THE BACTERICIDAL ACTION OF VOLATILE SUBSTANCES

PART I. DISINFECTION OF POWDERS CONTAINING SPORES, BY MEANS OF GASEOUS FORMALDEHYDE

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WHILE many workers have endeavoured to assess the bactericidal efficiency of gases, the methods employed have often left much to be desired. In early experiments organisms were dried on threads or other materials and exposed to the gases but the results were mainly qualitative or at best contained only a rough quantitative element. Nordgren[1] attempted to make the method quantitative but his apparatus was too cumbersome to permit of continuous sampling and the results he obtained have little greater value than those of earlier workers. In all such methods the dried organisms are unavoidably coated with material of undetermined composition from the culture which may greatly affect the course of disinfection. Recent experiments have been carried out on the disinfection of air in small closed rooms or chambers[2,3,4], notably by glycols and epoxy compounds. In these investigations washed organisms suspended in water or other diluent were sprayed into the chambers containing the bactericidal gas. Air-borne organisms are usually, however, attached to or embedded in solid particles of dust and this adventitious material may considerably affect the course of disinfection. The effect of such enveloping solids is of interest not only when considering the disinfection of air but also when examining the possibility of sterilising powders by means of bactericidal gases. It was thought, therefore, of interest to study the action of such gases upon organisms contained in powders of known composition, under controlled conditions of humidity and temperature. It has previously been shown[5,6] that, by using a spray-drying technique, suitable powders can be obtained in which the organisms are evenly distributed and from which consistent replicate samples can be drawn. In the present experiments the powder to be tested was placed in a special glass chamber which could be opened for the withdrawal of samples and through which air containing controlled amounts of the bactericide and water vapour could be drawn in such a way that it passed through the powder. At intervals, samples were withdrawn, viable counts performed and the results plotted. It was thus possible to examine the course of disinfection under various conditions with considerable accuracy.

Formaldehyde was chosen as the bactericide to be used in the initial experiments because, while being an efficient gaseous bactericide, it can be generated at a constant concentration, is easily estimated chemically and gives reproducible results. This communication is therefore concerned with the factors affecting disinfection of powders by formaldehyde. It is hoped later to extend the observations to other gaseous disinfectants.

EXPERIMENTAL

The production of atmospheres of controlled humidity and formaldehyde content.

Apparatus. The apparatus used is shown in Figure 1.

A is a reaction chamber consisting of two No. 5 sintered glass filters of 3 cm. diameter fitted together by means of a ground joint. The powder to be tested was placed within the chamber and the bactericidal gas passed through it. The inlet and exit tubes were connected by ground glass

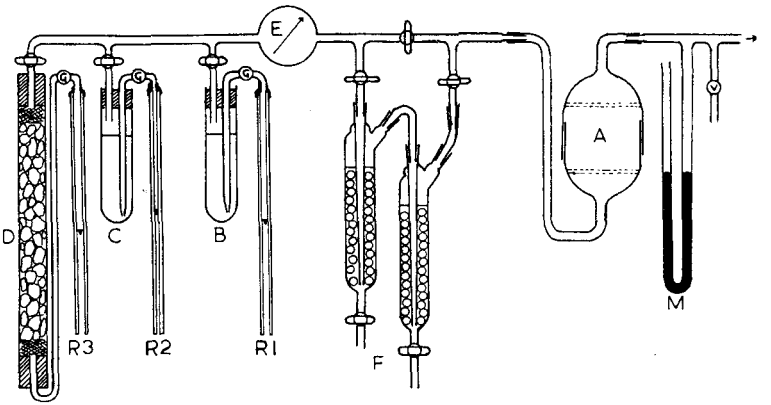

FIG. 1. Diagram of apparatus used.

joints to the rest of the apparatus so that the chamber could easily be removed for sampling purposes. B is a formaldehyde generator consisting of a boiling-tube containing an aqueous solution of formaldehyde and fitted with a short exit tube which passes *via* a stopcock to the main duct of the system. The inlet tube of B is long enough to reach nearly to the bottom of the tube and its end was drawn out to a fine jet: it was connected through a gas regulator G to a Rotameter flowmeter R1, placed in this position so that it always worked at atmospheric pressure. In the earlier experiments the tube was loosely packed with glass wool to assist saturation of the air but it was found that, provided the jet of the inlet was fine enough, results were equally good with or without the glass wool. C is similar to B but contained water. D consists of a calcium chloride tube also connected through a gas regulator to a Rotameter flowmeter R3. These 3 devices generated respectively, air nearly saturated with water and containing formaldehyde, air nearly saturated with water, and nearly dry air, when air was drawn through them. This was achieved by means of a vacuum pump connected to the opposite side of the reaction chamber, an adjustable outlet valve V, being introduced so that the total volume of air drawn through the apparatus could be regulated. Passage of the air through the sintered plates of the reaction chamber involves an appreciable pressure drop. A manometer M was therefore placed between A and V and the latter was adjusted so that in each experiment M showed a reading of 20 cm. E is a paper hygrometer through which the mixed gases passed

and which recorded the relative humidity of the mixture. F consists of 2 conventional gas absorbers connected in series for the estimation of formaldehyde.

Generation and estimation of formaldehyde. In most of the experiments formaldehyde was generated by passing air through solution of formaldehyde B.P. but in a few cases the solution was first diluted with water. Such solutions frequently contain small amounts of methanol but it was considered that the proportion of this in the air leaving B would be too small to affect the course of disinfection. A more serious contingency is the possibility of polymers of formaldehyde passing over. However, Spence and Wilde[7] declared that "polymerisation occurs only as a heterogeneous or solid phase reaction leaving the liquid unaffected" and Nordgren[1] assumed that air after passage through formaldehyde solutions contained only simple molecules.

Nordgren used Romijn's[8] method for the estimation of formaldehyde. This involved oxidation with alkaline iodine and back titration with thiosulphate. The method is not, however, sufficiently sensitive. The use of Schiff's reagent has been suggested[9] but the colour was found not to be permanent, and exact timing in reading is necessary. Rimini[10] utilised the reaction of formaldehyde with phenylhydrazine and ferricyanide which gives an intense red colour but the reagents must be freshly made. Moreover, reproducibility was not found to be very good. A very elegant method is that using chromotropic acid[11] (1 : 8-dihydroxynaphthalene-3 : 6 disulphonic acid) which gives an intense purple colour on heating with formaldehyde in moderately strong sulphuric acid. The colour is fully developed in 30 minutes and is stable for at least 36 hours[12]. Indeed experience showed that it was stable for even longer periods.

This is a great advantage as it avoids the necessity of carrying out too great a multiplicity of tasks while a disinfection experiment is in progress. The following modification of the method was used. 500 ml. of the gas from generator B was aspirated through 20 ml. of water in each of the absorption tubes. (It was found that absorption was complete in the first tube at the flow-rates used). The tubes were then disconnected and their contents washed out with water into a 200-ml. graduated flask, and the volume made up to 200 ml. with water (dilution N). After mixing, a suitable volume of this solution, usually about 1 ml., accurately measured, was transferred to a graduated test tube, 5 ml. of a solution containing 2 per cent. of chromotropic acid in 75 per cent. sulphuric acid was added and the volume made up to 10 ml. with water. The contents of the tube were mixed and the tube was heated in a boiling water bath for 30 minutes and then cooled. The colour was compared, using a Spekker photoelectric absorptiometer and a combination of blue-green and orange filters, with colours obtained similarly with known amounts of formaldehyde.

Regularity of evolution of formaldehyde. The work of Blair and Ledbury[13] showed that at a given temperature and for a given air flow the concentration of formaldehyde in air aspirated through a formaldehyde solution should be constant. While the results obtained in the present experiments were more variable than those of Blair and Ledbury, the

difference between successive samples was not found to be such that the irregularity of evolution of formaldehyde would affect significantly the course of disinfection. Moreover, the flow rates used appeared to have no effect on the concentration of gaseous formaldehyde attained and dilution of the aspirated air with measured volumes of air from C and D reduced the concentration in the calculated proportions. All the results are therefore directly comparable on a basis of the amount of formaldehyde issuing per minute. Table I shows the concentrations of formaldehyde obtained at different sampling times from formalin solutions containing approximately 40 per cent. of formaldehyde, at a temperature of 20° C. The aerial concentration of formaldehyde attained was 2·32 ± 0·167 mg./l.

TABLE I

TESTS FOR REGULARITY OF EVOLUTION OF FORMALDEHYDE FROM GENERATOR B AND EFFECT OF DILUTION WITH MOIST OR DRY AIR

Rate of flow, ml./minute			Period of sampling, minutes	Volume of dilution used ml.	Spekker reading	Concentration of formaldehyde in gas from B mg./l.
B	C	D				
100	—	—	5	1	0·283	2·38
100	—	—	5	1	0·290	2·44
100	—	—	5	1	0·270	2·27
100	—	—	5	1	0·270	2·27
200	—	—	2·5	1	0·270	2·27
200	—	—	2·5	1	0·241	2·03
50	—	—	10	1	0·319	2·68
50	—	—	10	1	0·286	2·41
50	—	—	10	1	0·242	2·04
100	200	—	5	1	0·278	2·34
122	121	—	5	1	0·345	2·42
103	—	27·5	5	1	0·297	2·43
100	—	50	5	1	0·282	2·37
103	—	110	5	1	0·268	2·19

PREPARATION AND TESTING OF SPORE-CONTAINING POWDERS

Preparation of powders. The test organism was the Marburg strain of *B. subtilis* (NCTC No. 3610) used in earlier experiments.[5] A suspension of spores was made as previously described and a spray-dried kaolin powder containing approximately 6,000,000 spores per g. prepared from it[6]. The powder was stored in a plugged jar over calcium chloride and used as a "master" powder from which dilutions were subsequently made in the following manner. 4 g. of the above powder was placed in a sterile, screw-capped, glass jar of 200 ml. capacity and 36 g. of a sterile diluent powder was added. In most cases this consisted of kaolin, but in certain experiments mixture of kaolin with spray-dried peptone were used. Sterile glass beads were added to the jar which was then rotated mechanically at about four revolutions per minute for six or seven days.

Method of performing viable counts. Viable counts were performed on the powder before and after exposure to formaldehyde. 0·1 to 0·2 g. of the powder was transferred by means of a flamed spatula to a sterile, tared test-tube, closed by means of an aluminium cap. The tube was re-weighed and a volume of sterile water having a weight 40 times that of the powder was added. The suspension was thoroughly mixed by means of a pipette and 2 serial 10-fold dilutions were made using sterile water as

diluent. Viable counts were performed on the final dilution by the roll-tube technique previously described[5].

Test for evenness of distribution and viability of spores. A comparison of the counts obtained on the diluted powders, prepared as above, with those on the "master" powder showed that the milling process had had no lethal effect on the spores. In order to decide whether the process had

TABLE II

TESTS OF EVENNESS OF DISTRIBUTION OF SPORES IN POWDERS

Powder	Mean counts	Variance ratio $\frac{\text{Difference between samples}}{\text{Difference within samples}}$	P
P1	142·4, 135·6, 134·6, 142·2, 135·0, 126·6	0·499	>0·2
P2	145·4, 152·2, 140·8, 140·2, 146·4 140·0, 153·8, 140·8, 140·8	0·763	>0·2
P6	153·8, 160·4, 159·2	0·366	>0·2
P7	150·0, 142·2, 148·0, 142·2, 150·8 150·2, 149·8, 147·2, 158·4	0·804	>0·2

Mean count per g. of master powder: 5,786,407.
Mean count per g. of diluted powder: 595,200.

distributed the spores evenly in the powder the counts obtained on successive samples were subjected to an analysis of variance as previously described.[5] The results are shown in Table II. For simplicity only the means of the 5 counts on each sample are shown, together with the ratio of the variances due to differences between and to differences within samples. The mean counts recorded are those of the samples taken at the commencement of each disinfection experiment. They therefore show not only that the spores were evenly distributed but also that they suffered no significant mortality over the period during which each powder was used.

EXPOSURE OF POWDER TO DISINFECTANT ATMOSPHERE AND SUBSEQUENT EXAMINATION

Performance of disinfection experiments. Blair and Ledbury[13] found that when air was aspirated through aqueous formaldehyde solutions at 20° C., the partial pressure of formaldehyde rose until 20 to 25 l. of air had been passed, when the partial pressure became constant. Before placing any powder in the reaction chamber, therefore, air was drawn through the whole apparatus at 100 ml. per minute (generator B alone being in circuit) for 4 hours. The rates of flow through the generators were then adjusted to the required values. The reaction chamber was disconnected and opened so that 2 g. of the powder being tested could be quickly placed in it. It was then immediately closed and restored to circuit. At the same time a control sample of the same powder was weighed from the stock jar and a viable count performed on both. After a suitable interval A (Fig. 1) was disconnected and a sample of the powder withdrawn to a sterile, capped tube as before, A being immediately restored to circuit and the powder in it levelled by gentle tapping. The tube was shaken sharply from side to side to mix the powder with air and dilute out any

residuum of formaldehyde carried over from the reaction chamber. It was found that this whole operation could be completed in less than half a minute and as the sampling intervals were not less than 10 and were usually 20 minutes, it was considered that the break in continuity of the disinfection process would not have any significant effect, an expectation supported by the regularity of the curves obtained. A viable count as described above was performed on each sample of powder withdrawn.

In some cases dried, and in other cases humidified air was drawn through the powder prior to exposing it to formaldehyde. For this purpose the powder was placed in a second reaction chamber A′ connected to the pump in parallel with A, the inlet tube of this second chamber being connected either to C or D (Fig. 1). Drying or humidification of the powder could thus proceed at the same time as equilibration of the formaldehyde generator. When the process had proceeded for a chosen period a control sample of the powder was withdrawn and a viable count performed on it and the remainder of the powder was immediately transferred from A′ to A. Further test samples were then taken at recorded time intervals as above.

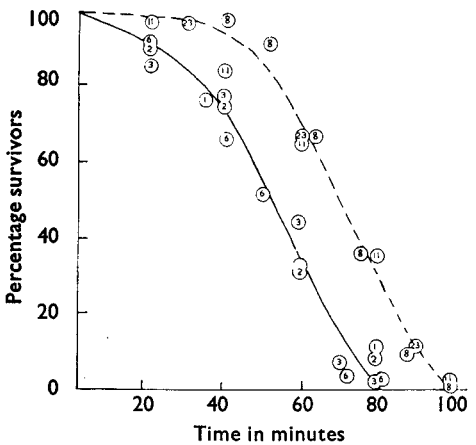

FIG. 2. Reproducibility of time/survivor curves of *B. subtilis* spores exposed to air containing 2 mg./l. of formaldehyde at 21° C. and 98 per cent. relative humidity.

— — — — = flow-rate of 50 ml./minute.
———— = flow-rate of 100 ml./minute.

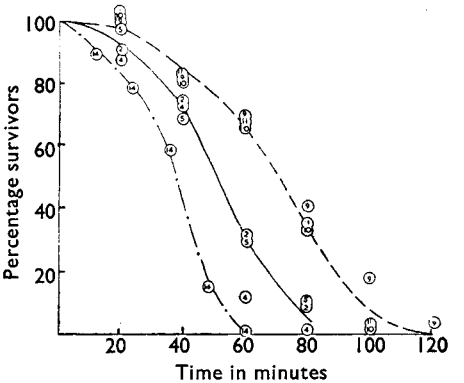

FIG. 3. Effects of rate of flow of gas and of concentration of formaldehyde on disinfection of kaolin containing *B. subtilis* spores.

— — — — = 0·1 mg. of formaldehyde passing through A per minute.
———— = 0·2 mg. of formaldehyde passing through A per minute.
——·——· = 0·4 mg. of formaldehyde passing through A per minute.

Numbers in circles are those of the experiments given in Table V.

Reproducibility of experimental results. Figure 2 shows the results of replicate experiments carried out with kaolin powders using flow rates of 100 ml. per minute and 50 ml. per minute. It appears from these graphs that reproducibility is satisfactory especially along the steeply descending portions of the curves. A proper assessment cannot be made, however, without statistical analysis. Such an analysis is facilitated if the regressions to be compared can be represented as linear. Withell[14] suggested

that, by plotting the probit values representing percentage mortality against the logarithm of time, sigmoid curves of the type obtained in the present experiments could be converted to straight lines. The results of this conversion and the statistical analyses are given in a later section. They show that above probit 4·3, representing 24 per cent. mortality, linearity of the regressions can be assumed, and that there is no significant difference between the replicates taken at either 50 ml. per minute or at 100 ml. per minute.

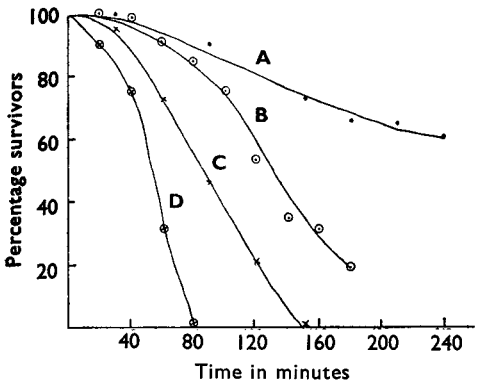

FIG. 4. Effect of relative humidity on disinfection of kaolin containing *B. subtilis* spores, by formaldehyde at 20° C.

A. Relative Humidity = 65 per cent.
B. ,, ,, = 78 per cent.
C. ,, ,, = 87 per cent.
D. ,, ,, = 98 per cent.

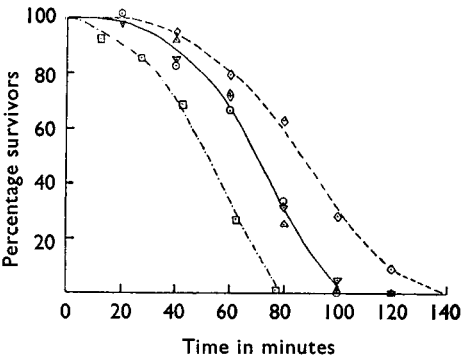

FIG. 5. Effect of previous humidification and drying on course of disinfection.

○ = control.
▽ = 1 hour's previous humidification.
△ = 1 hour's previous drying.
□ = 4 hours' previous humidification.
◇ = 4 hours' previous drying.

FACTORS AFFECTING THE COURSE OF DISINFECTION

(*a*) *Rate of flow.* The apparatus permitted the effective use of flow rates of air through each generator of from 25 to 250 ml./minute. It was found, however, that at flow rates at which the total volume of air passing through the reaction chamber exceeded 200 ml./minute the results appeared very uneven, presumably because "channelling" occurred with uneven penetration of the gas through the powder. Figure 3 shows results obtained with lower flow rates.

(*b*) *Concentration of formaldehyde.* The results shown in Figure 3 were obtained by passing air through the formaldehyde generator B containing either 100 per cent. or 50 per cent. of formalin B.P., which yielded concentrations of formaldehyde in the air of approximately 2 mg./l. and 1 mg./l. respectively. In some of the experiments the air from B was mixed with known volumes of air from the water generator C. It can be seen that, if the concentration of formalin in B and the air flow through it are kept constant, dilution of the issuing air with air from C has no significant effect on the course of disinfection. Reduction of the concentration of the formaldehyde solution in B and hence of the amount of formaldehyde per minute does, however, reduce the death-rate.

(*c*) *Humidity.* Figure 4 shows the results obtained when air passing through B at 100 ml. per minute was mixed with different amounts of dried air from D. There is a marked fall in the death-rate as the relative humidity falls from 98 per cent. to 65 per cent.

Experiments were also carried out in which either dried or humidified air was first passed through the powder before disinfection began. The results are given in Figure 5 and show that previous drying reduced the rate of disinfection while previous humidification somewhat increased it, though in each case it required more than 1 hour for the treatment to show any significant effect.

(*d*) *Effect of the enveloping powder.* Most of the powders used consisted of kaolin but a few contained peptone in addition. The effect of the kaolin alone cannot be directly assessed by the present technique. Figure 6 shows the results of some experiments in which the powder was first treated with a low concentration of formaldehyde till about 75 per cent. and 50 per cent. respectively of the original organisms had been killed. Normal disinfection was then carried out with 2 mg./l. of formaldehyde. If the kaolin had had no effect on the course of disinfection the modified curves should show no initial curvature. The fact that the curves were still sigmoid when more than 25 per cent. of the organisms had been killed suggests that the slower death-rate at the beginning of these experiments is at least partly due to the uptake of the formaldehyde by the kaolin.

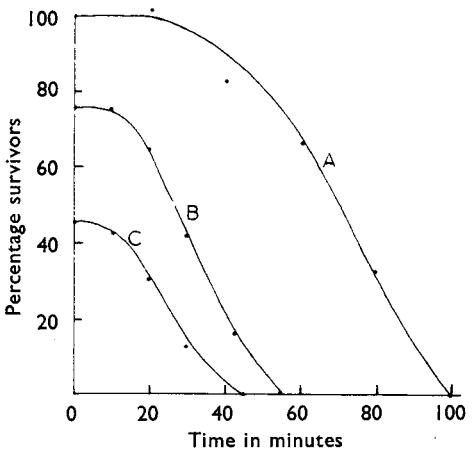

FIG. 6. Effect of uptake of formaldehyde by kaolin on death-rate of *B. subtilis* spores contained therein.
A = Disinfection throughout with 2 mg./l. of formaldehyde.
B and C = Disinfection by 2 mg./l. of formaldehyde subsequent to partial disinfection with 0·8 mg./l. of formaldehyde.

Figure 7 shows the effect of adding different proportions of sterile, spray-dried peptone to the kaolin powders. It is evident that the presence of even small amounts of peptone markedly inactivates the formaldehyde and there appears to be no significant difference between the effects of 10, 50 and 95 per cent. peptone, under the conditions of these experiments.

(*e*) *Temperature.* Figure 8 shows the results obtained at 21° C. and also when the reaction chamber was enclosed in an air oven at 58° C. In the latter case the high death-rate made it impossible to obtain an accurate evaluation of the mean temperature of the powder during the specified time interval, as the necessary opening of the oven and reaction chamber must affect the maintenance of the temperature. The results,

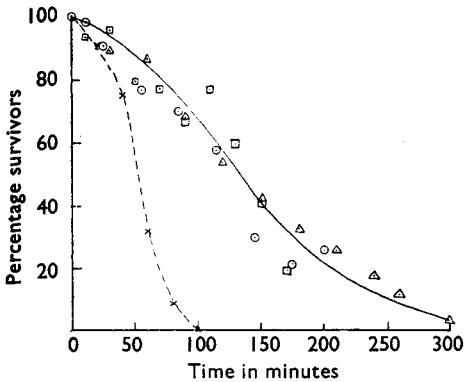

FIG. 7. Disinfection of powders containing varying proportions of kaolin and peptone.

□ = 10 per cent. peptone with 90 per cent. kaolin.
△ = 50 per cent. peptone with 50 per cent. kaolin.
○ = 95 per cent. peptone with 5 per cent. kaolin.
× = 100 per cent. kaolin.

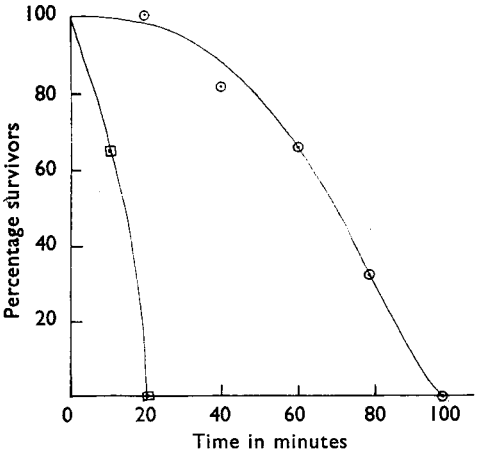

FIG. 8. Effect of temperature on disinfection by formaldehyde of kaolin powders containing *B. subtilis* spores.

○ 21° C. □ 58° C.

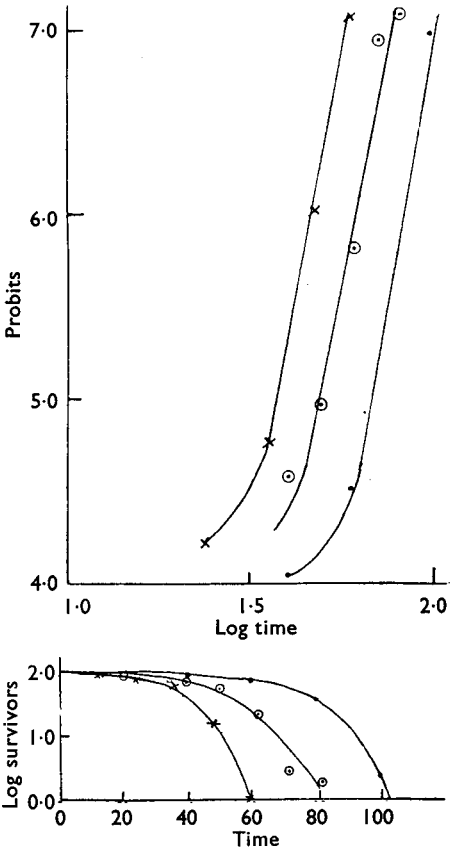

FIG. 9. Probit-log time and log survivor-time curves of *B. subtilis* spores contained in kaolin powders exposed to formaldehyde at 20° C.

• 50 ml./minute of air containing 2 mg./l. of formaldehyde.
○ 100 ml./minute of air containing 2 mg./l. of formaldehyde.
× 200 ml./minute of air containing 2 mg./l. of formaldehyde.

therefore, while being highly significant statistically, must be regarded as giving only a qualitative estimate of the effect of temperature.

STATISTICAL EXAMINATION OF RESULTS

Figure 9 shows the results of plotting probits representing percentage mortality against the logarithm of time. The results have been analysed in the following manner.

Tests for linearity of regressions.

In order to test for linearity, experiments have been examined in which the same sampling times have been used. An analysis of variance has then been carried out according to the method given by Berry and Michaels[15]. Tables III and IV show the results using flow-rates of 100 ml./minute from 100 per cent. formalin and probit values above 4. It is

TABLE III

DISINFECTION OF KAOLIN POWDERS AT 20° C. WITH AIR DRAWN THROUGH 100 PER CENT. FORMALIN AT 100 ML. PER MINUTE

Experiment	Logarithm of time			
	1·60206	1·77815	1·90309	
2	4·3318	5·4789	6·3852	16·1959
3	4·2108	5·1307	7·3263	16·6678
4	4·3628	6·2107	7·4573	18·0308
5	4·5295	5·5187	6·3469	16·3951
	17·4349	22·2390	27·5157	67·2896

TABLE IV

ANALYSIS OF VARIANCE OF RESULTS IN TABLE III

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Times	12·7059	2			
Regression	12·5422	1			
Remainder	0·1637	1	0·1637	0·8530	>0·2
Pooled error	1·7268	9	0·1919		

TABLE V

Group	Experiment	Flow rate			Concentration of formalin, per cent.	Relative humidity, per cent.	Temperature, ° C.	Special treatment
		B	C	D				
A	1	100	—	—	100	96	23	—
	2	100	—	—	100	98	20	—
	3	100	—	—	100	96	21	—
	4	122	121	—	100	98	20	—
	5	100	200	—	100	98	21	—
	6	100	—	—	100	98	20	—
	7	100	—	—	100	96	20	—
B	8	47·5	—	—	100	98	20·5	—
	9	50	50	—	100	96	20·5	—
	10	100	—	—	50	96	20·5	—
	11	50	—	—	100	97	21	—
	12	100	—	—	50	97	21·5	1 hour's pre-humidification
	13	100	—	—	50	97	21·5	1 hour's pre-drying
C	14	200	—	—	100	95	21	—
D	15	100	—	—	50	96	21	4 hours' pre-humidification
	16	100	—	—	50	97	20	4 hours' pre-drying
E	17	100	—	50	100	78	20	—
	18	103	—	27·5	100	87	19·5	—
	19	103	—	110	100	65	20	—
F	20	100	—	—	100	97	20	95 per cent. peptone
	21	105	—	—	100	97	21	10 per cent. peptone
	22	100	—	—	100	99	22	50 per cent. peptone

seen that the probability obtained is satisfactory and the regressions may be assumed to be linear. When probits below 4 were included the probability lay between 0·01 and 0·05 suggesting that in the region of probit 4 some degree of curvature must be assumed.

Similar results were obtained with results at flow rates of 50 ml./minute, the probability being above 0·2 using probits above 4·3, while the inclusion of probit values of 4 and below reduced the probability to 0·01 to 0·05. Subsequent analyses have therefore been carried out using probit values above 4 only.

Tests for common regression.

For this purpose the experiments have been separated into groups and classified as shown in Table V.

(*a*) *Test of common regression within Group A.* Table VI gives the regression data for the experiments in this group. x is the logarithm of the time in minutes and y is the appropriate probit value.

TABLE VI

REGRESSION DATA FOR GROUP A

Experiment							
1	x	1·54407	1·77815	1·90309			
	y	4·2778	5·4650	6·3966			
2	x	1·60206	1·77815	1·90309			
	y	4·3318	5·4789	6·3852			
3	x	1·60206	1·77815	1·84510	1·90309		
	y	4·2108	5·1307	6·5141	7·3263		
4	x	1·60206	1·77815	1·90309			
	y	4·3628	6·2107	7·4573			
5	x	1·60206	1·77815	1·90309	2·00000		
	y	4·5295	5·5187	6·3469	7·3263		
6	x	1·60206	1·69897	1·79239	1·85733	1·91381	2·00000
	y	4·5903	4·9799	5·8204	6·9431	7·0969	7·5121
7	x	1·65321	1·76343	1·85126			
	y	4·3287	5·0954	5·7655			

The analysis of the data from Table VI is given in Table VII and shows that the whole of the data could be represented by a common regression and that the death-rates in the various experiments are not significantly different. The method used is that described by Tippett[16].

TABLE VII

TEST FOR COMMON REGRESSION WITHIN GROUP A

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Residual SS from overall regression ..	3·5882	24			
Residual SS from individual regressions ..	0·9889	12	0·0824		
Remainder	2·5993	12	0·2166	2·628	0·05–0·1

(*b*) *Test of common regression within Group B.* Table VIII gives the regression data and Table IX the test of significance. Here again it can be assumed that the data can be represented by a common regression.

(*c*) *Test of common regression within Groups A and B.* In group A 0·2 mg. of formaldehyde was passed through the powder per minute and in group B 0·1 mg./minute. Within each group, concentration and flow-rate have been varied. It has been shown above that a common regression

TABLE VIII

REGRESSION DATA FOR GROUP B

Experiment					
8	*x*	1·80618	1·88081	1·94448	2·00000
	y	4·5821	5·3638	6·2930	7·3263
9	*x*	1·60206	1·77815	1·90309	2·00000
	y	4·1184	4·5239	5·2353	5·9116
10	*x*	1·60206	1·77815	1·90309	2·00000
	y	4·0259	4·5656	5·4427	7·4089
11	*x*	1·60206	1·77815	1·90309	2·00000
	y	4·0458	4·5070	5·3638	6·9954
12	*x*	1·77815	1·90309	2·00000	2·07918
	y	4·4201	5·4761	6·6546	7·5758
13	*x*	1·77815	1·90309	2·00000	
	y	4·4112	5·6620	6·5220	

TABLE IX

TEST FOR COMMON REGRESSION WITHIN GROUP B

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Residual SS from overall regression ..	4·8847	21			
Residual SS from individual regressions ..	2·0636	11	0·1876		
Remainder	2·8211	10	0·2821	1·5037	>0·2

TABLE X

TEST FOR COMMON REGRESSION BETWEEN GROUPS A AND B

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Residual SS from overall regression ..	17·4825	47			
Residual SS from individual regression ..	8·4729	45	0·1883		
Remainder	9·0096	2	4·5048	23·9235	<0·001

TABLE XI

REGRESSION DATA FOR GROUP D

Experiment					
15	*x*	1·62325	1·79239	1·88649	1·98677
	y	4·5211	5·6068	6·3285	7·4573
16	*x*	1·90309	2·00000	2·07918	2·14613
	y	4·6495	5·5740	6·3408	7·7478

can be calculated for each group and it is necessary to know whether the regressions so obtained differ significantly from one another. The test of significance is given in Table X. It can be seen that the difference is highly significant.

(*d*) *Test of common regression between Groups D and B.* Group D contains 2 experiments, Nos. 15 and 16, representing the effects of 4 hours' pre-humidification and pre-drying respectively. It is necessary to test these two regressions separately against the combined regression of group B. Table XI gives the experimental data for group D and Tables XII and XIII the tests of significance.

TABLE XII

TEST FOR COMMON REGRESSION BETWEEN EXPERIMENT NO. 15 AND GROUP B

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Residual SS from overall regression ..	7·2240	25			
Residual SS from individual regressions ..	4·974	23	0·2163		
Remainder	2·250	2	1·125	5·2011	0·01–0·05

TABLE XIII

TEST FOR COMMON REGRESSION BETWEEN EXPERIMENT NO. 16 AND GROUP B

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Residual SS from overall regression ..	7·2812	25			
Residual SS from individual regressions ..	5·0929	23	0·2214		
Remainder	2·1883	2	1·0942	4·942	0·01–0·05

TABLE XIV

REGRESSION DATA FOR GROUP F

Experiment								
20	*x*	1·92942	2·06070	2·16137	2·24304	2·31175		
	y	4·4813	4·8134	5·5476	5·8381	6·0494		
21	*x*	2·04139	2·11394	2·17609	2·23045			
	y	4·5351	4·7570	5·2404	5·8853			
22	*x*	1·95424	2·07918	2·17609	2·25527	2·32222	2·38021	2·43136
	y	4·5183	4·9122	5·2198	5·4705	5·6495	5·9463	6·1850

It can be seen that the probability of obtaining a common regression is, in each case, less than 0·05 and is nearer to 0·01. It is probable, therefore that the differences are significant. Increased replication of the results would make this clearer. It is evident, in any case that the difference between the two treatments themselves is significant and that therefore the effects must be taken as real.

(*e*) *Test of common regression within Group F.* Group F consists of experiments in which various amounts of peptone were incorporated in the powder. Tables XIV and XV give the necessary data, and show that no significant differences can be detected between the experiments. This is probably in part due to the greater irregularity of the counts obtained

TABLE XV

TEST OF COMMON REGRESSION WITHIN GROUP F

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Residual SS from overall regression ..	0·8052	15			
Residual SS from individual regressions ..	0·4259	11	0·0387		
Remainder	0·3793	4	0·0948	2·449	0·1–0·2

TABLE XVI

TEST FOR COMMON SLOPE

Source of variance	Sum of squares	N	Mean square	Variance ratio	Probability
Residual SS assuming common slope ..	7·9015	44			
Residual SS for individual slopes	3·9437	29	0·1360		
Remainder	3·9578	15	0·2639	1·9404	0·05–0·1

with these powders when compared with those obtained with the plain kaolin powders.

Test for common slope.

If the probit/log. time regressions can be regarded as linear, comparison can be made between different regressions if they can be regarded as parallel. The test used is that described by Tippett[16]. Table XVI gives the analysis of variance of the results obtained with all the experiments which were carried out at the same humidity and temperature on kaolin powders. It shows that when the conditions are standardised in this way parallelism of the regressions can be assumed. When groups E and F were included in the analysis the probability fell below the 0·05 level but it would require greater replication to decide the significance of this.

DISCUSSION

In most disinfection experiments attention has been paid to the concentration rather than to the total amount of bactericide to which the organisms have been exposed. Indeed, in the usual type of experiment, in which the organisms are confined in a liquid or gas in which they move freely, it is difficult to separate one factor from the other. The present experiments, however, permit of variation independently of the concentration of formaldehyde and of its rate of flow through the powder. The total amount of the gas by which the powder has been permeated can be represented by the product of these two factors. Consideration of Figure 3 and of the results given in Table V shows that at a constant concentration of formaldehyde gas the death-rate increases with the rate of flow. When the latter is kept constant the death-rate increases with increasing concentration of formaldehyde. In certain cases the air from generator B was mixed with air from C. This reduced the concentration of formaldehyde in the air without altering the amount of formaldehyde passing through the powder in a given time. It can be seen that dilution in this manner does not significantly affect the death-rate, over the ranges of concentration and time used. In other experiments the concentration of formalin solution in B was reduced so that the concentration of formaldehyde gas in the air

issuing from B was halved. The effect was the same as if the flow rate had been halved. In all cases where the products of flow-rate and concentration of formaldehyde were equal the death-rates were equal and the death-rate increased with increase in the value of the product. It appears, therefore that under these conditions there is a quantitative relationship between the degree of disinfection and the total quantity of formaldehyde drawn through the powder. This might be expected in the early parts of an experiment but it held when the gas was passed for 2 hours or longer.

Contradictory results have been obtained by different workers with regard to the effect of humidity on the disinfectant activity of formaldehyde. Most workers appear to have found that the presence of moisture in the air increases the efficiency of the formaldehyde, but others regarded "dry" formaldehyde gas, obtained by autoclaving formalin with calcium chloride, as the better agent[1]. It was considered by Nordgren[1] that the discrepancy was due to the failure of the latter workers to prevent condensation of water in their apparatus when they were using moist formaldehyde gas. In the present experiments complete saturation with water vapour of the air emanating from the generators was not obtained and as the reaction chamber was always at a temperature equal to or above that of the generators no such condensation occurred. Figure 4 shows that, under these conditions, and with passage of equal quantities of formaldehyde per minute, the death-rate decreased rapidly as the relative humidity fell from 98 per cent. to 65 per cent. At the latter humidity only 60 per cent. of the organisms had been killed after 4 hours and it must be concluded that at humidities below this, formaldehyde is not an efficient aerial disinfectant at room temperature.

The manner in which bactericides react with bacteria must be extremely complex. It has often been held that the bactericide dissolves in condensed moisture present on the surface of the organism before reacting with the bacterial substance[17]. This could of course in any case only be one stage in the process of disinfection and it is difficult to reconcile with the above mentioned fact that in dynamic experiments it is the total quantity rather than the concentration of formaldehyde in the vapour phase which is important. It is also difficult to reconcile this view with the previously expressed view that virtually dry formaldehyde has a not inconsiderable bactericidal effect and with the fact illustrated in Figure 4 that even when the humidity is reduced below 70 per cent., some disinfection occurs even if it is so little as to be unsuitable for sterilisation. King[19] has shown that the rate of diffusion of water vapour through a keratin membrane is proportional to its water content and Lidwell[17] suggested that the increase of efficiency of certain hydroxyacids with rise in relative humidity was due to their increased rate of diffusion through the salivary particles in which his organisms were embedded. If this is so it would not be unreasonable to suppose that the same may be true of the bacterial cell wall, though it might be expected that the changes thus brought about would take some time to produce. The experiments the results of which are given in Table XI and Figure 5 were therefore carried out to test the

suggestion. They show that prior drying of the organisms reduced the subsequent death-rate only if the period of drying exceeded one hour. Prior humidification increased the rate of disinfection but only if a similar period was allowed. This evidence is consistent with the view that changes in humidity affect the permeability of the cell wall to formaldehyde. It is difficult to reconcile the facts with the view that under these experimental conditions the formaldehyde first dissolves in a film of moisture the presence of which is the operative factor governing the rate of disinfection. It must further be remembered that these dynamic experiments suggest that in most work, equilibrium between the powder and the vapour phase has probably never been established and it is possible that the humidity is controlling the rate of uptake of formaldehyde by the powder as well as influencing such factors as cell permeability. The observed effects of rise of temperature tend to support this view. Raising the temperature may have at least 2 effects. It will decrease the solubility of formaldehyde in water and it will probably increase the rate of reaction between the formaldehyde and the cellular substance. If the equilibrium between the formaldehyde in the vapour with that dissolved in a film of moisture on the surface of the organisms is the controlling factor, then one would expect that raising the temperature would decrease the death-rate. As this conflicts with the experimental evidence it appears necessary to consider other factors.

The efficiency of formaldehyde as a practical disinfectant is severely limited by the ease with which it is inactivated. This is shown in Figure 7 from which it can be seen that quite small amounts of peptone greatly reduce the death-rate. The irritant nature of formaldehyde also militates against its use though it has been used for sterilisation of surgical instruments[1] and dressings[20]. In the present experiments, however, formaldehyde provides a useful index of the efficiency of the method used, as a sterilisation process. It has been shown that the powders can be evenly penetrated by the gas and that, particularly at higher temperatures, sterilisation can be achieved in a relatively short time. If a gas could be found that possessed the efficiency of formaldehyde without its disadvantages the method might easily be adapted for the sterilisation of small quantities of powders. Investigation of this is proceeding and it is hoped to report upon it later.

The present investigation was not designed for the study of the fundamental nature of the time/survivor relationship. Indeed it has been suggested above that the initial part of the curves obtained are affected by the presence of the kaolin. The sigmoid nature of the curves obtained by plotting percentage survivors against time is more pronounced than in most recorded data. When the logarithm of the percentage survivors is plotted against time, as in Figure 9, the regressions produced appear to be smooth curves rather than straight lines even in the proximal portion where the effect of the kaolin is probably insignificant.

When probits representing percentage mortality are plotted against the logarithm of time the regressions show curvature up to about probit 4·5 and above that appear to be linear. This is confirmed by the statistical

analysis given in the text which also shows that all the regressions obtained at 96 to 99 per cent. relative humidity with kaolin powders were parallel above probit 4·5, enabling comparison to be made at any mortality level above 30 per cent. Other workers have obtained similar results when working with liquid disinfectants[15]. If therefore conditions were standardised so that the effect of the kaolin was constant the method could be used for comparing the efficiency of different gaseous bactericides, and more information could be obtained about the fundamental nature of the time/survivor relationship. It is hoped to investigate this, but for a satisfactory interpretation of the results further examination is necessary of the equilibrium conditions obtaining in the powders in respect of the formaldehyde concentrations.

SUMMARY

1. An apparatus has been described which utilises streaming gases for the disinfection of powders. An examination has been made of the results obtained.

2. The factors affecting the disinfection by formaldehyde of powders containing spores of *B. subtilis* have been examined. It has been shown that under the fairly wide range of conditions used the death-rate is a function of the amount of formaldehyde permeating the powders in unit time rather than of concentration of the disinfectant in the vapour phase. The role of moisture in disinfection by formaldehyde has been investigated and discussed.

3. Practical applications of the method have been discussed.

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DISCUSSION

The paper was presented by MR. E. A. RAWLINS.

MR. T. D. WHITTET (London) referred to a passage in the Report of the Central Health Services Council dealing with the findings of a Committee on "The Injection of Wrong Solutions into Hospital Patients." It had been recommended that formaldehyde tablets in a warm moist

atmosphere should be used for the sterilisation of the outer surfaces of ampoules and suggested this would occur within an hour. He did not think this likely.

DR. W. MITCHELL (London) remarked that ethylene oxide was being used successfully in America to render powdered spices relatively bacteria-free. An obvious disadvantage was the explosive risk.

MR. G. SYKES (Nottingham) said the authors had referred to the work of Blair and Ledbury, who said that formaldehyde was released into air bubbled through its solution at a fixed rate depending on the air flow rate, and then had contradicted this statement. Despite a reference in the paper to results in Table V, this table gave only the conditions of experiments and no results. It would have been more useful had Table I included more information regarding samples and concentrations over different sampling periods. They had carried out experiments on the effect of humidity on disinfection rates, from 98 per cent. down to 65 per cent. relative humidity, and had assumed that progress at lower humidities continued in the same linear fashion. That seemed to him to be a dangerous assumption. The authors had suggested that partial disinfection at low formaldehyde concentrations might be partly due to uptake of disinfectant by the kaolin because the disinfection graph still proceeded in a sigmoid form. Mr. Sykes suggested that the form of disinfection was altering by increasing the concentration, and that it might have nothing at all to do with the kaolin-formalin reaction. Ethylene oxide was used fairly extensively for disinfection. It had been used for the sterilisation of penicillin powders. The time of disinfection was much longer than with formaldehyde, periods of 18 to 24 hours being necessary.

DR. K. BULLOCK, in reply, said the problem appeared to be much more difficult than had been hoped. One would expect the rate at which bacteria died to be proportional to the concentration and not to the amount of gas passing through the powder. He could think of no physico-chemical mechanism which would explain their results, unless it were visualised that the powder was an inert mass and the bacteria were exposed to only a certain amount of the gas, taking up the whole of that in their vicinity. Then the rate at which one changed the atmosphere by passing the gas through was controlling the rate of disinfection. More work would have to be done on the subject, but the results so far obtained had been definite and had therefore been published. They were concerned, not so much with the concentration of formaldehyde which should be used, but with the mechanism of its action.

MR. RAWLINS, in reply, said that from his experience with paraformaldehyde it seemed doubtful whether sterilisation could be achieved in the circumstances described by Mr. Whittet. One difficulty with paraformaldehyde was that it volatilised as formaldehyde molecules and then polymerised again on the substances which were to be sterilised. The gas which the authors proposed next to investigate was ethylene oxide. It had been extensively used for sterilising spices, and some American workers had investigated general sterilisation in closed chambers with it.