has exceeded the critical micelle concentration. The agar diffusion technique for the investigation of antibody-antigen reactions[11] involving high molecular weight entities indicates that large molecules can diffuse through agar gels. The molecular weights of most antibacterial quaternary ammonium compounds lie between 300 and 400 and these are below the molecular weights of common antibiotics, for example, streptomycin base (582), chlortetracycline hydrochloride (516), oxytetracycline hydrochloride (497) and tetracycline hydrochloride (481).

It is concluded that the inactivation of antibacterial quaternary ammonium compounds by agar is not sufficient to preclude the investigation of preparations containing these compounds by the conventional

seeded agar plate methods. By refrigerating the plates after adding the material under test, but before incubation, the size of the zones of inhibition can be increased and the use of such a technique should enable the testing of antibacterial quaternary ammonium compounds to be undertaken in a manner similar to that employed for materials containing antibiotics.

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After Mr. Groves presented the communication there was a DISCUSSION.