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Disinfection of woollen blanket

By W. G. CREWTHER and A. B. McQUADE
Division of Protein Chemistry, C.S.I.R.O., Wool Research Laboratories, Parkville, N. 2 (Melbourne), Victoria, Australia

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INTRODUCTION

Following the demonstration that conventional laundering does not adequately reduce counts of bacteria from blankets (Steingold, Wood & Finch, 1954; Jerram, 1958) there has been concern that blankets may be important reservoirs of pathogens in hospitals (King Edward's Hospital Fund for London, 1959). Methods have been developed for sterilizing (Humfeld, Elmquist & Kettering, 1937; Gillespie & Alder, 1957; Finch, 1958; Foter, 1960; Stratford, Christie & Dixson, 1960; Caplan & Dickinson, 1961), and for boiling woollen blankets (Cunningham, 1956; Dickinson, Wagg & Fairchild, 1959; Pressley, 1960). Although boiling of blankets on a large scale has been routine practice for several years at hospital laundries in Australia (Cowling, 1959; Standards Association of Australia, 1962), and boiling has the advantage that mycobacteria may be destroyed, it has not yet been universally accepted in other countries. In view of this and the observation that conventional laundering with soap is very ineffective in removing bacteria (Blowers & Wallace, 1955; Frisby, 1957; Ravenholt, Baker, Wysham & Giedt, 1958; Schwabacher, Salsbury & Fincham, 1958; Thomas, Liddell & Carmichael, 1958; Larkin, Bridson, Grieve & Gibson, 1961; Dickinson, Wagg & Carter, 1962) the potentialities of washing with bactericides warrant closer investigation. Bactericides have not proved as effective in laundering trials with new woollen blankets as would be expected from the activities of the bactericides as measured by standard methods; the present paper is concerned with the possibility that this low efficiency of the bactericides may be due to their adsorption by the wool.

MATERIALS

Blanket

All-wool blanket was used; it was undyed and not treated for shrink-resistance. For all tests it was used as squares, area 25 cm.^{2} and weight about 1 g.

Cultures

Cultures of *Staphylococcus aureus* and *Escherichia coli* were used for contamination of blanket. These bacteria were isolated from disease sites, and were cultured in the following medium: (g./l.), concentrated beef extract (Oxo Ltd. London), 10; peptone (Bacto-peptone, Difco, Detroit), 10; yeast extract (Difco), 5; K_2HPO_4, 2; $MgSO_4.7H_2O$, 1; (pH = 7). Cultures (50 ml. in 250 ml. conical flasks) were incubated overnight at 37° C., and were started with 1 ml. inocula from similar, fresh cultures.

Bactericides and detergents

Bactericides used were P.C.M.X. (3,5-dimethyl-4-chlorophenol), C.T.A.B. (cetyl trimethyl ammonium bromide), Hibitane (chlorhexidine diacetate, i.e. bis-(4-chlorophenyl diguanido)-hexane diacetate), and detergents were Alkanate D (sodium dodecylbenzene sulphonate 82 %, sodium sulphate 15 %, water 3 % w/w) and Lissapol N 450 (ethylene oxide condensate compound of octyl cresol, Imperial Chemical Industries).

Antagonists

Antagonist for P.C.M.X. was Tween 80 (Erlandson & Lawrence, 1953), and for C.T.A.B. and Hibitane, a mixture of Lubrol W (ethylene oxide condensate compound of aliphatic alcohols, I.C.I.) and egg-yolk extract (Davies, 1949; Davies, Francis, Martin, Rose & Swain, 1954). The egg-yolk extract, supplied by British Drug Houses, was designated as 'Lecithin' 33 %, and the combined medium is known as Lubrol–Lecithin medium; it should be noted however that Davies *et al.* (1954) reported that egg-yolk was an effective antagonist of Hibitane whereas Lecithin was not.

METHODS

Loading of blankets with bacteria

Cells from four cultures were collected by centrifugation, mixed thoroughly with 1 g. of dried skim-milk powder (not sterilized), and freeze dried. The crisp plug of milk and bacteria so formed was transferred to a sterilized ball mill half-filled with glass balls (8 mm. diam.). Five blanket pieces (unsterilized) weighted with

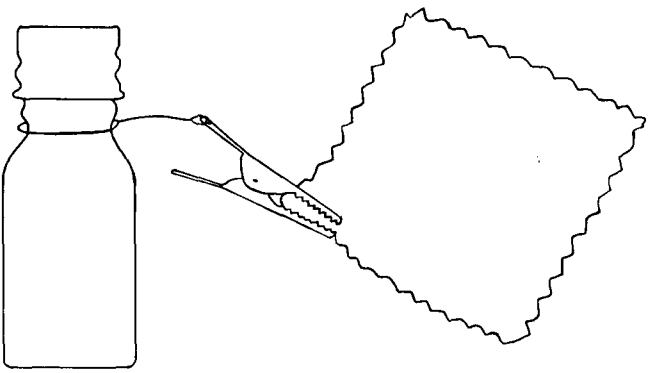

Fig. 1

sterile Macartney bottles (attached by wire and clips, see Fig. 1) were added to the ball mill, and the mill turned slowly for 6 hr. In this way the bacteria and milk formed a fine powder which impregnated the woollen fabric.

Preparation of wash liquors

Wash liquors contained 0·02 M sodium orthophosphate at pH 7·0, and detergents compatible with the bactericides. P.C.M.X. was maintained in solution with Alkanate D; it was dissolved in a minimal volume of ethanol, and added to solutions already containing Alkanate D at concentrations just sufficient to main-

tain solution of P.C.M.X. Lissapol N 450 was added with C.T.A.B. or Hibitane primarily as a washing agent, but its concentration was increased to dissolve Hibitane at high concentrations. For concentrations of agents see Table 1.

Table 1. *Concentrations of agents for pretreatment and washing of contaminated blanket*

	Concentrations of agents (mg./ml.)					
Treatment	PCMX	Alkanate D	CTAB	Lissapol N 450	Hibitane	Lissapol N 450
Pretreatments	4·0	8·0	4·0	0·5	3·2	0·1
Washes	0·5	0·5	0·5	0·5	0·1	0·5

Washing

All washing treatments were run under the following conditions: squares of blanket were immersed in 25 ml. of liquor contained in 100 ml. conical flasks, pre-heated to 50° C. and maintained at this temperature in a water-bath; preliminary tests showed that counts of survivors after washing with bactericides at 50° C. were lower than at 30° C. During washing the liquors and blanket pieces were shaken reciprocally at 130 r.p.m. with amplitude 4 cm. At completion of all washing treatments the blanket squares were rinsed in 25 ml. of water at room temperature (15–25° C.), and spin dried in a basket centrifuge (260 *g* for 90 sec.). In tests of bacterial inactivation care was taken to sterilize the neck of the flask after introduction of contaminated blanket, and operations after washing the blanket were performed to a rapid schedule in order to minimize post-washing kill.

Sampling bacterial populations on blanket

According to a modification of the method introduced by Frisby (1957), and elaborated by Jerram (1958), blanket pieces were cut aseptically to squares about 9 mm.^{2}, and disintegrated in 400 ml. of water by a top-drive macerator (Townson and Mercer, Croydon, England). Densities of bacteria in the supernatant liquor were determined by adding 1 ml. of appropriately diluted liquor to 15 ml. of the culture medium containing 1·5 % Difco agar and antagonist; for P.C.M.X., Tween 80, 1 % (w/v); for C.T.A.B. and Hibitane, Lubrol W, 1 % with egg-yolk extract ('Lecithin') 0·5 %.

Estimation of bactericides

For investigating the uptake of bactericides by the fabric the concentrations of P.C.M.X., C.T.A.B. and Hibitane remaining in the liquors were determined by adaptations of methods according to Folin & Ciocalteu (1927); Epton (1947) and Holbrook (1958) respectively.

EXPERIMENTS

Uptake of bactericides by blanket

Preliminary washes with bactericides at concentrations recommended for laundering indicated that reductions in counts of bacteria from 10^6 to 10^3 could be

expected for phenolic or quaternary ammonium compounds used under the present conditions. In trials with bacteria and bactericides, but without blanket, counts were reduced to zero, hence it seemed that the blanket was inhibiting the action of the bactericides. It is known that wool removes detergents from solution (Crewther, 1956; Goldsmith, Latlief, Friedl & Stuart, 1956); it was therefore considered probable that the bactericides were similarly being absorbed. Furthermore, it seemed possible that this supposed uptake could be inhibited by presaturation of the blanket with bactericides. Accordingly, pieces of blanket were

Table 2. *Loss of bactericides from solutions during pretreatment and subsequent washing of blanket*

		Concentration of bactericide after various times of treatment (mg./ml.)					
		Pretreatment liquor (min.)				Wash liquor (min.)	
Bactericide	Blanket piece no.	0	5	25	125	0	5
P.C.M.X.	1	0	0	0	0	0·5	0·00
	2	0·8	0·72	0·52	0·44	0·5	0·12
	3	1·6	1·4	1·2	0·88	0·5	0·24
	4	2·4	2·0	1·6	1·3	0·5	0·32
	5	3·2	—	2·3	1·5	0·5	0·44
	6	4·0	3·3	3·0	2·6	0·5	0·56
C.T.A.B.	7	0	0	0	0	0·5	0·12
	8	0·8	0·46	0·34	0·26	0·5	0·18
	9	1·6	1·0	0·76	0·65	0·5	0·26
	10	2·4	1·6	1·2	1·1	0·5	0·30
	11	3·2	2·1	1·8	1·5	0·5	0·35
	12	4·0	2·8	2·3	2·2	0·5	0·38
Hibitane	13	0	0	0	0	0·1	0·00
	14	0·8	0·04	0·03	0·00	0·1	0·00
	15	1·6	0·75	0·14	0·03	0·1	0·02
	16	2·4	1·4	0·73	0·44	0·1	0·09
	17	3·2	2·0	1·4	1·1	0·1	0·21
	18	4·0	2·5	2·9	2·9	0·1	0·30

washed in solutions containing bactericides at various concentrations for relatively long periods, and the concentrations of bactericides remaining in the wash liquors determined. The pretreated blanket samples were rinsed, and rewashed in solutions of bactericides at single concentrations for the standard time of 5 min. Bactericides remaining in the second wash liquors were also estimated. The data in Table 2 show that depletion of the bactericides from the wash liquors progressively decreased with increase in the concentrations of these agents in the pretreatments. In further tests with blanket pieces pretreated so that no bactericide was taken up in the second wash, if the second wash was repeated three times it was found that little if any bactericide was taken up in the repeated washes.

Washing of contaminated blanket

To determine whether the pretreatment of blanket with bactericide would lead to an increased bactericidal effect corresponding to the prevention of uptake of

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bactericide from wash liquors, washes were run with contaminated blanket. Blanket pieces were pretreated (125 min.), rinsed, dried, and with untreated pieces as blanks, were contaminated with bacteria. Two sets of pretreated and untreated samples were tested; one set was sampled before washing to check the possibility that bacteria on the treated samples might have been inactivated. This 2^2 factorial design was employed in six separate tests of the three types of bactericide and two species of bacterium. The concentrations of agents were as given in Table 1. The data presented in Table 3 indicate that washing without pretreatment reduced counts from around 10^6 to 10^3, whereas with pretreatment the counts decreased to zero.

Table 3. *Washing of contaminated blanket*

		Bacteria in liquor from macerate of blanket (no./ml.)			
		Blanket not pretreated with bactericide		Blanket pretreated with bactericide	
Bactericide	Species of bacterium	Not washed	Washed	Not washed	Washed
P.C.M.X.	*S. aureus*	1×10^7	3×10	2×10^6	0
	E. coli	5×10^5	2×10^2	6×10^5	0
C.T.A.B.	*S. aureus*	4×10^5	0	7×10^5	0
	E. coli	1×10^5	3×10^2	1×10^5	5
Hibitane	*S. aureus*	9×10^4	3×10^2	2×10^4	0
	E. coli	3×10^5	3×10^3	4×10^5	0

In conjunction with the observation that the pretreatments of the fabric prevented removal of the bactericides from wash liquors, these data indicate that the dense populations of bacteria loaded on to the blanket were virtually destroyed. It was possible, however, that the lowering of the counts was due, at least in part, to bacteriostasis, in spite of the inclusion of antagonists in the plate medium. To check this possibility the amounts of the bactericides leached out during maceration of pretreated, washed and rinsed blanket were determined; the concentrations in the liquors were about 3, 10 and 20 μg./ml. for Hibitane, C.T.A.B. and P.C.M.X. respectively. In controls where antagonist, bacteria (at low densities) and bactericide were added in that order to the agar medium, these quantities of the bactericides did not cause bacteriostasis in the presence or absence of the antagonists. As a further check for bacteriostasis a duplicate set of plates was prepared in all tests, and to the plates were added suspensions of bacteria at calculable densities (1 ml. of the corresponding dilution of the bacterial suspension derived from the untreated, unwashed blanket). Expected counts were obtained except with blanket which had been pretreated and washed; for this treatment combination the counts varied from the expected value to about one-tenth of the expected value. These variations in counts might have been due to bacteriostasis, but were negligible in comparison with the effects attributable to the treatments.

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DISCUSSION

There have been three outstanding difficulties in the problem of blanket hygiene in hospitals. Owing to the complexities of hospital epidemiology it has been difficult to assess the role of the blanket, and it has therefore not been possible to propose standards of laundering known definitely to be appropriate for minimizing cross-infection. Bound with this problem have been the related difficulties of estimating densities of micro-organisms held in fabrics, and the provision of cheap and efficient methods for disinfection.

Methods for sampling populations of micro-organisms on fabrics based on sweeping (Blowers & Wallace, 1955), percussion (McQuade & Sutherland, 1960), and contact (Rubbo & Dixson, 1960) all suffer the disadvantages that the permissible upper limit of count is about 10^3 colonies, and that the sampling fractions are not known. The finding by Frisby (1957) that the sweep-plate method samples 1 in 10^4 bacteria found by maceration points to the inadequacies of the percussion, sweeping and contact methods for analysing processes of disinfection. It follows that the reports that bactericides render blankets virtually sterile (Steingold *et al.* 1954; Blowers & Wallace, 1955) were not necessarily correct. It should be pointed out, too, that natural contaminants include spores, and because these were not distinguished from vegetative cells, the data of the above authors cannot be regarded as measures of the efficacy of the agents they examined, these agents (quaternary ammonium and non-ionic detergents) usually being regarded as virtually non-sporicidal.

The problem of determining the density of micro-organisms on blanket has not been completely resolved; at present no method can be claimed to provide unequivocal estimates of the absolute densities of micro-organisms held by a fabric where the organisms are initially applied as a powder. On the other hand, we were able to recover 100 % of bacteria applied as a broth culture, indicating that the cells were not adsorbed on the fibres, and giving reasonable ground for assuming that methods based on maceration of the fabric give fairly accurate estimates of the original densities of bacteria on woollen blanket.

In assessing the efficiency of disinfection by chemicals the maceration technique has the potential disadvantage that some bactericide may be leached from the treated fabric during maceration, and cause bacteriostasis in the cultures for counting. For this reason agents claimed to inhibit bacteriostasis were routinely added to the medium especially since Rubbo, Stratford & Dixson (1960) confirmed that bacteriostasis by Hibitane was reversed by Lubrol W and Lecithin added to broth cultures. However, under our conditions of culture none of the additives seemed to decrease bacteriostasis; the observed decrease in counts below the expected values may have been due to some cause other than bacteriostasis. It is interesting to note that impregnation of the blanket did not lead to appreciable lowering of recoveries of bacteria applied to the blanket as powder (compare columns 1 and 3 in Table 3) confirming the observation that under conditions of use no bactericidal effect can be expected against dry bacteria on woollen fabric impregnated with bactericide (Rubbo *et al.* 1960).

The demonstration that, in the presence of blanket, bactericides at conventional

concentrations are rapidly reduced to low levels, presumably by adsorption on the wool, provides an explanation for the low bactericidal efficiency of cetrimide found in laundering trials by Dickinson *et al.* (1962). The fact that three chemically different bactericides were adsorbed suggests that bactericides generally may be adsorbed on blankets freshly exposed to these agents. The further observation that pretreatment of the blankets lowers the rate of removal of bactericide from subsequent wash liquors points to the possibility of conserving the activity of bactericides in wash liquors by presoaking the fabrics in concentrated solutions of these agents. On introducing blankets to use, the presoaking could be delayed until immediately before the first wash and it might not be necessary to repeat the pretreatment. In unpublished tests we found also that repeated washing of blanket with bactericides at conventional concentrations led to a progressive increase of residual bactericides in sequential wash liquors; this observation may explain the improvement in cleaning noted by Frisby (1957) on repeated washing of blanket with a quaternary ammonium compound.

Sweep-plate counts for used hospital blankets are generally of the order of 10^3 for an area of blanket approximately 2500 cm.^{2}, and assuming the sweep-plate method samples 1 in 10^4 of the total population in the volume of blanket sampled, the densities would be around 10^5 per 25 cm.^{2}. The densities we achieved by artificial contamination, being of the order of 10^8 bacteria per 25 cm.^{2}, were much higher than would be expected in use; this observation in conjunction with the high kills observed suggest that bactericides properly used might prove very effective in the bacteriological cleaning of woollen fabrics. However, laundering trials would be necessary to determine the practibility of this suggestion because laboratory trials of bactericidal activity must be interpreted with caution (Sykes, 1962). Furthermore, the compatibility of the bactericides with the washing agents must be determined, and optimal conditions for the activity of the bactericides should be found. It is also preferable for the laundering of wool that the pH of the liquors should be kept below 7, and for frequent washing the fabric must be treated for shrink-resistance (Pressley & Morris, 1962).

SUMMARY

The efficiencies of bactericides in destroying bacteria on woollen blanket were investigated on a laboratory scale. The bactericides were not effective when tested with new blanket; this low efficiency was found to be related to the rapid adsorption of the bactericides by the wool. Pretreatment of the wool with concentrated solutions of bactericides depressed the rate of depletion of the bactericides from subsequent washing liquors with bactericides at customary concentrations, and led to more satisfactory rates of kill (inactivation factors about 10^6).

The test organisms, *Staphylococcus aureus* and *Escherichia coli*, were applied to the blanket as a powder, and the relative densities of bacteria on the blanket were determined using a procedure based on maceration of the fabric. The bactericides, 4-chloro-3,5-xylenol (P.C.M.X.), cetyl trimethyl ammonium bromide (C.T.A.B.) and bis-(4-chlorophenyl diguanido)-hexane diacetate (chlorhexidine diacetate) were tested in the presence of appropriate detergents.

Cultures were kindly supplied by Dr Hildred Butler of the Royal Women's Hospital, Melbourne.

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