J. Pharm. Pharmac., 1971, **23**, 699–704 *Received May* 19, 1971

The effect of suspensions on the bactericidal activity of *m*-cresol and benzalkonium chloride

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The bactericidal activity of experimental aqueous suspensions of light kaolin with *m*-cresol as preservative agreed closely with the activity of aqueous *m*-cresol solutions not brought into contact with kaolin (i.e. aqueous reference solutions). In contrast the activity of light kaolin suspensions with benzalkonium chloride was lower than that of aqueous benzalkonium chloride solutions because of adsorption of preservative by the kaolin. Suspensions containing benzalkonium chloride possessed a greater activity than the corresponding supernatant solutions removed from contact with the kaolin, owing to some of the bactericide adsorbed on the kaolin becoming available to the bacteria. Suspensions of procaine penicillin with benzalkonium chloride gave similar results to those for kaolin.

The report to the Swedish National Board of Health by Kallings, Ringertz & others (1966) on microbiological contamination of medicinal preparations, focussed attention on the possible need for preservatives in oral mixtures. Kaolinite and bentonite in suspension are capable of stimulating the respiration of some bacterial species (Stotsky & Rem, 1966) suggesting that under suitable conditions the presence of certain suspended solids can provide ideal environments for growth of spoilage organisms.

Loss in antibacterial activity of some antibiotics owing to their adsorption on suspended solid matter has been shown by Pinck (1962) and El-Nakeeb & Yousef (1968), and McCarthy (1969) has pointed to a possible loss in activity of preservatives arising from their adsorption onto solids commonly used as medicaments. The spoilage of the B.P.C. mixture of sulphadimidine for infants is attributed to the adsorption of the preservative, benzoic acid, by the suspended sulphonamide (Beveridge & Hope, 1967).

We have examined the effect of light kaolin on the preservative activity of *m*-cresol and benzalkonium chloride. We chose *m*-cresol since preliminary studies established that it was not apparently adsorbed by the kaolin; hence with this system we looked for the effect, if any, of the presence of suspended solid on bacterial death and particularly for a possible protective effect of the solid to parallel the stimulating effect of some solids on respiration of some organisms shown by Stotsky & Rem (1966) Benzalkonium chloride, in common with other quaternary ammonium compounds, was known to be adsorbed by kaolin (Batuyios & Brecht, 1957) and so the system benzalkonium chloride-light kaolin was chosen to illustrate the effect of preservative adsorption on antibacterial activity and this was compared with the system procaine penicillin-benzalkonium chloride where again adsorption of the quaternary ammonium compound occurred. We were particularly interested in these experimental suspension systems containing benzalkonium chloride to see if they possessed greater antibacterial activity than that found in the suspending medium after removal of the

suspended particles, an effect noted by Batuyios & Brecht (1957) for aqueous suspensions of talc with cetyl pyridinium chloride as preservative. A parallel effect to this was reported by the Conference on the Control of Antibiotics (Ministry of Health, 1957); suspensions of procaine penicillin preserved by cetrimide showed 80% adsorption of preservative and the suspension was more bactericidal than the supernatant from the suspension.

MATERIALS AND METHODS

Materials

Kaolin. Light kaolin B.P. was sterilized by dry heat at 160° for 1 h and stored in an air-tight container. *Procaine penicillin* (Glaxo Laboratories Ltd), potency: 1000 i.u./mg. No microbial contamination was detected. m-*Cresol*, laboratory grade, was redistilled and the fraction boiling at 201–203° was used. *Benzalkonium chloride* (BAK) was a 50% w/v solution (Koch-Light Laboratories). *Polysorbate 80* (Honeywill-Atlas Ltd). *Calcium chloride* was the Analar anhydrous salt. *Penicillinase* (Boots Pure Drug Co Ltd) 1 ml inactivated 800 000 i.u. penicillin in 60 min at room temperature.

Methods

Adsorption of preservatives by solids. The procedure was basically as described previously for the adsorption of phenol on carbon (Bean & Dempsey, 1967). For the adsorption of BAK on procaine penicillin 3 h was sufficient for equilibrium to be established; for the light Kaolin-BAK system, equilibrium was attained in 6 h but for convenience the system was left overnight before assay, as was the *m*-cresol-kaolin system. Supernatant concentrations of BAK were determined using the colorimetric method of van Steveninck & Maas (1965); *m*-cresol was assayed by measuring the absorbance at 271 nm.

Bactericidal activities. The extinction times of the reaction mixtures were determined against *Escherichia coli* NCTC 5933 (20×10^6 organisms/ml) according to Bean & Dempsey (1967) for the *m*-cresol systems. For the BAK-procaine penicillin suspensions a 3 h equilibration period was allowed before commencing the bactericidal reactions. A single tube of indicator broth (Bean & Dempsey, 1967) containing 0·1% w/v Lubrol W non-ionic surfactant was used for the inactivation of each BAK sample, since preliminary experiments showed 0·1% Lubrol to be an effective inactivator for this purpose; in addition, for procaine penicillin-BAK systems, 0·1 ml of penicillinase was added to each tube of recovery medium. For systems containing BAK, 1 ml of reactant sample transferred to 24 ml of recovery medium resulted in a concentration of BAK below the minimum inhibitory concentration (M.I.C.) in the Lubrol indicator broth (M.I.C. 0·014% w/v compared to a value of 0·002% w/v in indicator broth without Lubrol).

The suspension systems tested were essentially experimental and for the light kaolin systems concentrations of suspended solid greater than 5·0 g/100 ml were not investigated since at concentrations greater than this considerable sampling difficulties arose.

RESULTS

The removal of BAK from solution by light kaolin (Batuyios & Brecht, 1957; McCarthy, 1969) followed the L-type isotherm described by Giles, MacEwan &

others (1960); with 2·5 g kaolin/100 ml solution and over an initial BAK concentration range of 37·5 to 56·0 $\times 10^{-3}$% w/v there was approximately 80% adsorption which fell within the plateau region of the isotherm. The adsorption was reversible, at least in part. In contrast, there was no detectable adsorption of *m*-cresol by light kaolin (2·5 g/100 ml) over the range of concentration used (i.e. 0·315 to 0·40% w/v).

The BAK concentrations used for the antibacterial investigations in the presence of light kaolin resulted in flocculated suspensions as shown by sedimentation volume studies (Martin, 1961). Aqueous *m*-cresol appeared to have no flocculating effect upon kaolin and it was decided to produce flocculation by addition of 0·1% w/v calcium chloride to the *m*-cresol systems (Anderson & Fitzgerald, 1967) so that subsequent bactericidal investigations were on suspensions in a similar state of aggregation for both *m*-cresol and BAK systems.

The bactericidal activities of *m*-cresol in the presence of flocculated light kaolin suspensions and the activities of the corresponding supernatants are shown in Table 1.

Table 1. *The bactericidal activity of* m-*cresol against* E. coli *at* 25° *for aqueous suspensions and the corresponding supernatants of light kaolin flocculated by addition of* 0·1% (w/v) *calcium chloride.*

Initial concn *m*-cresol (% w/v)	2·5 g kaolin/100 ml Extinction times (min)		5·0 g kaolin/100 ml Extinction times (min)		Extinction times (min) for aqueous control soln (*m*-cresol/0·1% calcium chloride)
	Suspension	Supernatant	Suspension	Supernatant	
0·315	200	216	220	220	200
0·350	90	92·5	88	85	91·25
0·375	44	43·75	47	51·25	40·0
0·400	30	25	30	29	27·5
0·435	—	—	15	11·75	17·0

Little or no difference existed between suspension and supernatant activity for both 2·5 and the 5·0 g/100 ml kaolin systems; the activities of the aqueous control and the suspension systems were almost identical as was to be expected since there was no obvious adsorption of preservative by the suspended solid.

The suspension system BAK-light kaolin (2·5 g/100 ml) was more active than the corresponding supernatant from the suspension (Table 2). Because BAK flocculated

Table 2. *Bactericidal activity of benzalkonium chloride against* E. coli *at* 25° *in aqueous suspensions and the corresponding supernatants of light kaolin* (2·5 *g*/100 ml).

Initial concn BAK (% (w/v) $\times 10^{-3}$)	Aqueous phase (i.e. equilibrium) concnBAK (% (w/v) $\times 10^{-3}$)	Extinction time (min)		Extinction times (min) of aqueous control BAK at concentrations equal to aqueous phase of suspensions
		Suspension	Supernatant	
38·0	3·75	100·0	>120	83·0
40·0	5·20	79·5	>120	53·7
44·0	8·10	35·06	115	28·8
47·5	10·70	17·6	57·4	19·5
50·0	12·50	10·67	50	15·9
52·5	14·35	—	38·4	12·9
56·0	16·90	<1·0	14	10·2

the light kaolin, its activity was compared with that of a BAK-light kaolin system (2·5 g/100 ml) deflocculated by addition of 0·1% w/v Tween 80. The discrepancy between the suspension and supernatant activities persisted even in the deflocculated system; hence flocculation and therefore possible entrainment of the bacteria within the floccules was discounted as a possible reason for the discrepancy.

The effect of different concentrations of light kaolin suspension on activity is illustrated in Fig. 1 where the logarithm of aqueous phase concentration of BAK, found from adsorption data, is plotted against logarithm of extinction time.

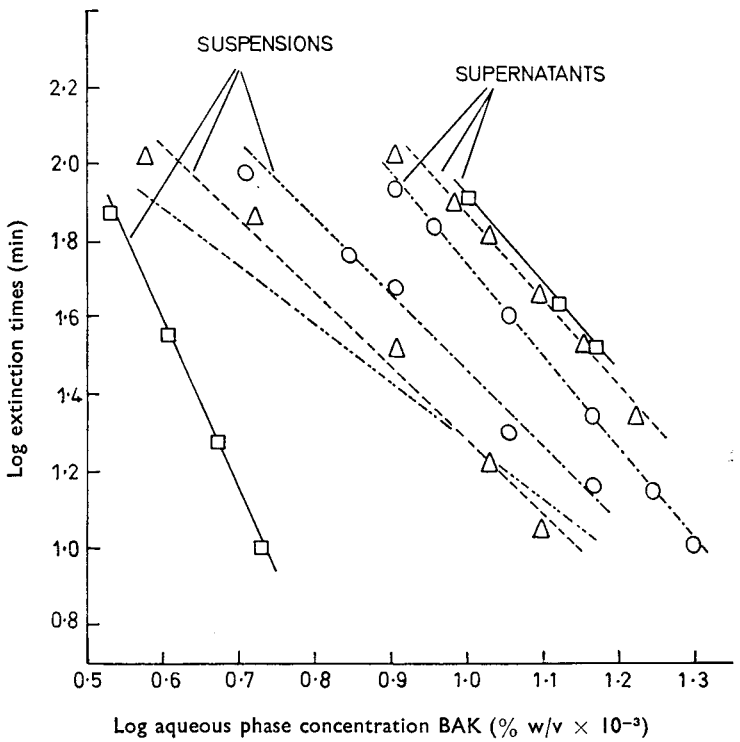

FIG. 1. Activities of suspensions and corresponding supernatants of system BAK/light kaolin against *E. coli* NCTC 5933 (20 × 10^6/ml) at 25°. Aqueous reference: —··—··— Light kaolin concentrations (g/100 ml): —·—○—·—, 0·5; — — —△— — —, 2·5; ——□——, 5·0.

For a fixed aqueous phase concentration of BAK, the antimicrobial activity of the suspension systems increased with increase in amount of suspended solid (i.e. increase in amount of adsorbed BAK), whereas the activity of the corresponding supernatant solutions from the suspensions decreased. The decrease of activity of the supernatants was presumably due to the inactivating effect of cations on quaternary ammonium compounds (Ridenour & Armbruster, 1948), the cations arising by cation exchange when the BAK was adsorbed on the kaolin (van Olphen, 1966); thus the greater the concentration of suspended solid the greater the adsorption of BAK and the greater the number of inorganic cations liberated into solution.

Two different suspended solid concentrations of procaine penicillin were investigated, viz. 1 and 5 g/100 ml. At 5 g/100 ml, the adsorption of BAK averaged 40% over an initial concentration range of 7 to 25 × 10^{-3}% w/v BAK; the adsorption was

in part reversible. An increase in suspended solid concentration resulted in an increase in bactericidal activity for any fixed aqueous phase concentration of BAK. Also, for a fixed aqueous phase concentration of BAK, the activities of the supernatants from the suspensions were less than those of the corresponding suspensions. The supernatant solutions removed from contact with the procaine penicillin had approximately the same activity for a fixed aqueous phase concentration of BAK, irrespective of the initial procaine penicillin disperse phase concentration.

Procaine penicillin in solution had an inactivating effect upon BAK; for a fixed aqueous phase concentration of BAK, the activity of aqueous BAK solution not brought into contact with procaine penicillin was approximately two to two and a half times greater than that of the procaine-BAK supernatant solutions withdrawn from the suspended solid (Fig. 2).

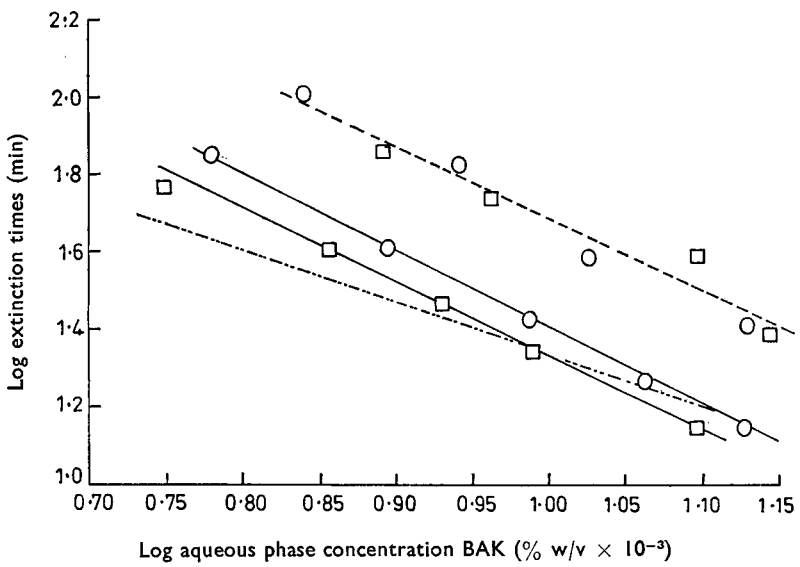

FIG. 2. Activities of suspensions and corresponding supernatants of system BAK/procaine penicillin against *E. coli* NCTC 5933 (20×10^6/ml) at 25°. Aqueous reference: —··—··—. Suspensions: ———. Supernatants: — — — —. Procaine penicillin concentrations (g/100 ml): ○, 1·0; □, 5·0.

DISCUSSION

The *m*-cresol-light kaolin system showed no significant difference between the activity of the suspension and simple aqueous solutions of *m*-cresol, indicating the lack of effect upon preservation of the suspended solid (cf. Stotsky & Rem, 1966) when no adsorption of the preservative was apparent. Where adsorption did occur, as with BAK on both light kaolin and procaine penicillin, the preservative activity of the system was less than that of an aqueous solution of the same total concentration since at least part of the adsorbed BAK was not available to the bacteria. The resultant antibacterial activity of the suspension systems was composed of a contribution from the aqueous residue of BAK plus a contribution from the adsorbed phase. If the activity of the BAK systems had been solely determined by the aqueous phase concentration of the preservative, then the activity of the suspension systems should have agreed with that of the supernatant solutions from them, but in fact, the

suspensions had a greater activity than the supernatants. This phenomenon could be attributed to a release of some of the adsorbed quaternary ammonium compound from the suspended solid since we have found that the inoculum of *E. coli* adsorbs BAK and, since the adsorption of BAK by the solids was in part reversible, the concentration of BAK in the aqueous phase might be partially restored by release from the solid following uptake by the bacteria. In this way the adsorbed phase of BAK on the solids would be acting as a reservoir of preservative. However, the results in Fig. 1, where an increase in suspension activity is shown for increase in suspended solid concentration at fixed equilibrium concentration of BAK, infer that there may have been a direct contribution to activity by the adsorbed phase of BAK quite apart from any activity in the aqueous phase.

It is difficult to predict whether adsorption of preservatives on suspensions will occur, but the surface activity of the preservative may be a guide in this respect; thus BAK is highly surface-active and was highly adsorbed by kaolin whereas the non-surface-active *m*-cresol was not adsorbed.

Acknowledgements

We are indebted to Glaxo Laboratories, Greenford, for the sample of procaine penicillin.

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