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Tests on self-disinfecting surfaces

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INTRODUCTION

It might help to reduce the spread of some infectious diseases if surfaces which are liable to bacterial contamination could be treated so as to make them able to kill organisms subsequently deposited on them. That this was so could be shown only by properly conducted field trials, but some information on the self-disinfecting properties of surfaces can be found from suitable laboratory tests. Some tests which have been used for this purpose are unsatisfactory because they do not simulate sufficiently closely the conditions under which the disinfectant has to act in practice. We have therefore attempted a critical evaluation of such tests, and have used a selected technique to assess the self-disinfecting properties of a variety of surfaces and treatments. Our methods are derived largely from those previously described by Klarmann, Wright & Shternov (1953), Lester & Dunklin (1955), and Morris & Darlow (1959). A recent discussion of techniques is given by Walter & Foris (1963).

Because of the small amounts of disinfectant available on a surface, no self-disinfecting surface is likely to be effective against massive contamination. Small drops of liquid contamination will dry rapidly, and dust settling on to a surface will be already dry. Thus, though it is useful to know the effect of the disinfectant on liquid inocula, the results with dry inocula are more generally relevant. These two types of inoculum give different results. If dry materials fall on to a dry, non-volatile, disinfectant layer, solid phase diffusion must take place before the disinfectant can reach the organisms, and this is a very slow process. Where a gaseous disinfectant such as formaldehyde is evolved (Hoffman, Kay & Feazel, 1959; Kingston, Lidwell & Noble, 1962) this limitation does not apply. With a liquid drop, some of the disinfectant will be dissolved and have a chance of reaching the organisms before the drop dries. Thus liquid inocula are often killed more readily than dry inocula. Also, care is needed in sampling since the disinfectant may be reactivated on wetting.

Dry pathogenic bacteria ordinarily occur in the environment associated with dried body fluids, and often on skin scales or textile fibres. Contamination is never likely to be with unprotected organisms, since the median equivalent diameter of airborne particles which carry bacteria is 10–14μ, indicating that there is much associated material (Noble, Lidwell & Kingston, 1963). In ordinary use surfaces

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rapidly become covered with small amounts of dust, and this will give still further protection to bacteria settling on them. For these reasons we thought that a fine cotton dust impregnated with organisms in broth suspension would be a suitable dry test inoculum.

For liquid inocula we used drops of broth culture, since contamination will ordinarily be with infected body fluids and so contain protein materials.

Bacteria can grow only at humidities above about 90% (Scott, 1957). Thus substances which merely inhibit growth will not reduce the numbers of bacteria on a dry surface. We have therefore used techniques designed to test specifically for bactericidal action. This limitation may not apply to fungi, and treatments and test methods used for them must therefore be critically examined before being used for bacteria.

MATERIALS

Organisms

Staphylococcus aureus (NCTC 9789). Phage type 80/81, isolated by Dr P. M. Rountree from a maternity infection.

Escherichia coli. Antigenic structure O.128:B.12:H.2, isolated from a case of infantile gastro-enteritis, and supplied by Dr Joan Taylor (her reference E56/59).

Before the start of the investigation a batch of ampoules was prepared for each organism, by distributing and drying a blood-broth culture.

Media

Broth. Oxoid nutrient broth No. 2.

Agar. Serum agar prepared from Oxoid blood agar base by the addition of 5% horse serum.

Diluent. Physiological saline with 5% broth added.

Resuspending fluid. Digest broth with 0·1% Tween 80 and one egg yolk in 500 ml. (Morris & Darlow, 1959). Tests for each disinfectant with three times the greatest amount which could be eluted showed that, when this fluid was used as described, there was unlikely to be killing during the elution of the bacteria or inhibition due to carry-over of disinfectant on to the plate.

Cotton dust

This was C4 white cotton flock, which is pure cellulose and corresponds to a bleached cotton fabric; it was kindly supplied by Messrs Hutchinson (Ramsey) Ltd.

Test surfaces

The blanket was cut into 1 in. squares, and the other materials into strips $\frac{5}{8} \times 1\frac{5}{8}$ in. (area 1 in.^{2}).

Blanket. 'Aertex' cellular cotton blanket.

Waxed wood. $\frac{1}{8}$ in. thick hardwood (Ramin) strip sprayed with dilute french polish and then coated with Traffic-Wax Paste (Messrs S. C. Johnson and Son Ltd.).

Painted wood. Similar strip painted with one coat of Dulux undercoat white 101 and two coats of Dulux gloss finish white 101 (Imperial Chemical Industries Ltd.).

Floor tile. A plastic floor tile kindly supplied by Marley Floorings Ltd.

Polythene. $\frac{1}{16}$ in. thick sheet (Tenaplas Ltd.).

Chipboard. $\frac{1}{8}$ in. thick strips cut from an old specimen tile. Since they were absorbent, they were coated with urea-formaldehyde resin for experiments with liquid inocula. (Chipboard is made from wood chips bonded with this plastic.) This material evolves formaldehyde (Kingston *et al.* 1962).

PFR (phenol-formaldehyde resin). $\frac{1}{16}$ in. thick laminated paper sheet.

Rubber. One of a series of experimental latex sheets kindly supplied by the British Rubber Producers' Research Association, selected because it produced a wide zone of inhibition on an agar plate seeded with *Staph. aureus*.

The waxed and painted wood, the floor tile and the blanket were used as vehicles for the disinfectant treatments. The floor tile was slightly absorbent, and thus provided an intermediate between the wood surfaces and the blanket. The other materials were tested without any disinfectant, because of the possibility that they might be inherently self-disinfecting, except for the Polythene which was included because it was thought to be unlikely to affect survival.

Disinfectants

Permachem (The Permachem Corporation Ltd.). Bis-tri-*n*-butyl tin oxide in association with various quaternary ammonium compounds. This was supplied in various formulations and the appropriate one used at the strength recommended by the manufacturers. This gave the following concentrations of the tin compound: wax, 0·1 %; blanket rinse, 0·2 g./kg. of dry blanket; dipping water for floor tile and painted wood, 0·004 %.

O-Syl (Lehn and Fink Products Corporation, U.S.A.). 12 % *o*-phenyl phenol in solution with potassium ricinoleate and glycols. Used at a 1/40 dilution. (There is a quite different British product of the same name.)

Savlon (Imperial Chemical Industries Ltd.). 0·3 % chlorhexidine digluconate and 3 % cetrimide. Used at a 1/40 dilution. In some experiments the effect of a moistening agent was investigated by adding 1 % glycerine to the solution used.

The blanket was given a final rinse in the disinfectant solutions, the other strips were dipped in them. The strips were then left in the open until dry.

METHODS

Inoculation of the surfaces

With liquid culture

A fresh ampoule of the dried organism was opened into broth. After overnight incubation a loopful of this culture was used to inoculate a 50 ml. bottle of broth. This broth after 18–24 hr. incubation provided the test inoculum. (All incubations were at 37° C.) Examination of the undried broth cultures by phase-contrast microscopy showed that the *Staph. aureus* culture consisted mainly of groups of two and four cocci, that of the *Esch. coli* of single bacilli. One drop (*c.* 0·02 ml.) from a standard dropper was allowed to fall on to each of the test strips; it was not spread deliberately, but on some of the materials it sometimes did so naturally. Two strips were sampled individually for each determination of survival.

With infected dust

A fresh ampoule of the dried organisms was opened into broth, incubated, and the culture flooded on to four agar plates. After overnight incubation the surface growth on these plates was suspended in 20 ml. of broth and mixed thoroughly with about 20 g. of cotton dust. The dust was then dried *in vacuo* over fused calcium chloride for 7–8 hr., and kept overnight in a chamber with saturated zinc nitrate solution to approximate to its humidity to 42 %. Before dispersal it was ground in a Waring blender and sieved in an exhaust-ventilated cabinet.

The survival during preparation was measured on one occasion and was found to be 17 % for *Staph. aureus* and 0·9 % for *Esch. coli*. Phase-contrast examination of the original suspension and of the suspension eluted from the dust showed that for *Staph. aureus* they both consisted of a wide range of aggregates of cocci, some of considerable size, whereas both of the *Esch. coli* suspensions consisted of single bacilli.

The prepared dust was blown into the top of a chamber 3 ft. × 3 ft. × 7½ ft. high, near a powerful fan. During this period the 60–80 test strips, which were set out on the floor of the chamber, were kept covered with a metal lid attached to the floor by hinges at one end. When the dust had been dispersed the fan was turned off, and after a short pause to allow violent air movement to die down, the cover was raised from outside the chamber. After allowing settling to take place for ¼ hr., the cover was lowered over the strips and a preliminary disinfection of the chamber carried out with ultra-violet light. (This method of disinfection is ineffective against bacteria screened by dust particles, but would kill any particles small enough to remain airborne for any length of time.)

There was considerable variation between the size of the inocula in different experiments; the counts were of the order of $0{\cdot}5 \times 10^6$ organisms per strip, corresponding to 1 mg. of dust. Within an experiment the variation of the counts was in excess of the theoretical minimum (Poisson), and gave a coefficient of variation of the order of 25 %. Accordingly, not fewer than five strips were sampled individually for each determination of survival.

It was possible to inoculate strips with the dust by sieving it manually on to them in a protective cabinet. The weight of dust was about 30-fold greater, but the uniformity, which was controlled by eye, was similar. Since the previous technique required special apparatus, it was thought that this might provide a more convenient alternative.

Estimation of survival

The strips were put into screw-cap 1 oz. bottles ('universals') containing 10 ml. of egg yolk Tween broth and shaken vigorously. The blanket strips were squeezed out and re-wetted several times. Appropriate dilutions were made in broth-saline and five or ten drops from a standard dropper were inoculated on to the surface of well-dried serum agar plates. Colonies were counted after overnight incubation at 37° C. The drops of inoculum were not spread on the medium.

Storage at constant humidity

The inoculated strips were stored at a constant humidity of 42 % and in a very dim light. Those with the dust inoculum were placed in the humidity chambers as soon as the ultra-violet disinfection of the settling room was completed. Owing to the amount of work involved in tests with the drop inocula, these strips were left at laboratory humidity until the 2 hr. sample had been taken.

The strips treated with different disinfectants were kept in different chambers, but there was no evidence that any except the formaldehyde-evolving compounds gave off toxic vapours. With the surfaces which evolved formaldehyde, organisms on control strips in the same chamber were killed and the rate of kill on the test strips was increased. Survival of these surfaces was therefore measured in the open in a well-ventilated room in a dim light. A continuous record was kept of the humidity in this room, and during the experiments it did not differ greatly from 42 %.

The constant-humidity chambers were trays 15 in. × 20 in. × 5 in. deep closed with heavy sheets of glass lying on sponge rubber gaskets. The humidity was controlled with saturated zinc nitrate solution containing excess solid; this is in equilibrium with a relative humidity of 42 % at 20° C. (O'Brien, 1948). The solution had a total surface area of 110 in.^{2}, and the specimens were kept on racks $1\frac{1}{2}$ in. above the surface. Tests showed that under these conditions adequate humidity control was likely to have been achieved, the criticisms of Martin (1962) applying mainly where large masses of absorbent materials are present.

The choice of humidity is important as it affects the natural death rate of the organisms, the rate at which the disinfectant can diffuse to them and the bactericidal activity of the disinfectant. Indoor measurements taken day and night throughout the year do not seem to be available. Daytime readings for the winter months in offices in Newcastle-upon-Tyne and in London (Lidwell & Williams, 1961) gave limits for a series of interquartile ranges as 31 and 62 %. Hourly outdoor wet and dry bulb readings taken for 8 years at Birmingham airport, and kindly supplied by the Meteorological Office, when converted to internal humidity values by an expression inferred from the previous data (internal R.H. = external millibars × 2·8 +26) suggested an interquartile range of 46–60 %. Thus the majority of humidity measurements in centrally heated buildings in England would probably lie between 35 and 60 %. Since zinc nitrate was readily available we adopted 42 % as the standard.

Zones of inhibition

Serum agar plates were flooded with a 1/100 dilution of an overnight broth culture of the organism. As soon as surface moisture had been absorbed, the inhibitory material was placed on the surface of the agar and the plates placed at once in the incubator, stacked not more than two deep. The effect of the liquid disinfectants was tested by using squares of blanket appropriately treated and dried, of the other substances by using strips of the test material itself. The zones of inhibition were examined after 18–20 hr. incubation and are reported as the width of the zone from the edge of the strip.

We think that this method of testing for self-disinfecting activity may be most misleading. However, some tests were done with it so that the results could be compared with those obtained by the other methods.

RESULTS

Control surfaces

Table 1 gives a summary of the percentage survivals found with dust inocula, Table 2 of those with liquid inocula. Preliminary inspection showed no significant difference between the survivals of drop inocula on Polythene, waxed wood, painted wood and floor tile, and these have accordingly been pooled. The values for the mid-point and the scatter of the different distributions, expressed as the median and the interdecile range, were found by plotting cumulative distributions on prob-

Table 1. *Percentage survival of dust inocula on untreated surfaces*

Interval from inoculation	Floor tile		Blanket	
	Median	Interdecile range	Median	Interdecile range
		Staph. aureus		
24 hr.	32	10–54	73	63–104
4 days	5·4	0·3–16	23	8–37
		Esch. coli		
24 hr.	27	7–47	25	18–33
4 days	15	4–25	13	2–23

The median is the point above and below which equal numbers of determinations lie. The interdecile range is the range within which 80% of the determinations lie, i.e. the range excluding the highest and lowest tenths.

Number of determinations for each percentage survival: *Staph. aureus*: floor tile, 6; blanket, 5; *Esch. coli*: floor tile, 7; blanket, 4.

Table 2. *Percentage survival of drop inocula on untreated surfaces*

Interval from inoculation	Other than blanket*		Blanket	
	Median	Interdecile range	Median	Interdecile range
		Staph. aureus		
0 hr.	100	82–117	106	56–156
2 hr.	126	87–165	58	11–140
5 hr.	157	101–213	32	0·6–130
24 hr.	157	101–213	23	4·3–120
5 days	141	68–214	3·9	0·3–11
		Esch. coli		
0 hr.	100	85–115	62	37–87
2 hr.	131	37–190	0·63	0·07–5·6
5 hr.	55	13–150	0·089	0·04–0·3
24 hr.	18	0·2–66	0·040	0·01–0·2
5 days	3·4	0·1–23	0·0072	0·003–0·02

Number of determinations for each percentage survival: blanket, 11; other surfaces, about 35.

* Polythene, waxed wood, painted wood, floor tile.

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ability paper. For the dust-borne organisms the numbers of determinations were small. However for *Staph. aureus* there were, from another series, nine determinations of survival at 24 hr. on floor tile. When these were included the distribution was not significantly changed. Thus the data presented are probably representative.

The results show that determinations of survival carried out under conditions practicable for routine tests give results which are more variable than is often assumed. The survivals tended to be consistently high or low for any one run. This suggests that it would be better to compare the survival on a test surface with the survival on its individual control. However, in the present series of experiments it was found that the reproducibility of duplicate estimations found in different experiments was similar whether comparison was made with the individual or the pooled values. In general, this variability indicates that a number of replicate determinations are needed before it is safe to say that different strains or different conditions give different survivals.

Time of drying

The drops ordinarily dried between the 2 hr. and the 5 hr. sample; the time of drying on the blanket was not determinable.

Table 3. *Survival of dust inocula on treated surfaces as percentage of survival on untreated surfaces*

Treatment	Interval from inoculation	*Staph. aureus*				*Esch. coli*			
		Floor tile		Blanket		Floor tile		Blanket	
Permachem	1 day	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	4 days		+ + +		+ + +	+ + +	+ + +	+ + +	+ + +
Savlon	1 day	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	4 days	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
Savlon + glycerine	1 day	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	4 days	+ +	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+ + +
O-Syl	1 day	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	4 days		+ +		+ + +	+ +	+ + +	+ +	+ + +

		Staph. aureus					*Esch. coli*				
		Rubber		PFR		Chipboard	Rubber		PFR		Chipboard
Untreated miscellaneous surfaces	1 day	+ + +	+ + +	+ + +	+ +	− +	+ + +	+ + +	+	−	
	4 days	+ + +	+ + +	+ + +	+ +	+	+ + +	+ +		−	

+ + +, Survival greater than 50 % of control; + +, survival between 50 and 10 % of control; +, survival between 10 and 1 % of control; −, survival less than 1 % of control. Each of these represents the results of a separate experimental determination. PFR, Phenol-formaldehyde resin.

Disinfectant surfaces

The results for dust inocula are given in Table 3, for liquid inocula in Table 4. They are given in terms of the median values set out in Tables 1 and 2. Since only big differences were considered significant the results have been grouped and tabulated in the following manner: + + +, survival greater than 50 % of control;

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Table 4. *Survival of drop inocula on treated surfaces as percentage of survival on untreated surfaces*

Treatment	Interval from inoculation	*Staph. aureus*									*Esch. coli*									
		Waxed wood		Painted wood		Floor tile		Blanket			Waxed wood		Painted wood		Floor tile		Blanket			
Permachem	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+	++	+++	+++	+++	++	+++	+++	+++	+++	
	5 hr.	+++	+++	+++	+++	++	++	+++	+	−	++	++	++	++	++	+++	+++	+++	+++	
	1 day	+++	+++	+++	+++	++	++	+++	+	−	++	+	++	+++	+++	+++	++	+++	+++	
	5 days	++	++	++	+++	++	+	+	+	−	++	+++	+	+	++	+++	+++	+++	+++	
Savlon	2 hr.	+++		+++		+++		−	−	−	+++		+++		++		+++	+++	⩽ +	
	hr	+++		+++		++		−	−	−	++		+		++		++	⩽ +	⩽ +	
	1 day	+++		+++		++		−	−	−	+++		++		+++		++	⩽ +	+	
	5 days	++		+++		++		−	−	−	+++		+++		+++		⩽ ++	⩽ ++	++	
Savlon+glycerine	2 hr.	+++	+++	+++	+++	+++	+++		++		+++	+++	+++	+++	+	+++		++		
	5 hr.	+++	+++	+++	+++	−	++		+		+++	+++	+++	+++	++	+++		++		
	1 day	+++	+++	+++	+++	+	++		+		+	+++	+	+++	−	+++		++		
	5 days	+++	+++	+++	+++	−	+		−		++	+++	++	+++	−	+++		++		
O-Syl	2 hr.	+++	+++	+++	+++	−	++		−		+	+++	+	+++	⩽ +	+++		⩽ +		
	5 hr.		++		++		−		−			++		++		+		⩽ +		
	1 day	+++	++	+++	+++	+	+		−		−	++	−	+	−	++		⩽ +		
	5 days	+++	++	++	+++	+	+		−		−	+++	−	−	−	−		⩽ ++		

Treatment	Interval from inoculation	*Staph. aureus*				*Esch. coli*			
		Rubber		PFR	Chipboard	Rubber		PFR	Chipboard
Untreated miscellaneous surfaces	2 hr.	+++	+++	+++	++	++	++	+++	++
	5 hr.	+++	+++	+++	+	+	++	+	−
	1 day	+++	++	+++	−	+	++	++	−
	5 days	+++	++	+++	−	++	++	+	−

+++, Survival greater than 50% of control; ++, survival between 50 and 10% of control; +, survival between 10 and 1% of control; −, survival less than 1% of control. ⩽ Equal to or less than the survival shown; no colonies were found at the dilutions tested, and the value given is that which would have resulted from there being three. Each of these represents the result of a separate experimental determination.

+ +, survival between 50 and 10 % of control; +, survival between 10 and 1 % of control; −, survival less than 1 % of control. Thus + + + implies that survival was similar on the control and the test surfaces, but + and − indicate some action of the disinfectant. In assessing the value of the results it must be remembered that to be useful, a reduction must be at least comparable with that brought about by cleaning.

Dust inocula

The results with dust inocula are clear-cut; none of the surfaces showed any useful lethal effect except the chipboard, which evolves formaldehyde. A more extensive investigation of the ability of surfaces which evolve formaldehyde to kill organisms present in dust has been reported in a previous paper (Kingston *et al.* 1962). The addition of glycerine to the Savlon might have facilitated the diffusion of the disinfectant into the bacteria by remaining liquid, but no increased kill was found.

Liquid drop inocula

With these inocula there is more chance of the disinfectant reaching the organism, but the degree of kill will be dependent on the amount of disinfectant available. On the wood surfaces there was no indication of useful kill with *Staph. aureus* suspensions, and on *Esch. coli* only O-Syl had a marked, though somewhat irregular, effect. Tests had shown that *Esch. coli* survived less well if spread over the surface, and since the surface-active agents sometimes caused the drop to spread, some of the slight kill observed with this organism may be due to an effect of spreading.

In contrast to the wood surfaces, which even if wetted retained only a thin film of disinfectant, the floor tile absorbed an appreciable amount, and substantial kills were sometimes found, generally when the drop had spread. (A drop will come into contact with more of the disinfectant on spreading.) Some examples are given in Table 5. A drop was said to have spread if the normal drop outline was not present. In such cases the drop had usually spread across the width of the strip. The effects were much greater than those found when drops were spread in the absence of disinfectant.

The blanket strips absorbed the disinfectant and allowed the drops to spread, and as expected good kills were found (Table 4). The results for *Esch. coli* were rather irregular, in part because this organism survived very poorly when applied to blanket in a drop of broth (Table 1), so that extra kill due to disinfectants was difficult to show. In addition, quaternary disinfectants, which are part of the formulation of Savlon and of Permachem, are less effective against intestinal organisms than against pyogenic cocci. Permachem gave rather irregular results with both organisms, probably because it sometimes prevented the drop of inoculum from being absorbed readily.

The chipboard gave good kills with drop inocula as well as with dust inocula, but the rubber and PFR showed no significant effect.

These results can be summarized by saying that under the conditions of test used here, liquid inocula were killed where there was enough disinfectant absorbed,

particularly if the ratio of disinfectant to inoculum was increased by the drop spreading. Dust inocula were not killed, except by those surfaces which evolved formaldehyde, and these surfaces also killed drop inocula. The addition of glycerine to the Savlon solution did not potentiate kill.

Table 5. *Effect of spreading on the survival on treated floor tile; survival as percentage of inoculum*

Organism	Treatment	Time from inoculation	Percentage survival	
			Drop	Spread*
Staph. aureus	Savlon + glycerine	2 hr.	84	8
		5 hr.	61	⩽ 5
		1 day	31	*c.* 0·004
	O-Syl	2 hr.	61	4
		5 hr.	1	⩽ 0·3
		1 day	6	0·3
		5 days	6	⩽ 0·003
Esch. coli	Savlon	2 hr.	45	1
		5 hr.	17	0·3
		1 day	9	⩽ 0·4
		5 days	2	*c.* 0·008
	Savlon + glycerine	5 hr.	100	1
		1 day	73	0·02
		5 days	25	0·05
	O-Syl	1 day	2	⩽ 0·03
		5 days	*c.* 0·03	*c.* 0·001

⩽ Equal to or less than the survival shown; no colonies were found at the dilutions tested, and the value given is that which would have resulted from there being three.

* The spread drops are those which had spread naturally. The area over which this had occurred was variable, but generally of the order of $\frac{1}{3}$ in.^{2}.

Table 6. *Width of zone of inhibition*

Material	*Staph. aureus*	*Esch. coli*
Blanket	0	0
Blanket (Permachem rinse)	3	0
Blanket (Savlon rinse)	2	0
Blanket (O-Syl rinse)	Trace	Trace
Rubber	6	3
PFR	0	0
Chipboard	3	0

The zone width is given in mm. and is the width from the edge of the strip. The lawns were prepared by flooding serum agar plates with a 1/100 dilution of overnight broth cultures.

Zones of inhibition

The results of this test are given in Table 6. The width of the zone bore no relation to the killing effect as judged by other tests. Thus rubber, which gave no useful kill in the other tests, gave good zones of inhibition on lawns of both organisms. Chipboard, which killed both organisms, gave a zone with *Staph. aureus* only. Tests with other surfaces which evolved formaldehyde gave similar zones, thus confirming

that they were due to formaldehyde and not to other constituents of the chipboard. Blanket treated with O-Syl, which killed both organisms in drop inocula but neither in dust inocula, gave very small zones with both organisms.

The inhibition zone test does not distinguish between bactericidal and bacteriostatic action, and there are two other features which make it liable to indicate unjustifiably that materials tested might have a self-disinfecting action: the bacteria are in good liquid contact with the disinfectant and they may be actively multiplying. Conversely, if the disinfectant were diluted out by the comparatively large bulk of the agar, useful materials might be missed.

The widths of the zones found with different specimen sheets of rubber suggested that the constituent responsible was zinc dimethyl-dithio-carbamate or its homologues.

DISCUSSION

Many authors have found that liquid inocula can be killed by surfaces coated with dried disinfectants; for example, Klarmann *et al.* (1953) who treated a number of surfaces with a variety of disinfectants and then spread loopfuls of culture over them, and Rountree (1946) who impregnated blankets with cetyl pyridinium bromide and sprayed them with streptococci. Both these techniques increase the amount of disinfectant available to the organisms by dispersing them over a large area, and this may explain the high degree of kill these authors found. It is also probable that the elution technique of Morris & Darlow (1959) is more satisfactory than the methods of culturing used by many of the earlier workers, since it is easier to make sure that there has been no kill during sampling, or inhibition of growth due to carry-over of disinfectant. Our results suggest that many surfaces do not absorb enough disinfectant for useful kills to occur, and that the amount of kill is never very large unless the drop spreads.

With dust inocula the only surfaces we found to show any useful kill were those which emitted formaldehyde. Morris & Darlow (1959) tested paints containing disinfectants with organisms settled on to them in 10–15μ diameter particles. They found that, particularly with cetavlon in the paint, good kills were obtained at 70 % R.H., but that unless the organisms were sprayed in glycerol-buffer solutions kills were very poor at 40 % humidity. Rubbo, Stratford & Dixson (1960) found that none of the treatments they tried, which included Permachem and quaternary ammonium compounds but not O-Syl, reduced the level of contamination on blankets in use in the wards. Finegold *et al.* (1962) found no self-disinfecting activity when floors were treated with quaternary ammonium compounds, Permachem, or Amphyl (a disinfectant similar to O-Syl). Our results support these findings. However, Lester & Dunklin (1955) were able to demonstrate some kill by surfaces treated with O-Syl at humidities down to 33 % and even with materials as poorly absorbent as glass. Their test contamination was spray-dried streptococci contained in particles less than 5μ in diameter and, more relevantly, sieved room dust. The reduction in counts they found was not large, but our slightly different test system did not give any useful effect at all. They also showed a large reduction under field conditions, but at humidities rather higher than are usual in England (Dunklin & Lester, 1959).

Claims have been made for rubber as a self-disinfecting surface (e.g. Nopitsch & Möbus, 1958; Dempster, Davy & Swanson, 1959), based chiefly on the ability of rubber either to produce a zone of inhibition on a seeded plate or to kill bacteria maintained in prolonged liquid contact with it. We believe such tests to be misleading.

The self-disinfecting surfaces which seem to us to show the greatest promise are those which slowly evolve formaldehyde. Hoffman *et al.* (1959) give details of a variety of compounds tried as formaldehyde donors, and conclude that paraformaldehyde is the most satisfactory. They also point out that these surfaces will kill spores as well as vegetative forms. Morris & Darlow (1959) found that paints containing paraformaldehyde killed less rapidly than those containing cetavlon. However, the lethal action of surfaces which evolve formaldehyde continues satisfactorily even after drops of broth culture have dried on them, and they are the only surfaces we have found to have a useful effect on dust-borne organisms. The data given by Kingston *et al.* (1962) show that such surfaces kill dust-borne organisms even after several months storage and after being washed.

It is interesting that Caplan (1962), examining the bacterial contamination of blankets after use in hospital wards, found lower levels on those which had previously been sterilized with formaldehyde. Dr V. G. Alder of Bristol (personal communication) has confirmed that blankets which have been sterilized with formaldehyde have self-disinfecting activity. Specially treated fabrics which evolve formaldehyde can cause dermatitis (Marcussen, 1959), though the workers at Bristol have not been troubled with this.

We think the tests we have used simulate the conditions under which self-disinfecting surfaces would need to act in practice, though we may have judged the level of contamination wrongly. However, even if such tests show a treatment to be promising, it does not necessarily follow that it will be useful. For example, cleaning may well be more effective than disinfection in reducing environmental contamination. Finegold *et al.* (1962, 1963) found that the reduction in environmental contamination brought about by certain disinfectant treatments appeared to be due to the cleaning involved in their use. They also found that the reduction in contamination did not bring about a reduction in morbidity.

SUMMARY

A variety of surfaces was tested for self-disinfecting action against *Staph. aureus* and *Esch. coli* at a humidity of 42 %. Surfaces which had been treated with disinfectants sometimes reduced the survival when the organisms were applied subsequently in drops of broth, but the reduction was very small unless the surface was able to absorb appreciable amounts of disinfectant and the drop of inoculum spread. When the inoculum was applied dried in cotton dust, only surfaces which evolved formaldehyde showed any activity against the bacteria. These surfaces also reduced the survival of bacteria in liquid inocula.

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