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The sterilization of a new building designed for the breeding of specific-pathogen-free animals

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SUMMARY

An attempt was made to sterilize a newly erected building of approximately 1200 m.^{3} (43,000 ft.^{3}), specially designed for the breeding of specific-pathogen-free mice, rats and guinea-pigs. Two methods of treatment were used, namely an ampholytic surface acting biocide/formaldehyde aerosol followed after 2 days by formaldehyde vapour. Bacteriological examination was made of 100 sites in the animal rooms, staff quarters and general service area before and after both treatments. Identification of the bacteria isolated was based upon their morphological appearance on laboratory media incubated aerobically and their reaction to Gram's stain. Organisms were isolated from 72/100 sites before treatment, from 50 sites after the first treatment, and from 13 sites after the second treatment. The bacteria that survived both treatments were of several species.

INTRODUCTION

Before stocking a newly erected specific-pathogen-free (S.P.F.) building it was necessary to reduce to a minimum the numbers of micro-organisms present. This need provided a good opportunity to examine the bacterial flora present and to observe the efficiency of conventional methods of fumigation designed to reduce bacterial contamination. Two techniques were adopted, first an ampholytic surface acting biocide with formaldehyde was employed as an aerosol. This was followed 2 days later by fumigation with formaldehyde vapour. The effectiveness of the two methods in reducing the number of contaminated sites is described in this paper.

MATERIALS AND METHODS

The building

The building (see Fig. 1) contains ten animal rooms, each approximately 70 m.^{3} (2500 ft.^{3}) in capacity, a general service area of 350 m.^{3} (12,300 ft.^{3}), a staff room and kitchen of 41 m.^{3} (1450 ft.^{3}), a store of 19 m.^{3} (700 ft.^{3}), an office of 19 m.^{3} (700 ft.^{3}), and two toilet/shower areas of 39 m.^{3} (1400 ft.^{3}). Together these rooms and areas constitute the 'clean' area. Entrance to this area by personnel is via the shower units and for materials via the 'dunk-tank' or autoclave. These portals of entry constitute the 'barrier'. The heating, lighting,

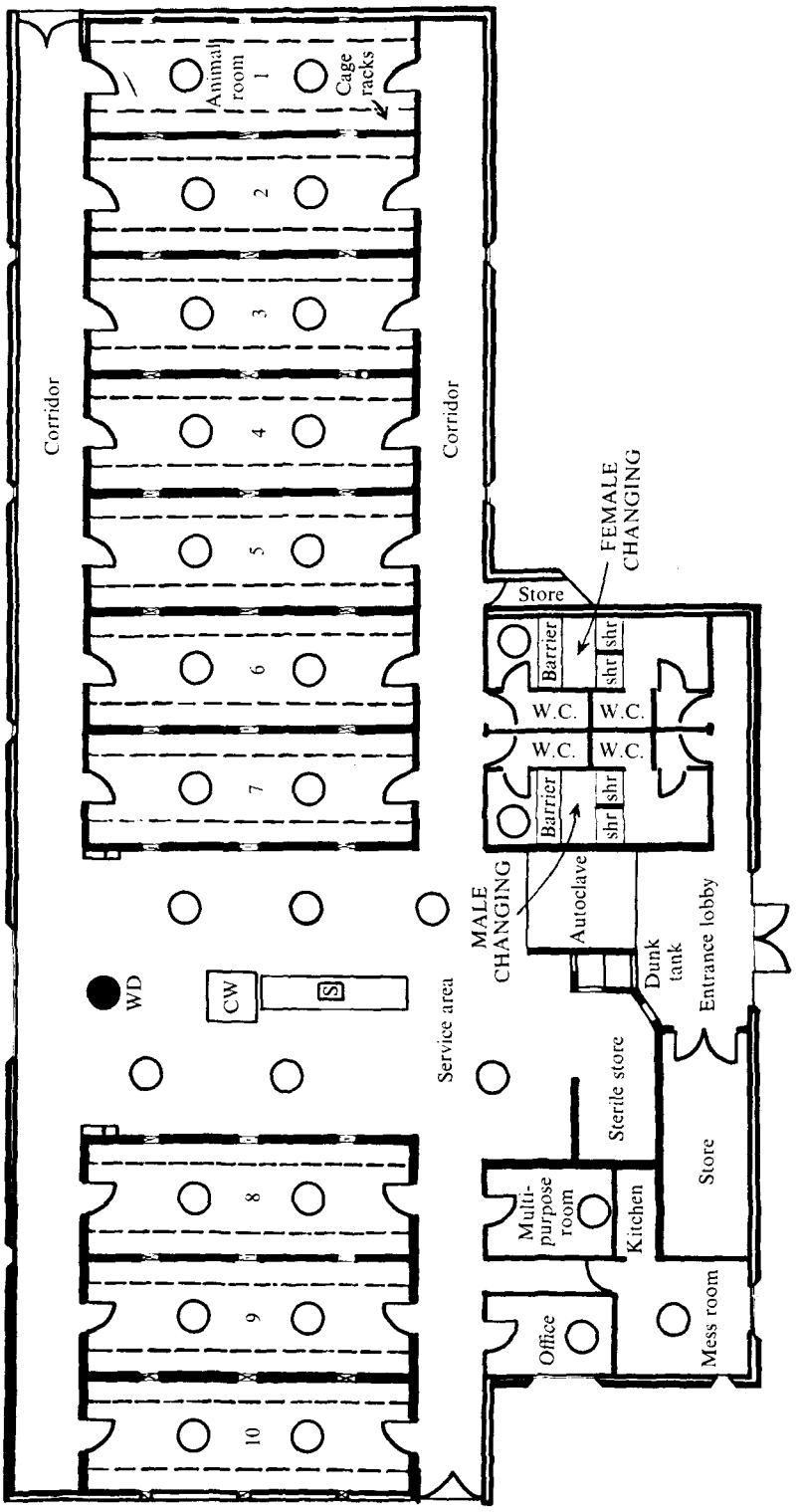

Fig. 1. The SPF animal production building. CW, cage washer; WD, waste disposal unit; S, sink; shr, shower; O, location of formaldehyde generation points.

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ventilation and air filtration services are separated from the 'clean' area and are related to it by ducting. A positive air pressure is constantly maintained within the 'clean' area. The building is situated on an open site in the country and is within 46 m. (50 yards) of a farm-animal isolation compound. The period of its construction was from June 1970 to October 1972. During this time there was ample opportunity for a build-up of bacterial flora, including potentially pathogenic bacteria, due to contamination by human contact and from wildlife vectors.

Preparatory cleaning

After completion the building was thoroughly cleaned to remove all gross dirt.

Bacteriological methods

Wet swabs were taken from 100 sites each of which was numbered with adhesive tape for subsequent identification (see Table 1). The swabs were broken into bottles containing 10 ml. Todd–Hewitt broth which was incubated at 37° C. for 36 hr. The cultures obtained were subcultured on 5% ox-blood agar plates which were incubated aerobically for 18 hr. at 37° C. Colonies were examined macroscopically and smears of them were stained by Gram's method and examined microscopically.

Fumigation procedures

First treatment

Before treatment the ventilation system was shut down to reduce the movement of air within the building. A commercially available preparation of an ampholytic surface-acting biocide and formaldehyde* was applied to the whole of the 'clean' area at a recommended concentration of 1% in water. A fogging apparatus was employed to produce a fine spray under pressure. This apparatus was held by an operator wearing a gas mask and suitable protective clothing: all surfaces including ceilings, walls and floors were carefully and systematically saturated with the agent. The doors of each room were closed after treatment; the ducting was also treated. The total time taken for the whole operation was 7 hr. The building was then sealed for 2 days with the air conditioning unit switched off. Two days later swabs were taken, from the sites originally examined, by personnel wearing gas masks and sterile protective clothing. The air conditioning was then switched on.

Second treatment

The temperature of the heating system was increased to approximately 21° C. and the entire area within the 'barrier' was saturated with water to produce conditions of high humidity. The air conditioning plant was then switched off. Measured amounts of potassium permanganate were put into 5 gal. lidless drums and placed in the positions shown in the figure. Measured amounts of formaldehyde, previously dispensed in sealed plastic bags, were poured onto the potassium

* Tegofectol supplied by Messrs Hough & Hoseasons, Manchester.

Table 1. *Results of the bacteriological examination of the building before and after fumigation*

Areas examined	Total no. sites examined	Before fumigation					After 1st fumigation					After 2nd fumigation				
		No. positive sites	Bacteria isolated*				No. positive sites	Bacteria isolated*				No. positive sites	Bacteria isolated*			
			Gm+Sp	Gm+b	Gm−b	Gm+c		Gm+Sp	Gm+b	Gm−b	Gm+c		Gm+Sp	Gm+b	Gm−b	Gm+c
10 animal rooms	50	26	12	10	4	1	25	7	5	3	11	8	2	0	3	3
General service area	20	16	4	5	2	7	8	2	2	0	4	1	0	0	0	1
Office	6	6	4	1	0	3	3	1	0	0	2	0	0	0	0	0
Mess area	6	6	5	1	0	5	5	3	1	0	2	1	1	0	0	1
Store	6	6	2	4	0	3	3	2	0	0	1	0	0	0	0	0
Showers																
Men's	6	6	2	2	1	4	2	2	0	0	0	2	0	0	0	2
Ladies'	6	6	4	2	0	5	4	4	0	0	0	1	0	0	0	1
Totals	100	72	33	25	7	28	50	21	8	3	20	13	3	0	3	8

Gm = Gram, c = cocci, b = bacilli, Sp = spore bearing bacilli.

* Totals of these columns may not coincide with that on the left because one site often yielded more than one organism.

permanganate by personnel wearing gas masks and suitable protective clothing. The amounts of potassium permanganate and formaldehyde used were based on the cubic capacity of each area so that a final concentration of not less than 2 mg./l. of formaldehyde vapour at 20° C. was obtained (Report, 1958). All doors inside the 'clean' area were left open to facilitate adequate fumigation of the corridors. This procedure required approximately 15 min., after which the building was sealed for 2 days. On the third day the air conditioning was switched on but personnel were not allowed to enter the fumigated area for a further 2 days, after which time the final swabs were taken from the sites originally examined.

RESULTS

Results of the three bacteriological examinations are recorded in Table 1.

Bacteria were isolated from 72 of the 100 sites before treatment, from 50 sites after the first treatment and from 13 sites after the second treatment. Nine sites which were originally negative gave positive cultures after the first treatment. This observation indicates the limitations of the swabbing technique, in that, although each site was clearly identified precisely the same site could not be sampled on all three occasions. Bearing in mind the limitations of the simple bacteriological identification methods used, there was no evidence that one type of organism was more resistant to treatment than another. It was apparent that there was less initial contamination in the animal rooms (out of 50 samples 24 were negative) than in the other areas where there had been more human activity (out of 50 samples 4 only were negative).

DISCUSSION

Because the two treatments were used consecutively, the effect of the second cannot be compared with the first. However, it may be said that the first treatment was totally inadequate, and it appears that the second treatment contributed significantly to the reduction in the number of contaminating organisms. The persistence of organisms after both treatments was surprising (13 positive out of 100 sites), in view of the long-standing belief that formaldehyde gas at the correct concentration and under optimum atmospheric conditions is lethal to bacteria. However, this work has indicated that it is probably impossible, with a single fumigation of the type employed, to sterilize completely a building of the size and type described.

I wish to record my gratitude to Mr K. Parsons for his technical assistance and to the staff of the S.P.F. animal building for their willing co-operation.

REFERENCE

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