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Delayed antimicrobial effects of skin disinfection by alcohol

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SUMMARY

Aqueous suspensions of *Staphylococcus aureus* were deposited on a Millipore filter and then exposed for a few seconds to 70% ethyl alcohol. Viable counts of bacteria extracted from the filter immediately after exposure to alcohol, and, in replicate experiments, after a further period of 3 h, showed that the mean immediate reduction of 97·6% in viable counts after treatment with alcohol was followed by a further mean reduction of 67·1% in the further 3 h holding time; the same bacterial suspensions allowed to dry on Millipore filters without exposure to alcohol showed a significantly smaller mean reduction in viable counts (34·3%) during a further 3 h holding time. These findings support the view that the reported further fall in numbers of bacteria on hands while wearing gloves for 3 h after alcohol disinfection can be explained by sublethal damage to some of the bacteria, from which they can recover only if promptly inoculated on culture medium.

INTRODUCTION

After disinfection with hexachlorophane, povidone iodine or chlorhexidine the skin has been shown to retain a residue of the antiseptic which can kill a large proportion of the bacteria in suspension subsequently deposited on the skin (Lowbury, Lilly & Bull, 1964; Lowbury & Lilly, 1973; Lowbury, Lilly & Ayliffe, 1974; Lilly & Lowbury, 1974). Ethyl alcohol, which evaporates rapidly from the skin during disinfection, has not shown this residual effect (Lowbury *et al.* 1974). However, when rubber gloves were worn for 3 h after disinfection of the skin, there was found to be a further reduction during this period in the yield of skin bacteria on quantitative bacterial sampling of the skin, not only after the use of antiseptics which leave an active residue on the skin, but also after the use of ethyl or isopropyl alcohol (Lowbury *et al.* 1974). It seemed that this result might be due to sublethal damage by alcohol to some of the bacteria, and that these damaged organisms might be resuscitated if they were immediately placed in a culture medium, but would die if culture was delayed for 3 h.

This hypothesis was tested in the experiments described here.

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MATERIALS AND METHODS

A strain of *Staphylococcus aureus* (77/3392) isolated from a patient's burn was subcultured on plates of horse blood agar. After 18 h incubation at 37 °C, the entire growth from each plate was carefully scraped off with a wire loop and suspended in 0·5 ml of sterile distilled water, to give a thick suspension. This suspension was dropped in 0·02 ml amounts from standard dropping pipettes onto the centre of sterile Millipore filters of pore size 0·5 μm ('Cellotate' EHWGO 2500, for filtering low molecular weight alcohols) lying in sterile petri dishes. The drops of suspension were spread evenly with a loop over the Millipore filter, leaving a margin approximately 2–3 mm around the edge for handling procedures, and allowed to dry; this took 30–40 min. The disks, in each experiment, were then divided into four groups which were treated as follows:

Group A

The disks were carefully transferred with sterile forceps to a Millipore Microsyringe Filter Holder (25 mm) with an extension barrel. The apparatus was assembled on top of a pyrex flask with a side tube attached to a vacuum pump; 5 ml of distilled water were then sucked through the filter disks, as in Groups C and D (see below). The disks were then extracted by shaking vigorously for 1 min in Universal containers with 10 ml sterile broth saline and glass beads. Colony counts were then obtained from tenfold dilutions in distilled water of the extracts by the method of Miles & Misra (1938) on blood agar plates.

Group B

Disks extracted as in Group A were allowed to stand at 37 °C in semi-darkness for 3 h before the tests for viable counts were set up, as described above.

Group C

Disks were transferred to the Filter Holder as described above, and 5 ml of 70% w/v ethyl alcohol was carefully pipetted on to the filter disk. Suction pressure was applied briefly, allowing 1–2 ml of alcohol to pass quickly through the disk and soak the deposit of bacteria; the remainder of the alcohol was allowed to pass through the filter more slowly (in about 5 s). Five ml of distilled water were then passed through the filter before it was dry, to wash away any residues of alcohol. Filter disks were then extracted and colony counts were obtained as described above.

Group D

Disks were treated as in Group C, but allowed to stand at 37 °C in semi-darkness for 3 h before extraction for colony counts.

In several experiments (1, 2, 3, 4, 5, 6, 13, 14, 16, 17, 18 and 19) single disks for Group A and Group B (control) tests were paired with two disks each in the Group C and Group D tests, the same bacterial suspensions being used for all tests.

TABLE

Viable counts of *Staph. aureus* on membrane filters

Experiment	(A) after drying from aqueous suspension	(B) = (A) followed by 3 h standing	(B) as % of (A)	(C) after drying from aqueous susp. following alcohol treatment	(D) = (C) followed by 3 h standing	(D) as % of (C)
1	329000	247000	75	1900	500	26
2				5400	1200	22
3	512000	401000	78	3200	950	30
4				7800	1100	14
5	286000	203000	71	9400	1750	19
6				2800	320	11
7	392000	273000	70	8500	2700	32
8	208000	127000	61	12600	4900	39
9	539000	365000	68	10400	2800	27
10	347000	227000	65	6800	1000	15
11	170000	75000	44	5200	1900	36
12	290000	180000	62	16300	14000	86
13	425000	312000	73	8500	2100	25
14				2000	5	0·25
15	583000	392000	67	6000	4500	75
16	540000	360000	67	10200	6500	63
17				17000	8000	47
18	625000	410000	66	22500	12500	55
19				13300	1700	13
20	340000	170000	50	13200	3000	23
21	283000	192000	68	6300	2000	32
Mean	408857	279381	65·7*	9014	3496	32·9*

* $P < 0{\cdot}01$ (Wilcoxon's test for two samples).

RESULTS

The Table shows, in each experiment, a large reduction in the numbers of bacteria on filter disks after treatment with alcohol. The further mean percentage reduction in viable bacterial counts on allowing filter disks to stand for 3 h after alchol treatment (67·1 %) was greater than the mean percentage reduction in colony counts from filters allowed to stand for 3 h after drying from aqueous suspension (34·3 %). The two series of percentages were ranked and compared, using Wilcoxon's test (Wilcoxon, 1945) for two samples. This showed that the further reduction in counts after exposure to alcohol was significantly greater than the reduction in counts after drying from aqueous suspension ($P \leqslant 0{\cdot}01$).

DISCUSSION

In earlier studies from this Unit it was found that the numbers of *Staph. aureus* deposited and spread on cover slips from deionized water did not fall, once the film had dried, over a period of 2 h at room temperature (Pettit & Lowbury, 1968). Over longer periods of time (60 h), however, an appreciable fall was found

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(Lowbury & Fox, 1953), and *Staph. aureus* deposited by the fingers of a carrier on a wall exposed to daylight showed a 90% reduction in 24 h (Ayliffe, Collins & Lowbury, 1967).

The experiments reported here show an appreciable reduction in the numbers of *Staph. aureus* deposited from an aqueous suspension on a membrane filter between the time when it appeared to be dry and 3 h later; it is probable that there was still some moisture on the filter when it was judged to be dry, so that the reduction in numbers of bacteria during the period of 3 h may be due, at least in part, to effects of evaporation of the residue of suspending fluid. Another factor may be the temperature (37 °C) at which the dried suspensions were held for 3 h. There was a significantly larger reduction in numbers of bacteria during the 3 h holding period when the bacteria had been exposed to alcohol. This result supported the hypothesis that the further continued death of bacteria on hands after disinfection with alcohol, shown in a previous study (Lowbury *et al.* 1974), is due to sublethal damage to some of the bacteria at the time of disinfection. The absence of any further reduction in counts, also shown in the previous study, after wearing gloves for 3 h following a wash with soap and water shows that such a fall in the numbers of bacteria on the hands would not be expected to occur in the absence of some mechanism active against the bacteria.

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