J. Hyg., Camb. (1967), **65**, 115
Printed in Great Britain

Experiments on terminal disinfection of cubicles with formaldehyde

By M. M. BEEBY, D. KINGSTON and C. E. WHITEHOUSE
Cross-Infection and Disinfection Reference Laboratories, Central Public Health Laboratory, Colindale Avenue, London N.W. 9

(*Received* 17 *October* 1966)

The report of the Committee on Formaldehyde Disinfection (Public Health Laboratory Service, 1958*a*) dealt mainly with the use of formaldehyde for sterilizing contaminated fabrics such as bedding. Their results, and the practical experience at the Microbiological Research Establishment, Porton, formed the basis for the fumigation procedures recommended in the note on the Practical Aspects of Formaldehyde Fumigation (Public Health Laboratory Service, 1958*b*). The work here reported was undertaken to explore further the advantages and limitations of this procedure in disinfecting isolation cubicles in hospitals.

The test specimens were prepared from *Staphylococcus aureus* and *Pseudomonas aeruginosa*, representing two important and very different pathogens. Dust was used to represent comminuted contamination into which the formaldehyde would readily penetrate. Dried broth drops represented substantial smears of contamination into which penetration might be more difficult. Really massive contamination was not represented as this should be removed mechanically. In addition, in some experiments the spore test piece of Beeby & Whitehouse (1965), suggested for the control of ethylene oxide sterilization, was included to see if it could serve as a control for formaldehyde fumigation.

The test specimens were exposed to formaldehyde vapour for 3 or 24 hr. under controlled conditions in the laboratory or in rooms into which formaldehyde had been liberated. Viable counts were carried out and the survivals after exposure compared with those in control specimens. In addition, measurements were carried out to determine the formaldehyde concentrations and humidities at different times.

MATERIALS AND METHODS

Media

Broth. Oxoid nutrient broth no. 2.

Serum. Horse serum no. 2. (Burroughs Wellcome and Co.).

Elution fluid. Broth or, for the spore test pieces, quarter-strength Ringer's solution with 0·1 % (v/v) Tween 80 (Honeywill-Atlas Ltd.).

Diluent. Quarter-strength Ringer's solution or broth-saline (physiological saline with 5 % (v/v) broth added).

Nutrient agar. Broth solidified with 1·2 % (w/v) Oxoid agar no. 3.

Serum agar. Broth solidified with 1·1 % (w/v) Davis New Zealand powdered agar with the addition of 5 % (v/v) horse serum before pouring.

Test contamination

Organisms

Staph. aureus NCTC 6571 ('Oxford'), *Ps. aeruginosa* NCTC 6749 and *Bacillus subtilis* NCTC 10073 (Camp Detrick strain) were used. Owing to the erratic survival of the *Ps. aeruginosa* it was used only in a limited number of experiments. The organisms were grown at 37° C.

Preparation

Dust. This was prepared by a procedure similar to that used by Kingston & Noble (1964). Two serum agar plates were flooded with a broth culture of *Staph. aureus* and incubated overnight. The surface growth was rubbed off in 10 ml. of broth and this suspension mixed into 10 g. of cotton dust. In some experiments the growth was suspended in horse serum. The impregnated dust was dried *in vacuo* over $CaCl_2$ for 7 hr. and allowed to stand over saturated $Na_2Cr_2O_7$ solution overnight to bring the water content towards equilibrium with a relative humidity of 52 %. The dust was then ground in a Waring blender. The prepared dust gave counts of the order of 10^9 organisms per g. (for detailed figures see Table 5, where the counts are given per 0·2 g. specimen, or per 0·1 g. for the 'bijou' specimens).

Dried drops. The suspensions of *Staph. aureus* were overnight broth cultures. For *Ps. aeruginosa* the surface growth on a nutrient agar slope after overnight incubation was washed off into 20 ml. of broth, and the resulting suspension was shaken by hand with glass beads for 1 min. to help break up the clumps. To prepare suspensions of the organisms in serum, the *Staph. aureus* culture was spun down and resuspended in about an equal volume of serum; the *Ps. aeruginosa* growth was washed off in it.

Strips of high-density polythene (4·1 × 1·6 × 0·008 cm.) were boiled for 5 min. in distilled water and allowed to dry. Each was inoculated with a drop (*ca.* 0·02 ml.) of the appropriate suspension. The drops were allowed to dry overnight over saturated $Na_2Cr_2O_7$ (52 % relative humidity). The prepared strips usually had counts of about 10^6–10^7 organisms (for detailed figures see Tables 1 and 5).

Spore test pieces. These were prepared by the method of Beeby & Whitehouse (1965). One drop (*ca.* 0·02 ml.) of a suspension of spores in 90 % (v/v) methanol in water was dropped on a piece of clean sterile aluminium foil (2 × 1 cm.). The suspension was used in two concentrations, containing respectively about 10^6 and about 10^3 spores per drop.

Exposure to formaldehyde

Desiccator experiments

The base of each desiccator contained about 150 ml. of an appropriate saturated salt solution with excess solid to control the humidity. The salts used and the corresponding relative humidities at 20° C. were as follows (O'Brien, 1948):

Table 1. *Survival of organisms in dried drops and of spore foils when exposed to formaldehyde in desiccators*

Each survival is given as a percentage of the count at the start of the experiment and, except as below, is derived from the mean of counts on 3 strips. The control survivals are derived from strips exposed at the same humidity in the absence of formaldehyde.

Relative humidity (%)	Formaldehyde (mg./l.)	Organism	Vehicle	No. of organisms per strip*		Percentage survival after exposure			
						Test strips		Controls	
				Thousands	% of inoculum	3 hr.	24 hr.	3 hr.	24 hr.
86	0·9	*Staph. aureus*	Broth	5000	83	0†	0	49	49
			Serum	2000	34	0	0	80	58
		Ps. aeruginosa	Broth	3000	5·0	0	0	33	⩽ 0·009‡
			Serum	200	3·8	0	0	⩽ 0·1	⩽ 0·1
		B. subtilis	—	400	N.D.§	⩽ 0·07	0	42	45
86	0·4	*Staph. aureus*	Broth	2000	12	0	0	13	87
			Serum	1000	17	⩽ 0·03	⩽ 0·03	240	22
		Ps. aeruginosa	Broth	20	0·055	⩽ 2	0	260	6·3
			Serum	10	0·13	⩽ 2	0	0·24	⩽ 2
		B. subtilis	—	500	N.D.	2	0	62	80
			—	0·3	18	90	0	81	170
58	0·6	*Staph. aureus*	Broth	3000	59	0·083	0·031	32	8·4
			Serum	3000	44	27	⩽ 0·01	56	14
		B. subtilis	—	0·3	N.D.	130	⩽ 9	170	120
32	0·7	*Staph. aureus*	Broth	3000	59	66	17	35	14
			Serum	3000	44	51	39	49	21
		B. subtilis	—	0·3	N.D.	89	0	160	120

* The number per strip is given in thousands and is the mean count of three strips estimated at the start of the experiment in the same way as the other survivals. It is also given as the percentage of the number of organisms per drop of the suspension used to prepare the strips. This percentage thus shows the loss in drying, etc., and the loss (if any) by adhesion to the strip.

† A zero indicates that there was no growth of the appropriate organism on the agar plates or when the strips were incubated with the remains of the eluate.

‡ ⩽ 0·009, etc., indicates survival equal to or less than the stated figure. There were no colonies formed from any of the dilutions plated, but at least one of the three strips incubated with the remains of the eluate showed growth of the appropriate organism. The stated figure corresponds to 3 colonies at the lowest dilution.

§ Not done.

KCl, 86%; NaBr, 58%; $CaCl_2$, 32%. Sufficient formaldehyde solution had been added to give the vapour concentration required.

The specimens, which were dried drops and spore test pieces only, were laid out on Petri dishes in a way that allowed free gas exchange with the saturated salt solution. Control specimens were exposed in identical desiccators to saturated salt solutions without formaldehyde. The desiccators were kept on the bench out of direct sunlight.

Room experiments

Dust was exposed in 0·2 g. lots in open unguent jars (5 cm. diam., 3 cm. deep), dispersed as evenly as possible over the base of each jar. Also, as a model for contamination to which the formaldehyde could penetrate only by diffusion, 0·1 g. of dust was placed in each of a number of $\frac{1}{2}$ oz. screw-capped ('bijou') bottles which had a 0·5 cm. diameter hole drilled through both cap and rubber liner, and four thicknesses of cotton gauze between the two. This test object was modified from that used by Dr R. M. Fry (personal communication), who used blanket in place of the gauze. Dried drops of broth were exposed by leaving the inoculated polythene strips in open Petri dishes, and spore test pieces were exposed similarly.

Cubicles at West Hendon Isolation Hospital with a volume of about 1500 cu.ft. were used. These had walls and ceilings covered with glossy paint, and floors of unpolished wood boards. Each contained an iron bedstead and a wooden locker, but no mattresses or curtains. One experiment was carried out in a room at the Central Public Health Laboratory with a volume of about 2000 cu.ft. The walls and ceilings were covered with glossy paint and the floor was of polished composition tile. There were ordinary laboratory benches of waxed teak. In preparing the rooms for fumigation the ventilators were closed and the cracks round the windows sealed with Sellotape. As soon as fumigation had been started the doors were also sealed with Sellotape.

Formaldehyde was vaporized by the reaction of formalin with potassium permanganate in the quantities recommended by the Public Health Laboratory Service (1958*b*). For the 1500 cu.ft. isolation cubicles, 255 g. of $KMnO_4$ were added to 750 ml. of formalin (40%, w/v, formaldehyde) in a small aluminium chamber pot standing in a large enamel basin in the middle of the floor. The reaction resulted in some spilling into the basin and the nearly complete evaporation of all liquid. Twice the recommended amount was used in the laboratory room.

In some experiments on the concentrations of formaldehyde and water vapour attained in the cubicles, formaldehyde solutions were boiled off from various electrically heated vessels.

Estimation of survival

Dust samples

These were shaken up with 10 ml. of nutrient broth and the number of organisms in the broth was estimated by inoculating suitable dilutions on the surface of serum agar plates. Colonies were counted after at least 24 hr. incubation at 37° C.

The mixture of broth and dust was not incubated, because it sometimes contained sufficient formaldehyde to inhibit bacterial growth.

Dried drops

The polythene strips were shaken up with 10 ml. of nutrient broth (control specimens) or 5 ml. (test specimens) and survival estimated in the same way as for the dust. In addition, the strips were incubated in the remains of the broth since there was not enough formaldehyde carried over to cause trouble. If the broth showed growth this was recorded as positive only if subculture showed the organism to be the test organism.

Spore test pieces

Each of these was shaken with glass beads in 10 ml. of quarter-strength Ringer's solution to which 0·1 % (v/v) Tween 80 had been added. Tenfold dilutions were inoculated on the surface of serum agar plates. An equal quantity of double-strength broth was added to 5 ml. of the elution fluid and this was incubated with the test piece for 2 days.

Carry-over of formaldehyde

Possible danger from this was investigated in the following experiments. Desiccators were prepared with relative humidities of 86 %, 58 % and 32 %, and formaldehyde vapour concentrations of 0·9 mg./l. (see section on Exposure to formaldehyde). Prepared strips with broth or serum drops were exposed in these for 2 days, removed, and inserted into screw-capped bottles containing 5 ml. of broth (cf. section on Estimation of survival). Each bottle was then inoculated with one drop of a suspension of *Staph. aureus* or of *Ps. aeruginosa*, containing 18 and 28 organisms respectively. Growth occurred with both organisms in all the bottles containing single strips—that is, under the conditions used for estimating survival. When pairs of strips were inserted growth still occurred, with the sole exception that pairs with serum drops after exposure to 86 % relative humidity inhibited both organisms. Under the same conditions pairs with broth drops did not inhibit. Owing to the uptake of water vapour, more formaldehyde will be carried over at high humidities, and these provide the most critical situation. It was thought therefore that as far as the strips were concerned there was unlikely to be trouble from the carry-over of formaldehyde.

In a further experiment, 0·2 g. samples of dust prepared from broth or serum suspensions were exposed similarly to formaldehyde. These were suspended in 5 ml. of broth—that is, half the amount used in estimating survival—and the suspensions inoculated with *Staph. aureus* and *Ps. aeruginosa*. The correct number of colonies grew when 0·1 ml. portions of this broth were spread without dilution on to serum agar plates, though no growth occurred in the broth itself. When 0·4 g. of dust was used the number of colonies of *Ps. aeruginosa* developing was reduced to about two-thirds, though there was no significant reduction for *Staph. aureus*. Since therefore signs of inhibition only started to appear with a fourfold concentration (twice the quantity of dust in half the volume of elution fluid), it was thought that there was an adequate margin of safety.

Estimation of formaldehyde

Twenty-five ml. of air was aspirated through a narrow piece of plastic tubing into a 30 ml. syringe containing 5 ml. of distilled water. The syringe was shaken vigorously for about ½ min. before the water was expelled into a screw-capped bottle (cf. Public Health Laboratory Service, 1958*a*). The formaldehyde content was estimated colorimetrically by the method of Nash (1953) within 5 hr. of the collection of the sample. It was shown that keeping the solutions on the bench for up to 24 hr. did not affect the formaldehyde estimation.

In sampling air from the cubicles about 2 ft. of the tubing was run between the door and the frame, between the upper edge and the lintel, the end being about 2 in. away from the door. In sampling from the desiccators about 9 in. of tubing was used, run in through the stopcock. The tubes were washed through with the air to be estimated before the sample was taken. The concentrations in the desiccators are the mean of determinations carried out just before removing the lid on at least two of the following occasions: before inserting the specimens, before taking the 3 hr. and before taking the 24 hr. sample. All samples were taken and estimated in duplicate.

Estimation of relative humidity

In the desiccator experiments, the relative humidity was assumed to be that in equilibrium with the saturated salt solution. In the cubicle experiments, the determinations before vaporization were made with a whirling hygrometer and subsequent determinations were made with an aspirating (Assmann) hygrometer which drew the air for the wet and dry bulbs through two rubber tubes run between the door and the lintel.

RESULTS

Desiccator experiments

The organisms were exposed on polythene strips in dried drops of serum or broth, with the exception of the spore test pieces (Table 1). At 86% relative humidity *Staph. aureus* and *Ps. aeruginosa* were satisfactorily killed by formaldehyde. With a concentration of 0·9 mg./l. there was no growth of the test organism from any of the strips after 3 hr. With a concentration of 0·4 mg./l. kill was not quite complete. At 32% relative humidity the formaldehyde had little significant effect on *Staph. aureus* or on *B. subtilis* spores. At 58% relative humidity there was a substantial kill, but this was never complete, all strips still showing growth after 24 hr. exposure. The spore test strips were included to see if they could be used to control formaldehyde fumigation. The suggestion from these experiments is that the disinfectant action of formaldehyde may be less dependent on humidity for the spores than it is for the other test strips. After 24 hr. all the spore strips at all the humidities failed to show growth, with the exception of 2 out of 3 at 58% relative humidity. With the exception of the highest humidity and formaldehyde concentration, all the spore strips survived fairly well after 3 hr.

Table 2. *Formaldehyde and water-vapour concentrations after vaporizing formalin in rooms*

(Expts 1–9: in isolation cubicles (vol. 1500 cu.ft.). Expt 10: in a room (vol. 2000 cu.ft.). Expts 1–5 and 10: vaporization by the addition of permanganate to formalin (occurred rapidly). Expts 6–9: formalin vaporized electrically. The time taken to vaporize was ½ hr., 1 hr., 1½ hr. and 2 hr. respectively, and the 0 min. samples were taken immediately after this.)

			Relative humidity (%)						Formaldehyde concentration (mg./l. air).						
				Time after vaporization:					Time after vaporization:						
Expt no.	40% formalin (ml.)	Extra water (ml.)	Before vaporization	0 min.	5 min.	15 min.	3 hr.	24 hr.	0 min.	5 min.	20 min.	30 min.	1½ hr.	3 hr.	24 hr.
1	750	—	26	—	—	52	36	—	—	—	1·9	1·6	—	0·3	—
2	750	—	70	—	83	—	64	—	—	0·9	—	—	—	1·2	—
3	750	—	57	—	86	—	64	—	—	1·6	—	—	—	1·0	—
4	750	—	65	—	86	—	—	56	—	1·4	—	—	—	—	0·1
5	750	—	55	—	82	—	—	56	—	1·7	—	—	—	—	0·3
6	375	750	54	83	—	—	—	45	0·6	—	—	—	—	—	0·05
7	750	1500	54	90	—	—	—	50	0·9	—	—	—	—	—	0·2
8*	750	1500	50	89	—	—	—	45	1·0	—	—	—	—	—	0·08
9	?†	?†	51	91	—	—	—	50	0·7	—	—	—	—	—	0·05
10*	2000	—	55	—	—	75	—	—	1·9	—	—	—	1·1	0·9	—

* A fan was run in the room or cubicle throughout the experiment.

† Vaporization (of 1500 ml. of formalin and 3 l. of water) was not complete, and the amounts actually vaporized are not known, since the proportions of formaldehyde and water in the residuum were not determined.

Cubicle experiments

Formaldehyde and water-vapour concentrations

It is not possible to predict the concentrations of formaldehyde and water vapour that will be achieved when formalin is vaporized in a room. A number of experimental determinations were therefore made and these are set out in Table 2. We discuss first the experiments (1–5) in which the formalin was vaporized by reaction with $KMnO_4$ in the quantities suggested by the Public Health Laboratory Service (1958*b*). The results show that the increase in the percentage relative humidity was never more than 25, and sometimes less. The 3 hr. determinations suggest that the humidity falls fairly rapidly. The formaldehyde concentrations remained adequate up to 3 hr. after vaporization (Expts 2 and 3). After 24 hr. only traces were left, probably below the level at which estimation was likely to be accurate (Expts 4 and 5). In Expt 1 the sealing of the cubicle was known to be unsatisfactory, and the concentration of formaldehyde fell rapidly.

Table 3. *Concentration of formaldehyde at top and bottom of door*

Experiment no.	Time of sample (hr.)	Concentration of formaldehyde (mg./l. air)	
		Top of door	Bottom of door
6 (no fan)	0	0·8, 0·7	0·4, 0·6
7 (no fan)	0	0·9, 1·0	0·8, 0·7
9 (no fan)	0	0·8, 0·8	0·5, 0·5
8 (fan)	0	1·2, 0·9	1·1, 0·9
10 (fan)	0	2·0, 1·7	1·8, 1·9
	1	1·4, 1·5	1·1, 1·4
	1½	1·2, 1·2	1·1, 0·9
	2½	0·8, 0·9	0·9, 0·9
	3	0·8	1·0

Experiment 10 was carried out in a room at the Central Public Health Laboratory with twice the suggested quantities of formalin and permanganate. The humidity rise was still only 20. The formaldehyde concentration was higher initially, but fell more rapidly, probably because pressure difference within the large building made the sealing less effective.

The results found when formalin solutions were boiled off electrically (Expts 6, 7, 8 and 9) cannot be compared exactly with those previously discussed, since vaporization by electrical heating took much longer. The water-vapour concentrations were much more satisfactory. Except in Expt 6 in which half quantities were vaporized, there was considerable fogging and condensation on the walls and window, showing that the air was saturated. The initial humidity measurements of around 90 % are therefore misleadingly low, probably owing to temperature variations. The formaldehyde concentrations of about 1 mg./l. are satisfactory, particularly as these levels are likely to have existed during vaporization. They were, however, rather low when half quantities were used or vaporization was not complete (Expts 6 and 9).

A check was made on the uniformity within the rooms by estimating the formaldehyde concentrations near the top and near the bottom of the doors, with and without a fan being run. Such determinations were carried out in Expts 6–10. The concentrations after 24 hr. were too low to give results of sufficient accuracy, but the other results are set out in Table 3. It can be seen that without a fan the concentrations found near the floor were lower, whereas no consistent differences were found when a fan was run. The differences were not, however, very large. Estimations of this sort are probably only capable of measuring concentration differences in the main body of the air in a room. The layer of comparatively still air next to a surface, the 'boundary layer', may have a concentration appreciably different to that in the bulk of the room air if the surface is absorbing or evolving formaldehyde, and it is in the boundary layer that the bacteria are to be found.

The interpretation of the formaldehyde concentrations found presents some difficulty. The maximum vapour-phase concentration of formaldehyde at 20° C. is about 2 mg. per litre of air, limited by the tendency of formaldehyde to polymerize to polyoxymethylene derivatives. (For a general account of the chemistry of formaldehyde see Walker (1964). The tables on pp. 113 and 150 summarize the data on the vapour pressures of formaldehyde over formalin and over paraformaldehyde respectively.)

The quantity of formalin suggested, 500 ml. per 1000 cu.ft., corresponds to 7·1 mg. of formaldehyde per litre of air. In fact a concentration of 2 mg./l. only was achieved in Expt 10 where twice the suggested quantities were used. Oxidation by the permanganate cannot be the explanation since, if this reaction follows the stoichiometric equation $4KMnO_4 + 3H_2CO = 4KOH + 4MnO_2 + 3CO_2 + H_2O$, 170 g. of $KMnO_4$ could oxidize only 24 g. of formaldehyde. Since there is always some air exchange through cracks in the floor boards, etc., ventilation might remove a substantial amount of formaldehyde during the comparatively slow vaporization from the electrically heated containers. If there were a steady concentration of 2 mg./l. and the improbably high ventilation rate of 1 air change/hr., 113 g. of formaldehyde would be lost. This is not quite enough. However this process would not explain the low concentrations found after the very rapid vaporization with $KMnO_4$. It seems probable the explanation is adsorption of formaldehyde on to the walls and furniture. This is known to occur (Harry, 1954). Adsorption would be reversible and give a buffering action against the removal of formaldehyde by ventilation. The small drop of formaldehyde concentration over 3 hr., which would correspond to an improbably low ventilation rate of about 0·1 air change/hr., suggest that this occurred. It was also found by Harry (1954). Such a buffering effect would also be produced by vaporizing sufficient formaldehyde to produce deposits of paraformaldehyde. The adsorbed or polymerized formaldehyde would have the effect of giving a slow kill on the surface after the concentration in the room had fallen (cf. Kingston, Lidwell & Noble, 1962), but possibly not to a useful extent. That slow evolution probably occurs does however mean that adsorption has stopped, so that the concentration of formaldehyde in the boundary layer will not be below that in the main bulk of the air.

Water vapour is known to be taken up in large amounts by many natural

materials, and the quantity of water required to raise the humidity in a room by a given amount cannot therefore be predicted. Air saturated with water vapour contains, at 20° C., 485 g. of water per 1000 cu.ft., or about 0·85 pints. The quantities suggested (Public Health Laboratory Service, 1958*b*) for vaporization by electrical heating are 1000 ml. of water and 500 ml. of formalin for each 1000 cu.ft., i.e. a total of 1300 ml. of water. This would saturate perfectly dry air 2·7 times. The results of Expts 7 and 8 show that, at least when starting with a relative humidity of 50 %, this amount is adequate. The recipe given for use with permanganate suggests using 500 ml. of neat commercial formalin for 1000 cu.ft., stating that owing to the formation of water from the formaldehyde no further water is needed. Since the specific gravity of commercial formalin is very nearly 1, 200 g. of formaldehyde and 300 g. of water are available. The stoichiometric equation shows that a negligible amount of water would be produced by the oxidation of the formaldehyde. Thus only about 300 g. of water would be available, enough to produce 62 % saturation of perfectly dry air. The measurements show that this much smaller amount is not adequate. The increase in the percentage relative humidity was only of the order of 25. Though the median relative humidity in centrally heated buildings in England is likely to be about 45 %, relative humidities down to 30 % will occur with reasonable frequency (see Kingston & Noble, 1964). An increase of up to 55 would therefore be necessary to raise the relative humidity to the optimum of 80–90 %. When the permanganate method was used with twice the suggested quantities the rise in the relative humidity was still inadequate (Expt 10). Since we found that if appreciable quantities of water were added to the formalin before adding the permanganate, the solution was not all boiled off, we conclude that the permanganate method does not vaporize enough water for optimum fumigation conditions.

Effect on test contamination

In Expt 1 (Table 2) neither the dried broth drops nor the dust samples were sterilized. The specimens exposed on the floor were least affected, survival of the organisms in dust being about 1 %, and in dried broth drops about 10 %. Conditions were unusually dry, and owing to inadequate sealing the formaldehyde concentration fell rapidly.

In the main experiment four cubicles were used (Table 2, Expts 2–5), two being given a 3 hr. exposure to formaldehyde, and two a 24 hr. exposure. The bacteriological results are set out in Table 4. Also in this table are the results of the experiment carried out in a room at the Central Public Health Laboratory (Table 3, Expt 10). In all these experiments the formaldehyde was vaporized by reaction with permanganate in the quantities recommended by the Public Health Laboratory Service (1958*b*), with the exception of Expt 10 in which twice the quantities were used.

The results show that on no occasion was the survival after exposure more than 0·1 %, and that it was generally much less than this. The specimens from which organisms could be recovered were not uniformly distributed, the majority of them occurring in the cubicle of Expt 3 (11 out of 17). Taking all the cubicles

Period exposed (hr.)	Ref. for Table 2 (Expt no.)	Organism	Nature of specimen	per prepared specimen (millions)	Survival as percentage of counts at start of experiment: Control*	Floor	Window-sill	High	Door	% for 3 cols.†
					Isolation cubicles					
3	2	*Staph. aureus*	Dust	100	35	N.G., —‡	N.G., N.G.§	N.G., N.G.	N.G., N.G.	0·0002
			Drop	20	120	0, 0‖	0, 0	0, 0	0, 0	—
		Ps. aeruginosa	Drop	20	18	0, 0	0, 0	0, 0	0, 0	—
3	3	*Staph. aureus*	Dust	100	35	N.G., N.G.	N.G., N.G.	N.G., 0·0001	N.G., N.G.	0·0002
			Drop	20	120	0·095, 0·056	0·069, 0	⩽ 0·002, 0	0, 0	—
		Ps. aeruginosa	Drop	20	18	⩽ 0·002, ⩽ 0·002¶	0·0060, 0·0060	⩽ 0·002, ⩽ 0·002	0, 0	—
24	4	*Staph. aureus*	Dust	100	15	N.G., N.G.	N.G., N.G.	N.G., N.G.	N.G., N.G.	0·0002
			Dust (Bijou)**	50	15	—, —	—, —	N.G., N.G.	—, —	0·0002
			Drop	20	93	⩽ 0·002, 0	0, ⩽ 0·002	0, 0	0, 0	—
		Ps. aeruginosa	Drop	20	18	0, 0	0, 0	0, 0	0, 0	—
24	5	*Staph. aureus*	Dust	100	15	N.G., 0·0002	N.G., N.G.	N.G., N.G.	N.G., N.G.	0·0002
			Dust (Bijou)**	50	15	—, —	N.G., N.G.	—, —	—, —	0·0002
			Drop	20	93	—, 0·0089	0, ⩽ 0·002	—, 0	—, 0	—
		Ps. aeruginosa	Drop	20	18	⩽ 0·002, 0	0, 0	0, 0	0, 0	—
					Laboratory room					
3	10	*Staph. aureus*	Serum dust	800	86	N.G., N.G.	—	Bench N.G., N.G.	—	0·00003
			Broth dust	300	37	N.G., N.G.	—	N.G., N.G.	—	0·0001
			Serum drop	2	82	0, 0, 0	—	0, 0, 0	—	—
			Broth drop	1	130	0, 0, 0	—	0, 0, 0	—	—
		B. subtilis	Spore foil	0·0003	—	0, 0, 0	—	0, 0, 0	—	—
			Spore foil	0·3	—	⩽ 0·09, ⩽ 0·09, ⩽ 0·09	—	0, ⩽ 0·09, ⩽ 0·09	—	—

* The control specimens give the survival found from specimens not exposed to the disinfectant procedure. They differ from the test specimens in the temperature and humidity to which they were exposed, as well as in the absence of formaldehyde.

† See under § below.

‡ Not done.

§ No growth when the specimens were plated out. These dust samples could not be incubated entire, as carry-over of formaldehyde caused inhibition. They might therefore be sterile or have a percentage survival equal to or less than the value given in the column headed '% for 3 cols.', which is that which would have resulted from three colonies being found at the lowest dilution plated.

‖ A zero indicates no growth when the elution fluid and the strip were incubated.

¶ ⩽ 0·002, etc.: survival equal to or less than the stated figure. There was growth of the appropriate organism when the strip and elution fluid were incubated, but no colonies were found from the lowest dilution plated. The stated figure corresponds to 3 colonies at the lowest dilution.

** The dust was in a screw-capped bottle with a small hole pierced in the lid, see text.

The specimens showed a percentage survival in preparation (cf. Table 1) as follows. Isolation cubicles: *Staph. aureus* drop, 100%; *Ps. aeruginosa* drop, 37%. Laboratory room (*Staph. aureus*): serum dust, 41%; broth dust, 34%; serum drop, 46%; broth drop, 20%.

together, 8 of the positive specimens came from the floor, 5 from the window-sill, 4 from the high shelf (all in cubicle 3) and none from the table near the door.

DISCUSSION

The results of our laboratory experiments (Table 1) can usefully be supplemented with the much more extensive series of the Committee on Formaldehyde Disinfection (Public Health Laboratory Service, 1958*a*). Since the significance of their results has sometimes been misunderstood, those which bear on the problem of terminal disinfection are summarized in Table 5. The test objects were cotton threads on to which about 10^4 organisms, either in 1 % gelatin or 90 % horse serum, had been dried. When these were exposed, hanging free in an atmosphere containing formaldehyde at a temperature of 20° C., times for rendering 45 % of the threads sterile were found, and are given in the table. It was also found that

Table 5. *Time for sterilization of* 45 % *of impregnated cotton threads*

(Public Health Laboratory Service, 1958*a*.)

Organism	Conditions	Vehicle	Time (min.)
Micrococcus	58 % R.H., 1 mg./l.*	1 % gelatin	29
Micrococcus	58 % R.H., 1 mg./l.*	90 % horse serum	276
Micrococcus	Vapour of 40 % formalin (1·75 mg./l.*)	90 % horse serum	54
Myco. tuberculosis (avian)		90 % horse serum	60
B. subtilis (spores)		90 % horse serum	173

Each thread had about 10^4 organisms dried on to it in the vehicle shown.
R.H.: relative humidity.
* Weight of formaldehyde per litre of air.

increasing the relative humidity from 58 % to 80–90 % approximately halved the time, and that exposing threads under three layers of blanket approximately doubled it. It was also found that cotton threads on which a 1 % suspension of variola major crusts in 90 % monkey serum had been dried were sterilized when exposed for 24 hr. in a disinfection cabinet with an initial concentration of about 3 mg. formaldehyde per litre, but that whole scabs were not. These results show that heavily contaminated test objects, even when moderately protected, were fairly readily sterilized by formaldehyde vapour, and that this disinfectant action was non-specific. A large mass of organic matter (whole smallpox scabs, dried horse serum) slowed the process down, presumably by delaying penetration. A high humidity potentiated the action of the formaldehyde, which was sometimes inactive at 32 % humidity.

The results of the formaldehyde and water-vapour concentrations found in the cubicles and laboratory room (Table 2) show that where the room was properly sealed the formaldehyde concentration was satisfactory up to 3 hr. after vaporization. The permanganate method did not vaporize enough water to achieve the optimum humidity, but even so a very substantial reduction in contamination was found (Table 4). Organisms in dust were more readily killed than those in dried drops. Specimens showing growth were not uniformly distributed.

Any evaluation of a disinfectant procedure is dependent on the tests simulating

sufficiently closely the conditions under which the disinfectant has to work in practice. If our tests were in fact good models, then our conclusions are that formaldehyde fumigation can be a satisfactory means of decontaminating an isolation cubicle, but that a number of points must be carefully attended to. The cubicle must be thoroughly sealed. In large buildings, considerable pressure differences may be set up by thermal convection and by wind, with the result that the vapour can be very readily sucked up ducting and through cracks under doors, etc. This may produce serious falls in concentration and make other parts of the building uninhabitable. Absolute sterility may never be reliably achieved, and really massive contamination will not be adequately disinfected and must be removed mechanically. Thus cleaning is desirable before fumigation. Bedding, particularly if opened out to the action of the vapour, would probably be effectively disinfected, but it would be preferable to remove it and deal with it separately.

We think that the permanganate method of volatilizing the formaldehyde usually will not vaporize enough water. If there is no alternative means of vaporization, twice the quantities suggested by the Public Health Laboratory Service (1958*b*) should be used, namely for each 1000 cu.ft. two lots of 500 ml. of formalin each reacted with 170 g. of $KMnO_4$. We think that it is preferable to boil off 500 ml. of formalin in 1000 ml. of water by some suitable heating system, or possibly to vaporize it by an atomizing spray. Unless the apparatus can safely be run dry, an electrical cut-out device which is unlikely to fail is necessary, since fogging may make it impossible to control the vaporization by eye. We think that it is probably an advantage to run a fan in a room being fumigated.

It would be convenient if there were some simple means of checking that the fumigation procedure had been satisfactory. Estimation of the formaldehyde and water vapour concentrations at the start of the fumigation and after 3 hr. would probably be the best method. If during this time the formaldehyde concentration did not fall much below 1 mg./l. of air, and if the humidity started at 80–90 % and did not fall below about 60 %, the procedure is likely to have been satisfactory. The technique we used for formaldehyde estimation seemed to us to be satisfactory; another has been suggested by Harry (1959). Bacteriological test pieces are tedious to prepare, difficult to standardize, and often erratic in their behaviour. We therefore included the spore test piece of Beeby & Whitehouse (1965) in some of our experiments, since this avoids many of these disadvantages. Unfortunately, it seemed possible that the action of formaldehyde on this test piece might, in comparison with the experimental contamination, be less affected by the humidity. Since we think the spore test piece to be a less good representation of naturally occurring contamination, we cannot recommend its use here without further investigation. The spore test pieces are made from a fairly resistant form exposed freely to the vapour, whereas natural contamination will be protected by dried fluids, skin scales and so on, through which the formaldehyde must diffuse before it can reach the rather more susceptible vegetative forms.

Lack of knowledge of the relative importance of the many different routes by which diseases can spread makes it impossible to lay down definite rules for deciding when terminal disinfection should be carried out. However, there are

some general principles to be considered. A source of specific recommendations is the report of the American Public Health Association (1965).

Disinfection is no substitute for cleaning. As has been pointed out, substantial pieces of contamination cannot be adequately dealt with by fumigation and must be removed mechanically. Since for other reasons also failures may occur, the more contamination that is removed by cleaning the better. Even the most thorough cleaning, however, will not remove all contamination, though it may be thought that the organisms remaining after a really thorough cleaning will be too inaccessible or too adherent to be likely to reach another susceptible host.

It is often pointless to try to achieve complete sterility. The ordinary hospital environment, however clean initially, is rapidly recontaminated with organisms. In particular, *Staph. aureus* and *Cl. welchii* are carried by about half the adult population and are therefore continually being disseminated. There is seldom any point in ensuring a total, but temporary, absence of these organisms. Thus the only occasions on which fumigation could be of value are if dangerous organisms of high infectivity are present, and if these organisms are not carried by a high proportion of normal people in hospital. It could be argued that strains of *Staph. aureus* which were thought to be unusually dangerous should be put into this category. Apart from the difficulty in deciding which strains are usually dangerous, there is some evidence that *Staph. aureus* is not readily acquired from fomites where it is present in small numbers (Gonzaga, Mortimer, Wolinsky & Rammelkamp, 1964). The only disease for which a general recommendation for fumigation has been made is smallpox (Ministry of Health and Scottish Home and Health Department, 1964). Where specially dangerous organisms are concerned, it may be advisable to fumigate before cleaning, as well as after, to give the greatest possible protection to the cleaners.

There are a number of special cases to be considered. Certain peculiarly susceptible patients have to be cared for in an environment which is as free as possible from bacterial contamination. Since extraordinary precautions are taken to reduce recontamination, there may be a case for ensuring that the room is as sterile as possible initially. Organisms capable of multiplying in the environment must be considered separately, since if any were left they might increase to serious levels again, whereas ordinary contamination is continually reduced by the natural death-rate of the organisms. Bacteria do not multiply in the dry state, and only those which are capable of multiplying in the more or less enriched tap-water of sinks and similar places can proliferate in the environment. The most important example is *Ps. aeruginosa*, but other organisms such as *Flavobacterium meningosepticum* can cause trouble in this way. However, since they will only be multiplying in localized areas, special measures directed against the damp places may be more appropriate.

This discussion has centred on the possible use of formaldehyde fumigation in the type of room in which the tests were carried out, namely isolation cubicles. Other buildings may differ in important ways, and it would be necessary to show for them whether or not conditions were satisfactory. The surface/volume ratio and the absorbancy of the walls and contents would affect the amount of formalde-

hyde and water needed to bring the air concentrations up to reasonable levels; this, however, would be checked by estimating the formaldehyde concentration and relative humidity. More seriously, a large mass of absorbent material might keep the formaldehyde and water vapour concentrations in the boundary layer over it, and hence over the bacteria, at a low level. The buildings must be capable of being effectively sealed. Temperature may be of importance, though the Public Health Laboratory Service (1958*a*) found little effect under their conditions. However, the equilibrium vapour pressure of formaldehyde over paraformaldehyde becomes low below 10° C. and this may impose a limitation, and there is the possibility of serious loss by condensation on cold surfaces even though the air temperature might be adequate. Attention must also be paid to the nature of the contamination, since formaldehyde will not readily penetrate large masses of organic material. For several of these points see Lancaster, Gordon & Harry (1954), Harry (1961) and Harry & Hemsley (1964).

SUMMARY

The conditions for satisfactory fumigation with formaldehyde were investigated using suspensions of *Staph. aureus* and *Ps. aeruginosa* dried as drops on strips of polythene or after mixing with cotton dust. In laboratory experiments with controlled concentrations of formaldehyde and water vapour, satisfactory results were obtained at 86% humidity, but at 32% humidity the formaldehyde was virtually inactive.

Measurements under field conditions showed that the permanganate method of vaporizing formaldehyde did not vaporize enough water to produce optimum conditions for sterilization, and that great care was necessary in the sealing of rooms. When sterilization was incomplete the surviving organisms were not uniformly distributed within the room.

In the limited circumstances in which fumigation with formaldehyde is thought to be essential, the process should be controlled by estimating the formaldehyde and water vapour concentrations initially and after 3 hr.

Our thanks are due to Dr J. C. Kelsey and to Dr O. M. Lidwell for helpful suggestions.

REFERENCES

American Public Health Association (1965). *Control of Communicable Diseases in Man*, 10th ed. New York: A.P.H.A.

Beeby, M. M. & Whitehouse, C. E. (1965). A bacterial spore test piece for the control of ethylene oxide sterilization. *J. appl. Bact.* **28**, 349.

Gonzaga, A. J., Mortimer, E. A., Wolinsky, E. & Rammelkamp, C. H. (1964). Transmission of staphylococci by fomites. *J. Am. med. Ass.* **189**, 711.

Harry, E. G. (1954). The influence of certain chemico-physical characteristics of formaldehyde on its use as a disinfectant. *Proc. Xth Wld Poult. Congr.* p. 217. Edinburgh.

Harry, E. G. (1959). A method of estimating the formaldehyde vapour concentrations used in egg fumigation. *Vet. Rec.* **71**, 842.

Harry, E. G. (1961). Surface disinfection of poultry buildings by formaldehyde and sulphur dioxide. *Vet. Rec.* **73**, 522.

Harry, E. G. & Hemsley, L. A. (1964). Factors influencing the survival of coliforms in the dust of deep litter houses. *Vet. Rec.* **76**, 863.

Kingston, D., Lidwell, O. M. & Noble, W. C. (1962). Self-disinfecting surface coatings which evolve formaldehyde. *Mon. Bull. Minist. Hlth* **21**, 246.

Kingston, D. & Noble, W. C. (1964). Tests on self-disinfecting surfaces. *J. Hyg., Camb.* **62**, 519.

Ministry of Health and Scottish Home and Health Department (1964). *Memorandum on the Control of Outbreaks of Smallpox*. London: H.M.S.O.

Lancaster, J. E., Gordon, R. F. & Harry, E. G. (1954). Studies on disinfection of eggs and incubators. III. The use of formaldehyde at room temperature for the fumigation of eggs prior to incubation. *Br. vet. J.* **110**, 238.

Nash, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.* **55**, 416.

O'Brien, F. E. M. (1948). The control of humidity by saturated salt solutions. *J. scient. Instrum.* **25**, 73.

Public Health Laboratory Service (1958*a*). Disinfection of fabrics with gaseous formaldehyde. *J. Hyg., Camb.* **56**, 488.

Public Health Laboratory Service (1958*b*). The practical aspects of formaldehyde fumigation. *Mon. Bull. Minist. Hlth* **17**, 270.

Walker, J. F. (1964). *Formaldehyde*, 3rd ed. New York: Reinhold Publishing Corporation.