DISINFECTION OF FABRICS WITH GASEOUS FORMALDEHYDE

By the COMMITTEE ON FORMALDEHYDE DISINFECTION*
of the Public Health Laboratory Service

(With 7 Figures in the Text)

Despite the fact that formaldehyde has been used as a disinfectant for some 65 years there is still no general agreement on its range of usefulness. In recent years there has been particular interest in its use for the disinfection of bedding in hospitals —with special emphasis on the killing of staphylococci—and for disinfection of articles made and sold by patients with tuberculosis. This paper reports a study made of the process of formaldehyde disinfection with these two uses in mind.

The investigation was begun in April 1950 at the suggestion of the former Chief Medical Officer, Ministry of Health, Sir Wilson Jameson.

HISTORICAL SURVEY

Serious interest in the use of formaldehyde as a disinfectant dates from the work of Trillat (1892), and in the next two decades a considerable amount of work was done on the bactericidal power of formaldehyde gas. In much of it no attempt was made to control the temperature or to measure the concentration of formaldehyde. Pottevin (1894), however, did work at several temperatures and also controlled the vapour concentration by varying the strength of the formaldehyde solution in the chamber. He found that, over formalin (i.e. 40 % formaldehyde), spores of *Bacillus subtilis*, dried on cloth, were killed in 1 hr. at 52° C., 2 hr. at 35° C. and 40 hr. at 15° C. At each temperature, a weaker solution gave slower killing. Perdrix (1906, 1907) measured the vapour pressure of paraformaldehyde at various temperatures and determined the time required to kill spores in the dry vapour; the times varied from 5 min. at 100° C. to 15 days at 18° C.

Many experiments were performed to assess the ability of formaldehyde to disinfect hospital wards and rooms (Oehmichen, 1895; Bosc, 1896; Roux & Trillat, 1896; Vaillard & Lemoine, 1896; Strüver, 1897; Petruschky, 1898; Lassablière, 1910). Tests were made with vegetative organisms and spores either dried on to fabrics or incorporated into pus or sputum which was variously exposed wet or

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after drying. The ease with which organisms were killed depended on the extent of their exposure. Freely exposed organisms were almost always killed in 24 hr.; the killing of organisms protected by one or two layers of paper or fabric was erratic and organisms in the centre of mattresses almost always survived. Perdrix (1907) found that formaldehyde penetrated fabrics more readily at higher than at lower temperatures, as had Sprague (1899) before him.

There was much argument over the relative merits of 'dry' and 'moist' formaldehyde, though these terms were seldom precisely defined. Roux & Trillat (1896) favoured 'dry' formaldehyde, whereas Rubner & Peerenboom (1899) stated that 'wet' formaldehyde was more effective than 'dry', but that in 'wet' gas dry objects were sterilized more readily than wet ones. Spengler (1903), on the other hand, reached the conclusion that moist objects were the more readily sterilized. Rubner & Peerenboom made the important observation that woollen articles take up more formaldehyde than cotton ones.

The conflicting results of these early observers led Nordgren (1939) to review the previous work and study the basic factors affecting the efficacy of formaldehyde gas as a sterilizing agent, i.e. concentration, temperature, humidity and physical or chemical protection of the organisms. He was able to carry out his experiments in an atmosphere containing measured concentrations of formaldehyde and water vapour, at a closely regulated temperature. The test objects were lengths of glass tube on to which a suspension of organisms was dried. After disinfection the tubes were freed from residual formaldehyde by washing in a solution of sodium sulphite (Hailer, 1921), and the remaining bacteria dispersed in agar for colony counts. Nordgren showed that at constant vapour pressures of formaldehyde and water the rate of disinfection of spores increased as the temperature was raised from 10° to 70° C. At constant temperature and formaldehyde vapour pressure the rate of kill increased with the relative humidity up to 50 %, but there was little increase as the humidity rose from 50 to 90 %. He showed that slow disinfection did occur even when the pressure of water vapour was less than 1·0 mm Hg and also that gross wetting of the objects retarded the killing. At constant temperature and humidity the rate of kill increased with the concentration of formaldehyde in the atmosphere. Nordgren concluded that the maximum rate of disinfection by gaseous formaldehyde approximated to that attained by immersion in a solution of formaldehyde giving the same formaldehyde vapour pressure.

Nordgren investigated the susceptibility of seventy strains of bacteria including members of the coliform and enteric group, staphylococci, human and bovine tubercle bacilli and aerobic and anaerobic spore-forming organisms. With every strain, formaldehyde vapour at 0·6–0·8 mm. pressure, and a relative humidity of 90–100 %, sterilized test objects in less than 1 hr.

In a study of the factors that in practice complicate the use of formaldehyde as a disinfectant, Nordgren showed that protection of the organisms by coating the test objects with blood or sputum reduced the rate of killing of spores. Coarse soil particles still contained viable organisms even after 24 hr. in the vapour of 35 % formalin at 21° C., but such particles could be sterilized in 2 hr. if the temperature was raised to 55° C. and the disinfection chamber evacuated before admitting the

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formaldehyde vapour. Neither elevated temperature nor preliminary evacuation alone sufficed to reduce the sterilization time to 2 hr. Later (Nordgren, 1941) he described an apparatus for sterilizing instruments and catheters which employed this 'new principle'.

The conclusions to be drawn from Nordgren's work are that the vapour of saturated formalin at room temperature will readily kill exposed organisms in 1 hr. but that when the organisms are protected by organic matter, such as blood, or where there is a physical impediment to the diffusion of the vapour, the time to sterility may be extended to 24 hr. or more. The killing of protected organisms can be accelerated by increased temperature and physical aids to the diffusion of the vapour. Paraformaldehyde evaporates so slowly that it is of little practical use and this slow evaporation may account for the ill repute of 'dry' formaldehyde as a disinfectant.

Most of the more recent published experimental work has been primarily concerned with disinfection of organisms on glass, or on surgical instruments and catheters. The Committee felt that more apposite information would be obtained if some fabric were used as the test object, and after a few preliminary trials short lengths of cotton thread were adopted. Early experiments showed that quantitative recovery of the organisms from the thread could not be attained and in all the experiments reported threads were classed simply as 'fertile' or 'sterile'. No attempt was made to enumerate the numbers of organisms on individual threads, although the mean number was estimated statistically by the method described below. Numerous tests were carried out on a laboratory scale to determine the effects of vapour concentration, relative humidity and temperature on the disinfection process. Later, tests were made of large-scale disinfectors in which control of these factors was more difficult or impossible, and in which there was the complicating factor of a large mass of fabric that absorbed formaldehyde.

METHODS

Formaldehyde

Formaldehyde vapour was obtained by evaporating appropriate dilutions (see Table 1 below) of standardized batches of commercial formalin—that is, a 40% solution of formaldehyde in water with about 10% of methanol added to prevent polymerization.

Test bacteria

For most of the experiments the test organism was a coagulase-negative micrococcus (N.C.T.C. 7944) that had been chosen by the British Disinfectant Manufacturers' Association Panel on methods for testing air disinfectants (Report, 1949). This organism grows readily on simple peptone-water media at 37° C. and does not form large clusters.

In later tests an avian strain of *Mycobacterium tuberculosis* (N.C.T.C. 8551) and a number of freshly isolated human strains of *M. tuberculosis* were also employed, as well as several strains of *Bacillus subtilis*, and the virus of smallpox.

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Test objects

The end of a 9 in. length of nichrome wire was bent round the centre of ten 10 in. lengths of white cotton (no. 36) (Fig. 1). The threads were treated with warm petroleum ether for 24 hr. to remove the dressing applied in manufacture, dried at 100° C. for 15 min., and suspended in boiling water for 30 min. They were allowed to dry and then pulled by means of the wire into pieces of glass tubing 6 in. in length and 5 mm. diameter. The ends of the tube were plugged with cotton-wool; the glass tube containing the threads was then placed in a plugged test-tube and autoclaved at 10 lb. for 10 min.

The test micrococcus was stored as a freeze-dried preparation. When required for use it was passed through two successive peptone-water cultures and then transferred to a peptone-water agar slope. Not more than five successive sub-cultures (on the agar slopes) were employed before using a new freeze-dried preparation; no culture was incubated for more than 24 hr.; and none was held in peptone-water for more than 24 hr.

In the first series of tests a gelatin solution was used for the final dilution of micrococci in which the threads were immersed for impregnation. Gelatin was dissolved in distilled water to give a 1 % solution and its pH adjusted to 7·6; it was then sterilized by steaming for 20 min. on 3 successive days, being held at room temperature in the meantime.

For use, the growth of the micrococcus on a peptone-water agar slope was washed off with quarter-strength Ringer's solution and diluted to an opacity equivalent to Brown's no. 1 tube. From this nine successive fourfold dilutions were made in the Ringer's solution. The original suspension and the nine dilutions were then all diluted tenfold in the 1 % solution of gelatin. These final dilutions are referred to as 'dilutions 1 to 10'.

The ten gelatin dilutions in 1 oz. screw-capped bottles were held in a water-bath at 25° C. Meanwhile, the glass tubes containing the bundles had been pushed through bored rubber bungs until a half-inch or so protruded. The bungs were of a size that would just fit the neck of the bottle containing the suspension. With the rubber bung in the neck of the bottle, the wire holding the hanks was then pushed down through the tube until the threads were completely immersed in the suspension. They were gently agitated for 1 min. They were then withdrawn, excess fluid shaken off, a paper-clip attached to the end of the threads and the bundles laid out horizontally in a special box (Fig. 2) which contained a layer of calcium chloride to dry the threads. The box was placed in the refrigerator at 4° C. for 24 hr. and the threads were stored under these conditions until required for use, but not for more than 7 days.

In order to provide greater protection for the cocci than was afforded by 1 % gelatin the cocci were, in later experiments, suspended in 90 % horse serum. Sputum, which might have been a more appropriate suspending medium, proved too difficult to manipulate.

When the avian type of mycobacterium was used, the suspension was made from growth on the solid medium of Ackart & Murray (1951), washed off and diluted

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with horse serum. In experiments with freshly isolated human strains of *M. tuberculosis*, the culture was grown on Löwenstein–Jensen medium, and the mass of cells was ground up in a glass mortar and suspended in horse serum. It was difficult to prepare a satisfactory suspension and the infected threads carried only 500–1000 viable units per half-inch length.

Cultivation of organisms on the threads

In order to determine the survival of the cocci on the threads quarter-inch lengths were cultured. The bundle of threads was pushed a short distance out of its ensheathing tube, a quarter-inch length was cut off and the twenty quarter-

Fig. 1

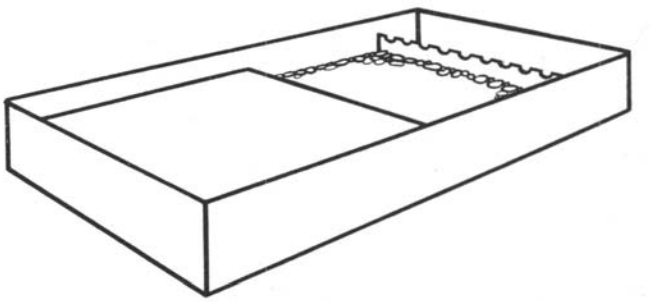

Fig. 2*A*

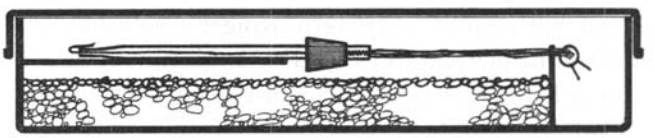

Fig. 2*B*

Fig. 1. Threads hanging from wire holder.

Fig. 2. Box used for drying threads. *A*, perspective view; *B*, longitudinal section to show threads held horizontally for drying.

inch fragments were allowed to fall on to a 4 in. diameter disk of sterile filter-paper resting on a large rubber bung. They were spread out over the paper with sterilized forceps and a 4 in. Petri dish containing moist peptone-water agar was then inverted over the filter-paper and pressed down; the paper was removed, leaving the threads attached to the agar. The plates were incubated for 4 days at 37° C. and the number of threads showing adherent growth of the typical form was counted.

Threads impregnated with the mycobacterium were cultivated individually on the surface of Ackart & Murray's solid medium or on glycerol-egg medium in 5 ml. screw-capped bottles, or in the liquid media of Ackart & Murray, Dubos or Kirchner in 30 ml. bottles.

Tests with spore-forming organisms

A strain of an aerobic spore-forming bacillus of the *B. subtilis* type was grown in broth for 3 days. The spores were collected by centrifugation, washed twice with

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distilled water, diluted in serum and used to infect threads. There was no significant difference between colony counts performed on the suspension before and after heating to 80° C. for 20 min. After disinfection the threads were cultivated individually in tubes of broth and incubated for 14 days.

Efficiency of impregnation methods

By several independent methods the threads were found to absorb, on the average, 0·001 ml. of the 1 % gelatin solution per quarter-inch length. In general the number of cocci taken up conformed to the expectation based on this volume. Survival after drying varied between 10 and 60 %. By the use of appropriate initial suspensions of the micrococcus there was, however, no difficulty in obtaining initial counts estimated to lie between 10,000 and 100,000 cocci per quarter-inch length of thread. (This estimate is based on the counts derived from threads impregnated from the more dilute suspensions.)

After drying, the cocci survived reasonably well on the threads (about 66 % surviving for 7 days), but initial counts were always made on the day of use in a disinfection test.

Technique of disinfection tests

The disinfection tests were, in almost all laboratory experiments, carried out in wide-mouth glass jars, 20 cm. high and 15 cm. in diameter (Kilner, 7 lb. fruit-preserving jars). The glass lid of the jar was replaced by a bakelite disk, which was perforated to allow the insertion of 6–9 glass tubes containing the threads; the tubes were held in place by rubber bungs (Fig. 3).

Atmospheres of known relative humidity and formaldehyde concentration were obtained inside the jar by means of saturated aqueous solutions of various salts, containing appropriate amounts of formaldehyde (see Table 1). About 100 ml. of the working solution and some of the solid salt were put in the disinfection jar and allowed to equilibrate for 2 hr.

A set of bundles of threads prepared from the ten dilutions was taken from the refrigerator immediately before use, allowed to reach room temperature and the individual threads were teased apart. In early experiments the dry threads were left to equilibrate to the required humidity over salt solution without formaldehyde, but this was later found to have no effect on the rate of disinfection and was abandoned. Quarter-inch lengths were cut from the bundles 7 to 10 and plated for the initial count. The glass tubes containing the bundles were then transferred to the disinfection jar and the wire to which the threads were attached was pushed out so that the threads hung free in the vapour (Fig. 3).

In experiments carried out at temperatures of 30° C. or 37° C. care was taken to keep the top of the jar warm, so as to prevent condensation on it.

At intervals, the appropriate thread bundles were withdrawn into the glass tube; the tube and bung were removed; quarter-inch lengths were cut for culture, and the tube and bung returned to the jar. The time between cutting and plating of the threads was usually less than 1 min. At least four bundles were tested at each time; as disinfection proceeded thread bundles with progressively heavier bacterial

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Table 1. *Formaldehyde vapour concentration over solutions of formaldehyde in water and in water saturated with various salts*

(Values in parentheses are of doubtful accuracy.)

Salt	Relative humidity (%)	Formaldehyde vapour concentration (μg./l.) for each 1 % formaldehyde in solution						
		0°	10°	15°	20°	25°	30°	40° C.
None	100	(16)	31	—	79	—	170	346
Na_2SO_4	93	—	—	—	127	—	—	—
KBr	84	—	—	—	132	—	—	—
NaBr	58	(10)	43	80	156	204	303	(437)
$CaCl_2$	32	—	—	—	214	—	—	—

This table is based on a series of measurements of the vapour concentration, made by the method described on p. 496, over the various salt solutions.

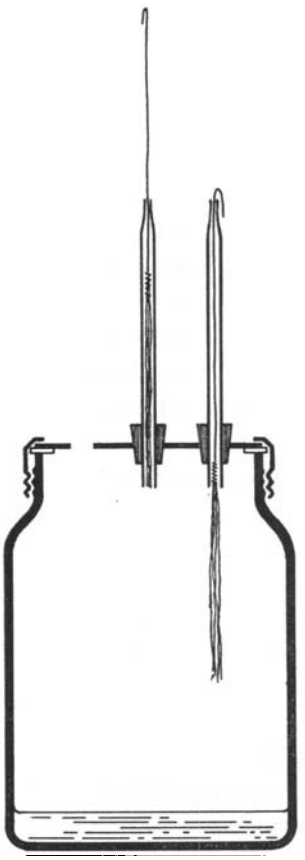

Fig. 3. Arrangement of jar disinfection experiments. During tests the threads hung free in the jar as shown on the right; they could be withdrawn into the glass tube (centre) and then the tube and rubber bung removed to enable samples to be taken.

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impregnation were used (see Table 2). In several experiments a duplicate set of threads was held over the saturated salt solution without formaldehyde for a second control count at the end of the experiment, but no appreciable decrease in the count was observed.

In some of the later experiments half-inch lengths of thread were cut instead of quarter-inch lengths.

Table 2. *Results of a typical disinfection test*

Dilution with which the tested bundles were impregnated	No. of fertile threads of 20 tested at specified times (min.)					
	0	10	20	30	40	50
1	—	—	—	—	10	5
2	—	—	—	17	6	3
3	—	—	19	13	4	3
4	—	20	16	8	4	4
5	20	20	11	3	2	—
6	19*	17	8	1	—	—
7	14	11	3	—	—	—
8	8	2	—	—	—	—
9	4	—	—	—	—	—
$\log_{10}$ count on threads in first bundle of the 4 used in computation	0·83	0·98	0·61	0·20	0·17	$\bar{1}$·87
$\log_{10}$ count on threads in bundle 1	3·85	3·39	2·41	2·26	0·17	$\bar{1}$·87

* Twenty threads from each of five bundles, impregnated with five successive dilutions, were tested at each sampling time. The count was computed from the four successive bundles indicated by the vertical line.

Penetration of formaldehyde

For experiments on the penetration of formaldehyde through blankets a special chamber was constructed, consisting of a tin-plate box 9 in. square by 13 in. high. To the under side of the lid was soldered a cylindrical box 3 in. diam. by 9 in. high, the sides of which were liberally perforated with quarter-inch holes. The requisite layers of blanket were wrapped around the inner box and secured with bands at the top and bottom. Through holes in the lid, thread bundles could be introduced either into the main chamber or into the centre of the blanket-enclosed inner box. A test was performed by putting a vessel of 40% formaldehyde in the bottom of the main chamber and by placing the thread bundles in position at once. In contrast with other experiments, no time was allowed for the air to become saturated with vapour before starting the test, and in consequence the disinfection was slower than that recorded in some other tests. Samples of threads were cut and plated at appropriate intervals. In tests of the large-scale disinfector C, the impregnated threads were laid between layers of blankets; a total of twenty-eight layers were set in a pile which was laid on the mattresses.

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Neutralization of formaldehyde in cultures

The possibility that sufficient formaldehyde might be carried over in the threads to inhibit growth on the culture plates was considered early in the Committee's work, and investigations were made of possible neutralizing agents (see Nash & Hirch, 1954). Numerous experiments were carried out without revealing that neutralization led to greater survival of cocci exposed to low concentrations of formaldehyde. The problem, however, became important when the threads were exposed to stronger formaldehyde concentrations. In some experiments 2% sodium sulphite was incorporated in the agar. In others (e.g. those in which disinfection of *M. tuberculosis* was tested) the threads were transferred to individual culture tubes or bottles containing at least 2 ml. of medium, which was calculated to afford sufficient dilution.

An alternative method was to transfer the threads from the disinfectant vapour first to the surface of a water-agar plate and subsequently to the nutrient agar. A thread made up in the standard manner with the organisms suspended in 90% serum was found to absorb about 80 μg. of formaldehyde per inch during 1 hr. over 40% formalin at 20° C. This fell to 30 μg. after 1 hr. in air, 20 μg. after 1 hr. over water in a jar, or 7 μg. after 1 hr. on water agar. When the threads were left overnight above formalin, 280 μg. per inch could be recovered and, even after soaking in water for 1 hr., the concentration was still 40 μg. per inch. Tests with infected threads showed that a negligible proportion of the organisms were left on the water agar.

A third method, used in the last set of experiments (Table 8), was to put the bundle of threads into a Petri dish and cover with a few drops of sterile 6% sodium sulphite solution; after 10–15 min. the bundle was shaken free of adherent solution, put into sterile water for 10 min., and finally plated in the ordinary way.

Neither gelatin nor serum could be shown to take up formaldehyde irreversibly under the conditions used in the disinfection tests.

Determination of formaldehyde vapour concentration

Bactericidal concentrations of formaldehyde are high enough for small samples of air to suffice for accurate measurement. Accordingly, samples were taken by means of a 30 ml. syringe, the needle of which was inserted into a piece of fine-bore polyvinyl chloride tubing leading to the chamber containing the formaldehyde vapour; 5 ml. of water were introduced into the syringe, which had a simple clamp so that the plunger always stopped at the 30 ml. mark. The sample was thus 25 ml. of air. The sample required dilution if the concentration was 2 mg./l. or over.

The formaldehyde had virtually all dissolved in the 5 ml. of water after standing for 5 min. or shaking for 1 min.

The colorimetric method used for estimating the amount of formaldehyde in the solution based on the Hantzsch reaction has been described elsewhere (Nash, 1953). The chromotropic acid method was not used because it had been found that

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commercial formalin contains a volatile impurity, trioxane, which reacts as formaldehyde with chromotropic acid, but which was shown to have little, if any, bactericidal effect.

Calculation of killing rates

General considerations

In the early experiments an attempt was made to estimate the mean number of viable cocci on the threads from the proportion of fertile threads observed in four sets impregnated with successive dilutions of the coccal suspension. The numerical calculations were those described by Fisher & Yates (1948) for estimating the numbers of viable organisms from dilution series. The 'count' so derived is referred to as the 'notional count' and was multiplied appropriately to give a notional count on the threads impregnated from dilution 1. For counts of the numbers of organisms initially present on the threads it was usually necessary to cultivate the threads impregnated from dilutions 7 to 10 of the micrococcus suspension. As killing of the cocci under the influence of formaldehyde proceeded, the threads from lower dilutions were examined (see Table 2).

If organisms were deposited on the threads randomly from the absorbed suspension then the chance of a thread being sterile would be $P = e^{-nv/z}$, where v ml. is the volume absorbed from a $1/z$ dilution of a suspension containing n organisms per ml. Under these conditions there would be a linear relationship, with unit slope, between log $(-\log P)$ and log z. The earliest experiments showed that this simple relationship did not hold even before exposure to formaldehyde and that the slope was less than unity, with successive dilutions appearing to differ by a factor less than that actually employed. Such a result would follow if the volume of suspension effectively absorbed on to a thread were subject to considerable variation, or if there were any other factors that might lead to large variation in the chance of organisms being found on the threads. If the distribution of values of v is lognormal with a variance σ^2 then the slope of log $(-\log P)$ against log z can be shown to be approximately $10^{-0{\cdot}32\sigma}$ at values of P around 0·5. The relationship is no longer strictly linear, although the curvature is not very great for values of P between 0·1 and 0·9 and for values of σ less than 1·0. For values of σ greater than 0·8 the probit of P plotted against log z (Grundy, 1951) gives a straight line with a slope equal to $\sqrt{(\sigma^2 + 0{\cdot}27)}$.

Bundles of threads impregnated in a series of dilutions of a suspension of cocci and plated out, as described in the previous section, before exposure to formaldehyde were found to show a slope for log $(-\log P)$ against log z of about 0·8, which corresponds to a value of σ of about 0·28. This value is very close to the figure of 0·31 found for the standard deviation of the logarithm of the volumes of dye solution absorbed into the threads, as estimated by colorimetric analysis, so that the variation in the volume of liquid taken up by the threads is apparently sufficient to account for the departures from the simple Poisson distribution shown by the untreated impregnated threads. It is possible, although less convenient, to impregnate threads in such a way as to reduce this source of variance considerably, e.g. by winding the bundles on frames which hold all the individual threads spaced from one another. This was not done, however, since in disinfection experiments

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it was found that the value of σ estimated as above increased steadily as disinfection progressed so that σ approximates to 0·28–0·34 log F, where F represents the fraction of the original organisms surviving. This additional source of variance might be due either to heterogeneity in the susceptibility of the cocci to the action of the formaldehyde, or, more probably, to varying accessibility of cocci embedded deeply in the threads.

Estimation of the numbers of cocci on the threads impregnated from a dilution series, either initially or after a period of exposure to disinfection, is considerably complicated in theory by the factors described above. The published methods of Fisher (1922) and Finney (1951) for estimating the numbers of organisms in samples from a dilution series are based on the hypothesis of a Poisson distribution of organisms in the samples. A rigorous evaluation is possible by the use of maximum likelihood methods based on the tables given by Grundy (1951) and extended by him to smaller values of σ (unpublished private communication), but this is extremely laborious. An approximate method can be devised, based on the fact that for $P = 0{\cdot}45$ the value of nvz is approximately equal to 0·8 irrespective of the value of σ, using any convenient interpolation to plot $f(P)$ against $f(z)$. In practice, however, Fisher & Yates's method appears to give values which are not significantly different from those obtained by the other possible methods so long as the condition is retained that the dilution series must extend from P near unity to P near zero.

Evaluation methods adopted

Killing-rate curves. From the results of testing batches of twenty quarter-inch lengths of thread cut from four different thread bundles (impregnated from successive dilutions) a 'notional count' for the bundle impregnated from dilution 1 was calculated. The logarithms of these counts were plotted against the time of exposure, and straight lines drawn by the method of least squares through the points over the time ranges 0–30 min. and 10–40 min. Killing rates (K) were then calculated from the relation

$$K = \frac{1}{N}\frac{dn}{dt} = \frac{2{\cdot}3(\log N_1 - \log N_2)}{t},$$

where t is the interval in hours between the times when N_1 and N_2 bacteria were found. (Logarithms to base 10 are used throughout.)

K values from 0 to 30 and 10 to 40 min. did not differ systematically up to values of the killing rate not greater than 5 per hour. With faster rates of disinfection the estimated killing rate was less over the latter period, an effect which might have been produced either by chance contamination or by the presence of a small proportion, not more than 5 %, of organisms more resistant to disinfection, either intrinsically or because of deeper embedding in the threads. Further reference will be made to this point in the following section but for most of the analyses values of K estimated over the first 30 min. of exposure have been used.

Sterilization of heavily infected threads. The killing-rate determinations made by the method just given describe the initial killing conditions. The killing rate often

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tended to decrease towards the end of the experiment, and this was confirmed by the observation that the value of K based on 0–30 min. tended to be higher than that based on 10–40 min. Experiments were therefore devised to examine the later part of the killing process. For this purpose the threads were impregnated from more concentrated suspensions of cocci.

Using the heavily impregnated thread bundles (estimated to carry 10^4–10^5 cocci per half-inch length of thread) it was found that the graph of the percentage of sterile threads plotted against the time of exposure had the sigmoid shape typical of many biological experiments in which a quantal response is observed. There was an approximately linear relationship between the probit of the percentage of sterile threads after a given exposure and the logarithm of the time of exposure (Fig. 4).

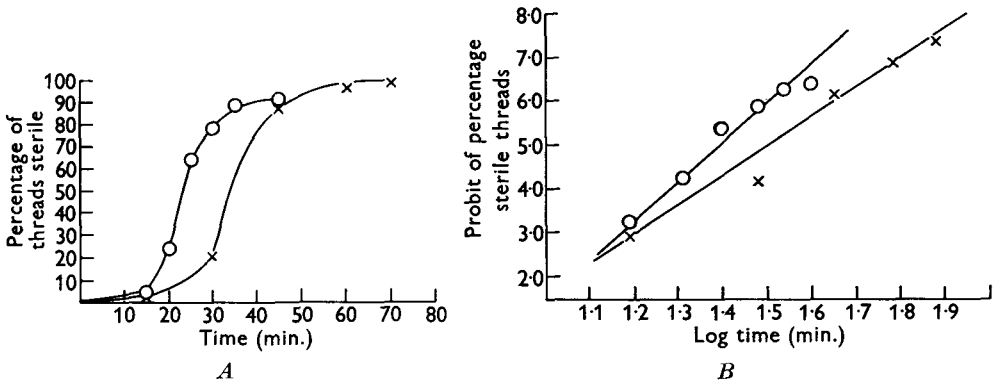

Fig. 4. Sterilization of heavily infected threads; formaldehyde 1·0 mg./l., at 20° C. and R.H. 58 %. *A*, percentage of sterile threads plotted against time; *B*, probit of percentage of sterile threads plotted against log time. ○ and × = results of two separate experiments.

It was noted earlier that, when the chance of an organism being found on a thread varies greatly from thread to thread, the probit of the proportion of sterile threads is linearly related to the logarithm of the dilution in a dilution series, i.e. to the logarithm of the expectation of the number of organisms per thread. We therefore have:

$$Y = A - S \log_{10} \epsilon$$

and, from the observations,

$$Y = a + b \log_{10} t,$$

where Y is the probit of the proportion of threads sterile at time t, ϵ is the expected number of cocci on a thread and A, a, S and b are constants.

Hence

$$\log \epsilon = \frac{a - A}{S} - \frac{b}{S} \log t,$$

or

$$2{\cdot}303 \frac{d \log \epsilon}{dt} = \frac{1}{\epsilon} \frac{d\epsilon}{dt} = -\frac{b}{S} \frac{1}{t}.$$

That is, the killing rate $\left(K = \frac{1}{N} \frac{dN}{dt} = \frac{1}{\epsilon} \frac{d\epsilon}{dt}\right)$ is not constant but, over this range, varies inversely with the time of exposure. In the absence of any satisfactory way of evaluating the constant S above, a value of ϵ—the expected number of cocci

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on a thread—can only be deduced for the time at which the proportion of sterile threads is 0·45, when ϵ is approximately equal to 0·8 independently of the value of S.

The time taken for disinfection to reach a stage at which the proportion of sterile threads is 0·45, and the average number of cocci per thread is 0·8, is conveniently referred to as ST_{45}; it represents something between a 99·99 and a 99·999 % kill of the cocci originally present on the thread, according as this number lay between 10^4 and 10^5.

The time required to sterilize with a defined degree of certainty a given number of threads can be obtained by extrapolation. It is, however, determined not solely by the ST_{45}, but also by the slope of the line, which is a measure of the standard deviation of logarithm of the time to sterilize individual threads. The time to sterility of the sample generally proved to be about 5 times the ST_{45}.

RESULTS

Laboratory Disinfection Tests

Tests with the Micrococcus

The effects of temperature, humidity and concentration of formaldehyde vapour on the killing rate

Reproducibility of results. Rather more than 100 sets of experiments were carried out in which bundles of threads impregnated from gelatin suspensions of the coccus at a series of dilutions were exposed to the action of formaldehyde vapour under various conditions. There were some forty-five estimates of the killing rate, K, at a temperature of 20° C. and a relative humidity of 58 %. These were carried out in six different laboratories; in some experiments the threads were equilibrated with the prevailing humidity employed before being exposed to the action of the formaldehyde (see Methods). An analysis of variance covering these factors was carried out using the logarithm of the killing coefficient ($k = \log_{10} K/C$, where C is the concentration of formaldehyde vapour in mg./l.) (Table 3). The assumption that K/C is constant, implicit in this analysis, is justified by the observations reported below. The between-laboratories variance did not significantly exceed the within-individual laboratories variance, and there was no significant effect attributable to prior equilibration with the prevailing humidity. The standard deviation of a measurement was high, being about 0·28 in $\log_{10}$ units, i.e. about +90 to −45 %.

Formaldehyde concentration. There was a clear increase in the killing rate with increased concentration at constant temperature and humidity (Table 4) and the figures show that there was no appreciable deviation from a linear relation between K and C, at least up to the value of C of 0·31 mg./l.

Relative humidity (R.H.). The effect of relative humidity on the killing rate is given in Table 5, based on all the experiments done at 20° C.; variations in formaldehyde concentrations have been allowed for by tabulating K/C. There appears to be a maximum in the killing rates around R.H. 80–90 %, but the magnitude of this peak is not easy to assess because of the large errors of estimation.

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Temperature. The number of observations at temperatures other than 20° C. was rather small so the results obtained at R.H. 58 and 100 %, which do not appear to differ significantly at 20° C., have been combined (Table 6). The errors of estimation are even larger than in the previous tables but with this limitation there was no evidence over the range 0–30° C. of any effect of temperature on killing rate.

Effect of temperature and formaldehyde concentration on sterilization time

The results described in the previous section were extended by the use of more heavily impregnated threads and determination of the time taken to sterilize 45 % of the threads (ST_{45}). It may be emphasized again that this proportion corresponds to an average of < 1 coccus per thread and implies a 99·99 % or greater kill.

Table 3. *Analysis of variance of values of the killing coefficient*

(At 20° C., 58% R.H.)

Laboratory	No. of expts. or degrees of freedom	$\bar{k}$*	Root mean square
T	10	1·764	0·197
S	7	1·636	0·150
C	6	1·410	0·271
P	5	1·601	0·509
H	16	1·658	0·242
M	1	2·104	—
Within laboratories	38 D.F.	—	0·266
Between laboratories	6 D.F.	—	0·341
Previously equilibrated (A)	32	1·652	0·286
Not equilibrated (B)	13	1·639	0·278
Between (A) and (B)	1 D.F.	—	0·040
Total	45	1·648	0·280

* $\bar{k}$ is the mean value of the values of k, the killing coefficient $= \log_{10} K/C$. K = killing rate as defined in the text; C = formaldehyde concentration, mg./l.

Table 4. *Effect of formaldehyde concentration on killing rate*

(At 20° C., 58 % R.H.)

Formaldehyde, mg./l. (C)	0·04	0·08	0·16	0·31
No. of expts.	5	10	22	4
$\bar{k}$	1·601	1·562	1·735	1·595
Mean K/C	40^{+13}_{-10}	36^{+8}_{-8}	54^{+8}_{-8}	39^{+15}_{-16}
Mean K	1·6	2·9	8·6	12·1

The ± figures given after the values for K/C in Tables 4–6 represent one standard deviation estimated from the value 0·280 given in Table 3 as the root mean square of k. Other conventions as in Table 3.

The first experiment in this series provided an opportunity of investigating the effect of the number of organisms per thread on the ST_{45}. From the results of ten tests it did not appear that the ST_{45} was appreciably influenced by the number of organisms present and, in fact, the coefficient of regression of the ST_{45} on log N was insignificant. A real relation between ST_{45} and log N may have been obscured by unsuspected differences in technique between laboratories, but it is more probable that, over the range of N observed, there was little relation between the

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two quantities, because other evidence suggests that the rate of killing was limited by the rate at which the vapour penetrated the gelatin-coated threads.

The results of these tests also illustrated the falling off in the killing rate as the disinfection proceeds. From Table 4 the value of K/C at 20° C. and 58 % relative humidity is approximately 46. Half an hour's exposure at 1·0 mg./l. of formaldehyde vapour should reduce the number of organisms on a thread by $(46 \times 0{\cdot}5)/2{\cdot}30 = 10$ powers of ten. In fact, the observed reduction in this time lay between 4 and 5 powers of ten. That is, the time to 99·99–99·999 % sterility was over twice what would be expected on the basis of the initial killing rate over the first 98–99 % of the organisms.

Table 5. *Effect of relative humidity on killing rate*

(At 20° C.)

Relative humidity (%)	32	58	84 & 93	100
No. of expts.	12	42	13 (10)*	22
$\bar{k}$	1·536	1·664	1·956 (1·817)	1·668
Mean K/C	34 ± 7	46 ± 5	90 ± 17 (66 ± 15)	47 ± 6

Conventions as in Tables 3 and 4.

* Figures in parentheses are means excluding three high rates all from one laboratory at one time.

Table 6. *Effect of temperature on killing rate*

(At 58 and 100 % R.H. taken together.)

Temperature (° C.)	0	10	15	20	25	30
No. of expts.	3	7	3	64	4	7
$\bar{k}$	1·861	1·665	2·007	1·665	1·832	1·907
Mean K/C	73^{+32}_{-23}	46^{+13}_{-10}	102^{+45}_{-32}	46 ± 4	68^{+26}_{-19}	81^{+22}_{-17}

Conventions as in Tables 3 and 4.

Table 7. *Effect of temperature and suspending medium on time to sterilize* 45 % *of threads* (ST_{45}, *min.*)

(Formaldehyde 1·0 mg./l.; R.H. 58 %. Initial number of cocci per thread = *c.* 10^4.)

Temperature (° C.)	Cocci suspended in 1 % gelatin	Mean	Cocci suspended in 90 % horse serum	Mean
10	20, 25, 41, 41, 44	34	108, 141, 301	183
20	19, 21, 24, 24, 27, 30, 32 35, 35, 40	29	144, 150, 168, 216, 415, 415, 425	276
30	8, 9, 12, 12, 13, 16, 20, 22, 28	16	89, 168, 228, 228	178

The effect of temperature at constant vapour concentration is illustrated in Table 7; the range of variation in the results was considerable and there is no consistent evidence of a substantial temperature effect within the range 10–30° C. The serum, however, had a clear protective effect on the cocci.

The formaldehyde concentration used in these experiments was only about two-fifths of the maximum that it is possible to reach by using saturated formaldehyde solutions. Exposure of similar threads to the vapour of 40 % formaldehyde effected a rapid sterilization even when serum was used as the suspending fluid (Table 8,

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Expt. 1). Under these conditions there is considerable carry-over of formaldehyde on the threads. A second experiment was carried out using the sulphite-wash method to neutralize this formaldehyde. The disinfection time appeared to be lengthened for the threads exposed at 20° C., but there was no detectable effect for those exposed at the higher temperature (Table 8, Expt. 2).

Table 8. *Time to sterilize* 45 % *of threads in the vapour of* 40 % *formaldehyde*

(Cocci suspended in horse serum; initial number per thread = *c.* 10^4.)

		ST_{45} (min.)			
		Expt. 1		Expt. 2	
Temperature (° C.)	Formaldehyde concentration (mg./l.)	Without neutralization of formaldehyde	Mean	With neutralization of formaldehyde	Mean
20	2·4	13, 18, 19, 23, 25, 26	21	12, 34, 40, 40, 42, 56, 66, 89, 107	54
30	6·8	7, 7, 9, 9	8	5, 9, 11, 12	9
37	*c.* 13·8	6, 6, 6, 6, 10, 10, 13	8	4, 5, 8, 9, 11, 13, 14, 17	10

Table 9. *Time to sterilize threads infected with* Mycobacterium tuberculosis *avian type in the vapour of* 40 % *formaldehyde*

(Organisms suspended in horse serum. Initial counts *c.* 10^4 bacilli/thread.)

		No. of experiments in which threads remained fertile to stated times				
Temperature (° C.)	No. of experiments performed	15 min.	30 min.	60 min.	120 min.	180 min.
c. 20	15	—	—	9 (50 %)	1	0
37	14	8 (46 %)	—	1	0	—

Figures in parentheses are the maximum percentage of fertile threads.

Table 10. *Time to sterilize* 45 % *of threads infected with spores in the vapour of* 40 % *formaldehyde*

(Spores of aerobic sporing-forming bacilli, suspended in horse serum, initial count > 10^4/thread.)

Temperature (° C.)	ST_{45} (min.)	Mean
20	135, 151, 159, 166, 170, 214, 219	173
37	18, 23, 23, 28	23

In contrast to the previous experiments, the formaldehyde solution used for both the experiments of Table 8 was the same at all temperatures, so that the vapour concentration increased with temperature. The shortening of the killing time at the higher temperatures was in part, at least, due to this. The same considerations apply to the results of Tables 9 and 10.

Penetration of formaldehyde through blankets

Nine tests were performed, and the results of two typical experiments on the penetration of formaldehyde vapour through blankets are shown in Fig. 5. In every instance the disinfection was appreciably slower within the blanket than it

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was outside and even a single layer increased the ST_{45} by 60 %. Measurements of the formaldehyde concentration outside and inside four layers of blanket amply confirmed the slowness of the penetration. Outside the blanket the concentration reached 1·76 mg./l. in 85 min. rising to 2·4 mg./l. at equilibrium, whereas inside the blanket it was only 0·60 mg./l. after 6 days.

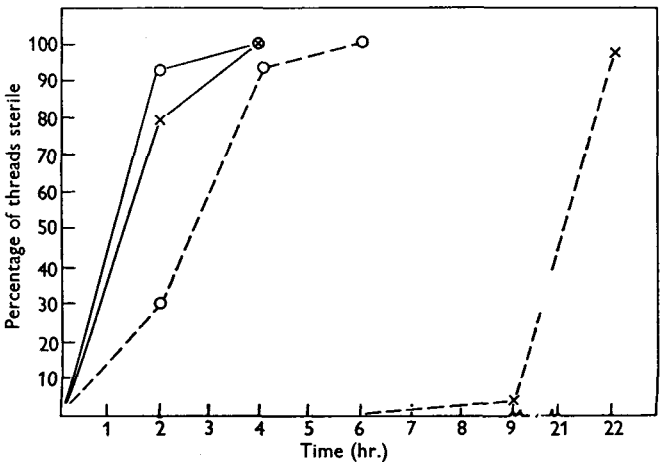

Fig. 5. Penetration of formaldehyde through blanket. Threads infected with micrococci suspended in serum, exposed over 40 % formaldehyde solution, at 20° C. O———O, outside three layers of blanket; O - - - - O, inside three layers of blanket; × ——— ×, outside fifteen layers of blanket; × - - - - ×, inside fifteen layers of blanket.

Tests with Mycobacterium tuberculosis

In twenty-nine experiments with the avian tubercle bacillus (Table 9) no threads were found fertile after 180 min. exposure at room temperature to the vapour of 40 % formaldehyde. At 37° C. none was found fertile after 120 min. Accurate estimation of the ST_{45} was not possible, but it would appear to be less than 60 min. at room temperature, and less than 15 min. at 37° C.

Nine similar experiments were carried out at room temperature with human tubercle bacilli. In two there were a few fertile threads at 60 min.; in the other seven all threads were sterile at 60 min., as were all threads in all nine experiments at 120 min. Four experiments at 37° C. yielded no fertile threads even after 30 min.

On five occasions threads were impregnated with sputum naturally infected with large numbers of tubercle bacilli and, after exposure to 40 % formaldehyde for 10, 40, 60, 120 and 120 min. respectively, were implanted under the skin of guinea-pigs. None produced any infection, although control animals receiving untreated threads all developed typical lesions.

Tests with Bacillus subtilis

A small number of tests were carried out with threads impregnated from a suspension of *B. subtilis* spores in 90 % horse serum (Table 10). The ST_{45} over 40 % formaldehyde solution was about 2 or 3 times that observed with the micrococcus.

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Tests in large-scale formaldehyde disinfectors

Tests were made in three formaldehyde disinfectors designed for the treatment of mattresses and bedding. Two of these, A and C, were commercially produced models and were tested under the conditions recommended by the makers.

Disinfectors

Disinfector A was a cabinet of 160 cu.ft. capacity, having a trolley capable of carrying seven mattresses; a complex solution supplied by the manufacturers, containing about 18 % formaldehyde, was heated in a closed external vessel until the temperature had reached about 200° C. and sufficient pressure was generated to open a valve and allow the solution to be discharged as a vapour or spray into the disinfection chamber. About 2800 ml. of the 18 % formaldehyde solution were used. The materials were exposed to the vapour for 2 or 20 hr., after which 600 ml. of ammonia were vaporized into the chamber before it was opened.

Disinfector B was built in the laboratory and consisted of a painted hardboard box of 80 cu.ft. capacity. A bank of eight 150 W. lamps on the floor of the chamber enabled the interior temperature to be raised to 45° C. Formaldehyde solution was boiled in an external vessel from which a tube led directly into the chamber. Impregnated threads could be inserted through other holes for determination of killing rates.

Disinfector C was a steam-jacketed autoclave of 130 cu.ft. capacity in which a vacuum of 20 in. Hg could be produced and which was fitted with a device for the introduction of formalin. The recommended quantity was 4 oz. (110 g.), which was just sufficient to saturate the air of the chamber at 45° C. without allowance for absorption by the load (see below). The high-pressure steam jacket enabled temperatures of over 100° C. to be attained on the surface of material exposed in the chamber. We are indebted to Prof. L. P. Garrod for the opportunity of testing this apparatus and for the loan of the blanket pile referred to below.

Disinfection tests

The test objects used in almost all the experiments were cotton threads impregnated with the micrococcus or an avian tubercle bacillus by the methods already described. Usually the threads were laid on the surface of a mattress in the disinfector. In a few experiments cotton squares smeared with sputum naturally infected with *M. tuberculosis* (and in one case further enriched with a culture of tubercle bacilli) were used, laid in Petri dishes and covered with a single layer of gauze. Threads were sometimes laid under a pillow to test penetration of the vapour, or wrapped in several layers of woollen blanket.

In the tests of disinfector C the impregnated threads were laid between layers of blankets; a total of twenty-eight layers were set in a pile which was laid on the mattresses.

Formaldehyde concentrations

In disinfector B the air was stirred with an electric fan to maintain an even concentration throughout. In A there was no such provision, and temperature

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measurements showed a considerable difference between the upper and lower parts of the box; there were probably similar differences in formaldehyde concentration.

The formaldehyde concentrations attained in disinfectors A and B are shown in Fig. 6. No method was available for extracting air samples from disinfector C. The profound effect of loading the chamber with mattresses is clear; other laboratory measurements have shown that woollen fabrics exposed in an atmosphere of

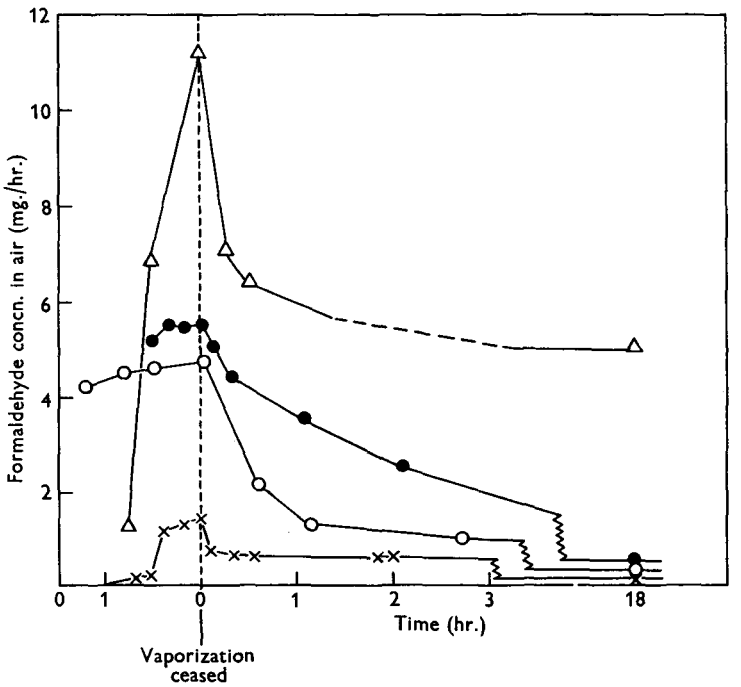

Fig. 6. Formaldehyde concentration in disinfectors A and B. The graphs are adjusted so that the times at which vaporization ceased coincide. ×, disinfector A with five or six mattresses, ambient temperature; △, disinfector B with no mattresses, 40–45° C.; ●, disinfector B with one mattress, 40–45° C.; ○, disinfector B with three mattresses, ambient temperature.

0·3 mg./l. formaldehyde can absorb up to 1 % of their weight of formaldehyde. A great excess over the quantity required to saturate the air space of the disinfector is therefore needed if any effective concentration is to be attained.

Results of disinfection tests

The results with disinfector A (Table 11) showed that threads impregnated with the gelatin suspension of micrococci and exposed on the surface of the mattresses were almost always sterilized. Threads impregnated with the serum suspension of the micrococcus, or with the avian tubercle bacillus were not consistently sterilized although the counts were greatly reduced. For example, the experiment in which 26 % of the threads proved fertile indicated a count of less than 1 coccus per thread, compared with the $1{\cdot}4 \times 10^6$ cocci estimated to have been on the threads before disinfection. Threads placed under a pillow were often apparently unaffected, and

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Table 11. *Results of tests in disinfectors A and B*

Disinfector	Formaldehyde: Introduced mg./l. of total air space	Formaldehyde: Max. air conc. attained (mg./l.)	Temp. in chamber (° C.)	Time of exposure (hr.)	Test bacteria	Suspending medium	Approximate initial count per ½ in. thread	Percentage of fertile threads after disinfection: results of individual experiments: Open	Under pillow	Under 2–6 layers of blanket
A	110	1·5	*c.* 20	2	*Micrococcus*	Gelatin	$1–33 \times 10^7$	0·8, <1·7	100, 100	—
A	110	1·5	*c.* 20	18	*Micrococcus*	Gelatin	$2–90 \times 10^7$	<0·8, 1·3	34, 95	<1·1, <1·2, 1·3, 4, 4, 40
A	110	1·5	*c.* 20	18	*Micrococcus*	10% serum	2×10^7	<0·8	100	<0·8, 3·3
A	110	1·5	*c.* 20	18	*Micrococcus*	90% serum	$2 \times 10^4 – 1 \times 10^7$	0·3, 0·5, 21, 26	—	—
A	110	1·5	*c.* 20	18	Avian T.B.	Broth saline	Not done	<0·9, 18, 38, 52, 68	—	—
B	90	2·5–6·0	44–49	22	*Micrococcus*	90% serum	$1 \times 10^3 – 1 \times 10^7$	0·3, <0·6, 0·8, 1·7, 3·3	—	—
B	90	3·2	48	22	Avian T.B.	90% serum	6×10^3	<1·7	—	—

Notes. Disinfector A was always tested with a load of about five mattresses and several pillows; B had one mattress. The concentration of formaldehyde in the air in disinfector A was not measured at each experiment. The number of threads tested varied between 40 and 400; in twenty-seven of the experiments 80 or more were used. <1·7 etc = no fertile threads found.

in one of three experiments with threads wrapped up in blankets the resulting disinfection was also reduced.

Two experiments were carried out in which infected sputum from patients with tuberculosis was smeared on half-inch squares of a cotton fabric, dried, and exposed in disinfector A. In the second experiment the sputum was reinforced with a suspension of the tubercle bacilli grown from the first experiment. For culture some of the cotton squares were treated with 10% sulphuric acid for 3 min., drained, transferred to 2% sodium hydroxide, drained again and then spread on slopes of Löwenstein–Jensen medium; other squares were cultured without preliminary treatment. In several cases viable organisms were recovered, although in small numbers.

It appears, therefore, that disinfector A was able to effect a great reduction in the bacterial count of test objects exposed in it, but could not be relied upon to sterilize them. It was less efficient with the tubercle bacilli than with the micrococci. The formaldehyde vapour had little power of penetration.

The tests in disinfector B (Table 11) were carried out to determine whether more reliable results could be obtained by raising the temperature to 45–50° C. In all experiments in which threads impregnated with the micrococci were tested there was almost complete sterilization.

Fourteen tests were carried out in disinfector C (Table 12); the chief difference between this disinfector and disinfectors A and B lay in the higher temperatures reached in C: on surfaces exposed to the free vapour in the chamber, temperatures of about 100° C. were reached, while under twenty-eight blankets the temperature (indicated by melting-point tubes) reached 50–60° C. The exposed surfaces must have been at a temperature of over 100° C. for most of the 2 hr. period; within the blanket pile the temperature rose slowly and the maximum was only reached at the end of the exposure period.

The results shown in Table 12 suggest strongly that heat and formaldehyde together disinfected more effectively than the heat alone. In six of ten tests carried out with formaldehyde more than 45% of the threads were sterile, even at the bottom of the pile, while with the heat alone only one of three tests gave this result. In these tests the temperature at the bottom of the pile did not exceed 60° C. and laboratory tests showed that, at 75° C. and under, very few threads were sterilized in 2 hr. At 80° C., 45% were sterilized in about 80 min., and at 95° C. 45% were sterilized in 40 min.

Two tests were carried out in disinfector C with threads impregnated with *Bacillus subtilis* spores in 90% horse serum to give either about 600 or about 2400 organisms per quarter-inch length. In both experiments most of the threads were fertile, indicating that not more than 90–99% of the organisms were killed. Although this may be partly due to the greater resistance to formaldehyde of the spores compared with the micrococci, it also suggests that much of the disinfection of the micrococci was due to the heat.

Since the part played by the formaldehyde in the disinfection of the micrococci was not clear, laboratory experiments were conducted using chambers either of 3 or of 14 cu.ft. capacity which could be raised to the same temperature as disinfector C.

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These experiments produced no consistent evidence that heat and formaldehyde together were more effective than heat alone in killing organisms in the depth of the blanket pile.

In another attempt to explain the apparent penetration of the small quantity of formaldehyde used in disinfector C, experiments were carried out to investigate the effect of temperature on the absorption of formaldehyde by blankets. The experiments were done using 125 ml. Buchner flasks, with a cotton-wool plug in the side tube and a stainless-steel sampling tube through a bung in the top. This tube protruded from the top of the oven in which the flask was kept at the required temperature.

Table 12. *Tests of threads impregnated with the micrococcus in disinfector C*

(Maximum temperature in chamber = 110° C.; 4 oz. 40 % formaldehyde in 130 cu.ft. chamber; time of exposure, 2 hr.)

Conditions of test	Year	No. of blankets above the layer at which approximately 45 % of the threads were sterilized*
Formaldehyde and vacuum	1956	0
Heat and vacuum	1956	24
	1957	1
		1
Formaldehyde, heat and vacuum	1956	29
		29
	1957	12
		29
		29
		2
		29
		20
		12
Formaldehyde and heat	1956	29

* 0 means that less than 45 % of the threads exposed on the surface of the top blanket were sterilized; 29 implies that not even the deepest-placed bundle had 55 % of fertile threads.

Pieces of blanket, 1 g. in weight, were put into the flask after they had been equilibrated at the temperature of the experiment. The flask was allowed to equilibrate for an hour at the given temperature and a control air sample taken by the usual syringe technique. It was then removed from the oven, a small amount of formalin introduced as quickly as possible, and the flask returned to the oven. Air samples were taken at intervals. In this way the approximate equilibrium vapour concentration of formaldehyde over the blanket was found for four different amounts of formalin and for four different temperatures (Fig. 7).

The dotted lines show the expected formaldehyde concentrations at two temperatures in the absence of blanket. It is apparent that the presence of blanket greatly reduced the formaldehyde vapour concentration, and that this reduction was less at higher temperatures. The formaldehyde added to disinfector C was equivalent to 2·4 mg. added to the 125 ml. flask used in the laboratory experiment;

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the weight of fabric corresponded approximately to the weight of blanket used in the experiment. Clearly the blankets retained less formaldehyde at the higher temperatures, but it is worth noting that even at the highest temperature, the amount of blanket used absorbed over 90 % of the formaldehyde supplied.

In one experiment at 106° C. samples were taken at successive hourly intervals to see whether there was any evidence of permanent combination, leading to loss of formaldehyde. The results of this experiment showed that such losses are negligible up to this temperature, for times up to 4 hr.

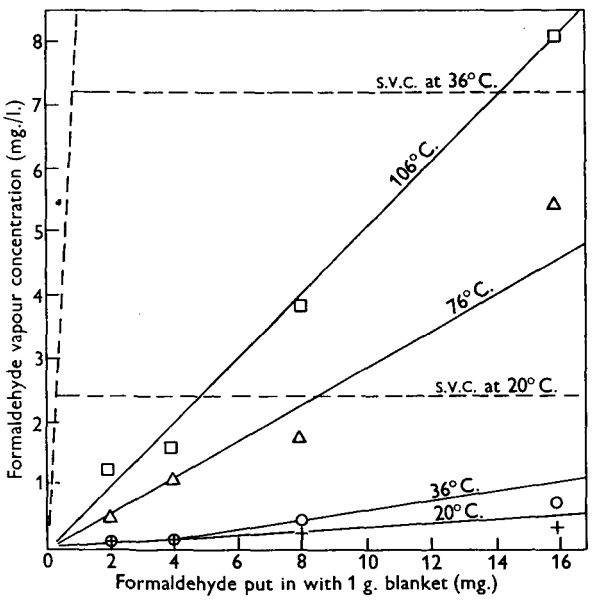

Fig. 7. Formaldehyde vapour concentrations in 125 ml. flasks containing various quantities of formaldehyde and 1 g. blanket at four temperatures. S.V.C. = saturated vapour concentration.

Disinfection of smallpox virus by formaldehyde

Two experiments were carried out by Dr F. O. MacCallum, of the Virus Reference Laboratory, Colindale, to test the efficacy of formaldehyde vapour in disinfecting the virus of variola major.

A 10 % suspension of variola major crusts in penicillin-streptomycin-broth saline was diluted 1 : 10 in Seitz-filtered normal monkey serum. Sterile threads similar to those used in the tests already described were soaked in virus suspension for 10 min., the excess fluid was squeezed out with forceps and the thread allowed to dry over calcium chloride in a desiccator at 4° C. overnight.

In the first experiment twelve quarter-inch pieces of the threads impregnated with virus, four scabs from the same lot of variola major as was used for thread experiments, and four scabs from alastrim patients were placed on pieces of clean linen on top of a mattress inside disinfector B, and formaldehyde vapour was introduced for 24 hr. The vapour was then neutralized with ammonia and the specimens were placed in Petri dishes and removed from the disinfector.

Four pieces of thread were placed on the chorio-allantoic membrane (CAM) of

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each of three 10-day-old chick embryos and left for 20 min. at 36° C. before adding 0·1 ml. of broth saline. The eggs were then gently rotated and placed in an incubator at 36° C. for 3 days. The two lots of untreated scabs were each ground and suspended in 0·5 ml. of broth saline containing penicillin and streptomycin, and 0·1 ml. was inoculated on to the CAM of each of three 10-day-old chick embryos.

No virus was recovered from the infected threads, even after blind passage of suspensions of the inoculated chorio-allantoic membranes harvested after 3 days' incubation. The control threads, not treated by formaldehyde vapour, produced confluent growth (400–500 pocks) on each egg membrane.

Both the treated variola and alastrim scabs yielded a growth of virus.

In the second experiment, twelve lengths of infected thread were supended over formaldehyde solution in a sealed Kilner jar and 2 lengths were removed after $\frac{1}{2}$, 1, 2, 4, 6 and 24 hr. Each lot of two threads was cut up into twelve quarter-inch lengths and placed on three eggs—four pieces on each egg—and allowed to settle for $\frac{1}{2}$ hr., after which 0·1 ml. penicillin-streptomycin saline was added to each egg.

No virus was isolated from any of the pieces of thread even after one pass on the CAM, although controls put up with infected threads produced growth along the line of the thread.

In other experiments carried out at various times on disinfector A, using variola scabs as the test object, virus was recovered even after 24 hr. exposure.

DISCUSSION

For studies of a disinfection process whose main usefulness lies in the treatment of fabrics that cannot be autoclaved, the use of some fabric as vehicle for the test organism seemed most appropriate. Quarter-inch lengths of cotton thread were convenient to use in large numbers, and it was possible to follow the disinfection by observing the proportion of threads sterilized, thereby avoiding any error due to the difficulty of recovering all the viable organisms from the test object. In most experiments we sampled at least 20, and often 100, threads for each experimental point.

The avoidance of any attempt to extract the test organism from the thread meant that an indirect method had to be used for quantitative study of the early part of the killing process. In the method adopted threads impregnated from successive dilutions of the suspension of test organism were exposed simultaneously to the disinfectant vapour, and at intervals samples were taken for culture from those bundles expected to be nearly sterilized. The early stages of disinfection were thus examined on the threads with the lighter inoculum, and the later stages on those with the heavier inoculum. Although this method has obvious theoretical objections, it seemed to give valuable information on the form of the disinfection process, and on the influence of temperature, humidity and formaldehyde concentration.

From a practical point of view, however, it is the later rather than the earlier stages of disinfection that are the most important; for the later stages we measured

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the time to sterilization of a set proportion of a set of 100 threads impregnated from a heavy suspension of the test organism. For theoretical reasons the time to sterilization of 45 % ('ST_{45}') of the threads seems the most useful, but it needs to be emphasized that this level corresponds to an average of less than one coccus per thread, and the time to reach this level is therefore the time taken to kill 99·99 % or more of the initial inoculum of 10^5–10^6 cocci.

The possibility that sufficient formaldehyde was carried over on the test threads to the culture plates was considered at several stages in our investigation, but it appeared to be important only in the later tests in which the threads were exposed to very high formaldehyde concentration. Even in these cases the adoption of one or other of various methods of neutralizing residual formaldehyde did not lead to any substantial alteration in the conclusions to be drawn from the tests.

One of the most notable features of the investigation has certainly been the difficulty of getting reproducible results when one experiment was carried out in several different laboratories. We were unable to overcome this completely even with careful standardization of all the methods and materials used and by frequent discussion of the interim results. Yet despite these difficulties a fairly clear pattern emerges from our results.

In the laboratory experiments there was a linear relation between the speed of disinfection and the formaldehyde concentration. The effect of humidity was somewhat equivocal, and although the optimum appeared to be about 80–90 % R.H. there was no great difference over the range between 58 and 100 % R.H.

Apart from the effect of concentration the most striking result was the protection afforded by factors that restricted the access of formaldehyde to the bacteria. One form of such restriction was the covering of the threads with layers of blanket, which absorbs formaldehyde and prevents its penetration. Another was the drying of the bacteria on to the threads from a serum suspension, so that the organisms were enclosed in a dried protein layer. These two aspects of the penetration of formaldehyde seem of great practical importance.

The question of rate of access is of interest also in connexion with temperature. In the steam-heated disinfector there seemed to be evidence of penetration through a great number of layers of blankets when the air temperature in the apparatus was 100° C. or more. It is difficult to be certain how much of the disinfection was due to heat and how much to the formaldehyde, but it seems probable that the formaldehyde was acting in situations in which it would have failed completely at room temperature. This gains in plausibility from the observation that blankets absorb less formaldehyde from air at higher temperatures than they do at the lower. At room temperature fabrics absorb so much formaldehyde that a great excess has to be supplied in a disinfector if even surface disinfection is to be obtained; at higher temperatures less is needed.

Our experiments do not enable us to reach firm conclusions on the effect of temperature on the disinfection process itself. In laboratory experiments increase in temperature from 10 to 37° C. had little effect on the rate of sterilization at constant formaldehyde concentration. Such constant vapour concentration was,

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however, obtained by reducing the concentration of formaldehyde in the solution used for generating the vapour at the higher temperatures, which in consequence reduced the equilibrium concentration of formaldehyde on the threads. Our results do not, therefore, conflict with the finding of a substantial increase in killing rate with temperature in experiments made in aqueous solutions of constant concentration (e.g. McCulloch & Costigan, 1936).

Our results substantially confirm those of Nordgren, and, like him, we should regard the best circumstances for formaldehyde disinfection as those providing high formaldehyde concentration, high temperature, and a reasonably high relative humidity. Our results suggest that there might be an optimum humidity somewhat below 100 %, but in fact this is probably unimportant because it would be difficult to obtain complete saturation with water vapour in practice. We have not explored the effect of a vacuum to any extent, but from our observations on the absorption of formaldehyde by fabrics at room temperature and, from the tests in disinfector C, we should favour the use of higher temperatures in preference to a vacuum, where penetration of formaldehyde is required.

Our difficulties in obtaining reproducible results, both in laboratory tests and with large-scale disinfectors, doubtless reflect a real irregularity in the action of formaldehyde vapour as a disinfectant in practical situations. It seems clear that disinfection by formaldehyde vapour should not be attempted when any other method is available. Blankets made of wool are better treated by immersion in a disinfectant solution or, if made of a suitable fabric, by heat sterilization. Hospital mattresses may be better enclosed in impervious covers which can be treated with fluid disinfectants. Objects made in occupational therapy departments of tuberculosis hospitals ought to be of materials that can be sterilized by heat or immersion. We should not be prepared in any case to recommend gaseous formaldehyde for the disinfection of fabrics infected with smallpox virus or anthrax bacilli.

For some time, however, there are likely to be situations in which formaldehyde vapour is the only disinfectant that can be employed. Our experiments have shown that, at room temperature, a reasonable degree of disinfection—though often not complete sterilization—of the exposed surfaces can be obtained if the material to be disinfected is fully exposed to the vapour for 2 hr., and if sufficient formaldehyde is provided to allow for the enormous amount absorbed by fabrics.

In the steam-heated formaldehyde disinfector (C) there was substantial disinfection even of material buried under many layers of blanket although the amount of formaldehyde used was very small. There must be some doubt as to how much the formaldehyde contributes to this disinfection but our experiments seemed to show that the disinfection was more reliable with formaldehyde present than without. Certainly the temperatures attained in the depths of the blanket pile were barely sufficient to disinfect; in such circumstances formaldehyde would afford a useful safeguard. There was no evidence that the temperatures employed in this machine had a deleterious effect on mattresses or blankets, though Marten & Speakman (1957) have recently stated that discoloration and degradation of wool takes place at dry-heat temperatures over 105° C.

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SUMMARY

For a study of the process of formaldehyde disinfection, cotton threads were used as test objects. These were, in most experiments, impregnated with a heavy suspension of a coagulase-negative micrococcus in 1 % gelatin solution or in 90 % horse serum, and dried. In other experiments *Mycobacterium tuberculosis*, *Bacillus subtilis* or smallpox virus was used. After exposure to formaldehyde, quarter-inch lengths of the threads were cut off and cultivated on agar plates; individual threads were recorded as fertile or sterile and the mean number of organisms per thread was estimated statistically. Neutralization of the formaldehyde was found to be unnecessary except when strong concentrations were used.

In laboratory tests there was a linear relation between the concentration of formaldehyde and the killing rate. Little effect was observed on the speed of disinfection from variation in temperature over the range 0–30° C. There was some suggestion of an optimum relative humidity for disinfection at about 80–90 %, but no great increase in disinfection rate was obtained by increasing the relative humidity above 58 %. Threads impregnated from a suspension of the cocci in 90 % serum were disinfected much more slowly than those impregnated with cocci suspended in a solution of gelatin. Covering the threads with several layers of blanket also slowed the killing rate considerably.

Tests were made of the sterilization of the threads in full-size disinfectors. Mattresses and other bedding absorbed a large quantity of formaldehyde so that the amount supplied had to be greatly in excess of that estimated from laboratory tests to provide a lethal concentration. A disinfector working at ambient temperature was found to kill over 99·99 % of the micrococci on threads exposed on the surface of mattresses. The destruction of micrococci on threads placed under pillows or blankets was less, as was that of tubercle bacilli on fully exposed threads.

A disinfector in which the air temperature could be raised to about 100° C. was found to be more effective, partly because the increased temperature allowed much greater penetration of fabrics by the formaldehyde vapour; but the relative contribution of the heat and the formaldehyde to the disinfection process could not be fully determined.

In both the laboratory and the field experiments it was found difficult to obtain completely reproducible results. This constitutes one of the most disconcerting features of disinfection by formaldehyde. The process is influenced by so many different factors that it is impossible to predict with real assurance the result under any given set of conditions.

It is concluded that disinfection by formaldehyde vapour should be used only when no other method is available. It certainly cannot be recommended for disinfection of fabric contaminated with smallpox virus or with anthrax spores, when complete destruction has to be ensured; and unless carried out with special care it is not really suitable for woollen garments and toys soiled with tubercle bacilli.

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