THE USE OF HYPOCHLORITES FOR AERIAL DISINFECTION

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(With 5 Figures in the Text)

Introduction

At the present time the three chief substances which it is claimed are useful germicidal aerosols for employing in the presence of man are (1) "Aéryl", a solution of resorcinol in watery glycerol (Trillat, 1938; Pulvertaft, Lemon & Walker, 1939), (2) hypochlorite solutions (Douglas, Hill & Smith, 1928; Masterman, 1938; Pulvertaft & Walker, 1939) and (3) a solution of hexyl resorcinol in propylene glycol, etc. (Twort, Baker, Finn & Powell, 1940). In our previous paper we (1940) discussed the relative merits of many phenolic germicides including "Aéryl", and here it is our intention to describe experiments carried out on hypochlorite solutions. We have also been interested in the mechanism by which hypochlorite solutions exert their action on bacteria, as a possible aid to an understanding of the action of germicidal aerosols in general.

Briefly, the technique, which we have previously described in detail (1940), consists in atomizing an equal volume of bacterial emulsion into each of two identical chambers, and the germicide under test into one of these. The colony counts on agar plates exposed in both chambers at definite intervals are compared, and the percentage of organisms surviving the action of the germicide recorded. The main test organisms for the present experiments have been *E. coli* and a white, saprophytic micrococcus ("F" coccus). The former was selected because other workers with hypochlorites have used it, and the latter was the organism mostly used by us in our standard tests of phenolic compounds.

Experiments

In the initial experiments with a proprietary antiseptic containing 1 % hypochlorite (1 % NaOCl, 16·5 % NaCl and small amounts of other Na, Ca and Mg salts), we were unable to obtain evidence of a definite kill when using concentrations up to 1 part of germicide mist in 5 million parts of air (Table 1*a*). It had been suggested (Masterman, 1938) that the germicidal action of hypochlorite mists is due to the liberation of HOCl gas by the action of the CO_2 in the atmosphere. Consequently, we repeated the tests in an atmosphere containing an additional 1 % of CO_2. This did not, however, produce any improvement in the kill (Table 1*b*). The antiseptic solution had been atomized from an "Atmozon" nebulizer, which we had utilized in most of our previous experiments on aerial disinfection, and which we had found to give mist particles with a maximum size of 1·8μ radius when using the hypochlorite solution. This method of atomization has already been shown to cause the loss of a volatile solvent (in the present instance water) during working, so it was possible that our failure was due to (1) the droplets containing less than a lethal dose of active substance, (2) the small droplets evaporating with such rapidity that they became completely dried before contact with the organisms, and (3) the amount of hypochlorite atomized not being truly indicated by the weight differences of the atomizer before and after use, due to distillation of water from the bulk of the fluid. It was subsequently demonstrated that the residue in the atomizer after use contained a higher concentration of NaCl than the original solution, but a lower concentration of NaOCl (0·9 %), due to the loss of HOCl.

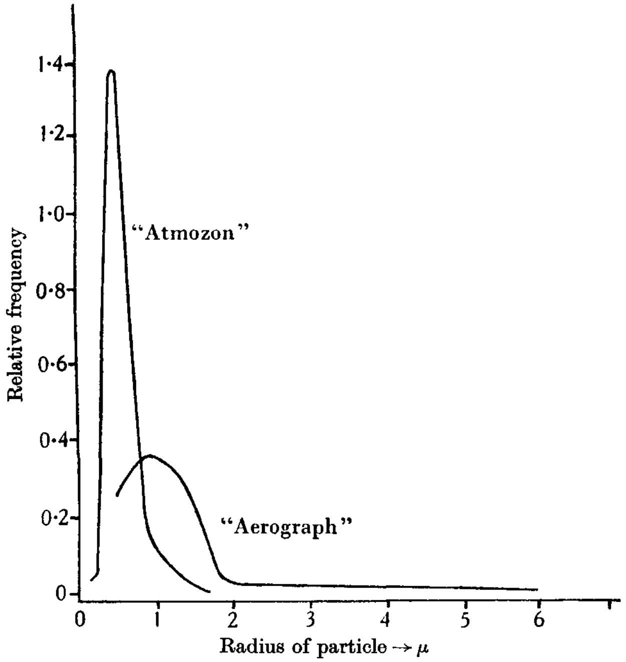

Fig. 1. Particle size distribution of 1 % NaOCl solution.

In order to obviate these objections the experiments were repeated, using an "Aerograph brush" ("A.E." Model) instead of the "Atmozon". This instrument not only gave a larger maximum size particle (6μ radius), but also avoided the distillation effect, since the whole of its charge can be ejected directly into the air, there being no continuous stream of air through the reservoir. The particle size distribution curves for both these atomizers, using the hypochlorite solution under consideration, have been determined by the method described in our original paper (1940, p. 279) and are shown in Fig. 1. The tests conducted with the "Aerograph" atomizer showed very good kills (Table 1*c*, *d*).

The difference in results obtained by the two methods of atomization led us to attempt to discover the mechanism of the action of hypochlorite, since, if the hypothesis of the liberation of HOCl gas were correct, then it was logical to assume that the smaller particles should result in a more rapid liberation of the gas with consequent increase in the killing rate. If, on the other hand, the postulate that molecular dispersions are relatively inactive is correct, as has been shown in connexion with phenolic bodies (Bechhold, 1935; Pulvertaft & Walker, 1939; Finn & Powell, 1939; Twort *et al.* 1940), then the action would seem to depend on the retention of the active principle in the mist droplets until these make contact with the suspended organisms.

Experiments were devised to test these hypotheses along the following lines:

(1) The addition of a weak acid to hypochlorite solution just before atomization.

(2) The utilization of a watery solution of HOCl and of gaseous HOCl.

(3) The utilization of chlorine gas.

(4) The utilization of a watery solution of sodium chloride equivalent to the NaCl concentration in the hypochlorite solution.

(5) The use of alkaline solutions of hypochlorite.

(6) The addition of glycerol to hypochlorite solutions.

(7) The use of hypochlorite in air free from CO_2.

(8) The effect of the concentration of NaOCl and NaCl in the solution atomized.

(9) Factors influencing the effective mist concentration.

The results of these experiments will be discussed separately. Throughout this series we have used a range of concentrations of the antiseptic (containing 1 % NaOCl) of from 1 g. per million ml. of air to 1 g. per 100 million ml. of air. When gaseous Cl_2 or HOCl, or a solution of HOCl gas has been used the concentrations were reduced to the equivalent of the 1 % hypochlorite solution, i.e. a $(5 \times 10^6)^{-1}$ mist of hypochlorite solution corresponded to 1 ml. of HOCl gas per $1{\cdot}7 \times 10^6$ ml. of air, and sufficient HOCl solution was used to yield this concentration of gas. We have adopted the convention of expressing a mist concentration of 1 g. per $y \times 10^6$ ml. of air as $(y \times 10^6)^{-1}$.

(1) *The addition of a weak acid to hypochlorite solution*

The object of the addition of a weak acid was to liberate the HOCl gas from the hypochlorite solution so that on evaporation of water from the mist practically all the HOCl would be liberated into the atmosphere. Glacial acetic acid (1 %) was employed, and added to the hypochlorite just before nebulization. A corresponding concentration of acetic acid was used in the control chamber. The results obtained against *E. coli* (10,000 bacilli per litre of air) with both atomizers are given in Table 1*e*, *f*.

The experimental results in Table 1 appear to show (distillation effect, pp. 566, 570) that large droplets of the hypochlorite solution were effective where the small ones were not, and the presence of the weakly ionizing acid (such as CO_2 or $\overline{HAc}$) did not appear to enhance the effect of the small ones, although it might be expected to do so were HOCl gas responsible for the lethal effect. On the other hand, a weak acid, either in the gaseous form (CO_2), or particularly

Table 1. *The effect of mists of hypochlorite solutions on nebulized broth emulsions of* E. coli

Germicide	Mist conc. $(y \times 10^6)^{-1}$	Percentage survivors after 5 min.	15 min.	30 min.	Mean	
(*a*) 1 % NaOCl	$y=20$	105	100	71	92	"Atmozon"
	13	100	100	83	94	
	5	109	75	60	81	
(*b*) 1 % NaOCl + 1 % CO_2 in air	20	103	69	77	89	
	13	75	57	65	66	
	5	75	79	100	85	
(*c*) 1 % NaOCl	20	72	76	32	60·2	"Aerograph"
	13	20	3·2	1·8	8·2	
	5	4·8	0·1	0	1·62	
	1	0	0	0	0	
(*d*) 1 % NaOCl + 1 % CO_2 in air	20	32	47	16	31·8	
	13	13	7·3	8·7	9·7	
	5	0·4	0	0	0·13	
(*e*) 1 % NaOCl + 1 % HAc	12	35	47	24	35·5	"Atmozon"
	5	107	113	168	N.K.	
(*f*) 1 % NaOCl + 1 % HAc	20	13	5·3	0·7	6·3	"Aerograph"
	13	7·1	4·5	0	3·9	
	5	0	0	0	0	

N.K. = no kill.

in the solution, seems to be beneficial where large droplets are concerned. This observation, again because of the longer retention of water, seems to suggest that droplets themselves containing free HOCl are the lethal agents or that rapid loss of water from small droplets allows no time for the absorption of CO_2. It must in fairness, however, be pointed out that the solution acidified with acetic acid and used in the "Atmozon" experiments was prepared at the beginning of the tests, and chemical estimations have since shown that such solutions lose their HOCl rapidly (which loss would be further accentuated by the distillation effect previously mentioned), whereas when the "Aerograph" tests were performed the acidified hypochlorite was prepared fresh for each test and used at once. It was not considered worth while repeating the "Atmozon" tests as the distillation effect would almost certainly vitiate any results obtained.

(2) *The utilization of* HOCl *solution and gaseous* HOCl

Analysis of the hypochlorite solution showed the product to contain chiefly 1 % W/W of NaOCl and 16·5 % W/W NaCl, which is in agreement with the statement of the manufacturer. The hypochlorous acid solution we prepared for test contained 1·52 g. HOCl gas per 100 ml. This was atomized from the "Aerograph" gun to give concentrations equivalent to the amounts of hypochlorite stated in Table 2. In this way we attempted to test the effect of the free gas as distinct from a solution of the gas in the form of a mist, on the assumption that complete evaporation of the water took place.

Table 2. *The effects of* HOCl *gas* (*solution*) *on nebulized broth emulsions of* E. coli

Mist conc. of HOCl sol. $(y \times 10^6)^{-1}$	Equiv. conc. of 1 % NaOCl sol.	Percentage survivors after 5 min.	15 min.	30 min.	Mean
$y=50$	$y=20$	47	46	53	48·8
30	13	45	89	44	59·5
12·3	5	3·1	2·0	7·0	4·4

It must be pointed out in connexion with the first two results shown in Table 2 that for the first test it was necessary to dilute the HOCl solution 1/5 to obtain a suitable volume of fluid for atomization. The additional water here, in slowing down evaporation from the bacterial mist, may account for the kill obtained being as good as that with the stronger mist of the second experiment (cf. p. 568).

If these results are compared with those obtained with the corresponding concentrations of NaOCl solution (Table 1*c*) it will be noted that the percentage of bacteria surviving is considerably higher; and in addition such kill as was obtained, unlike that with the NaOCl solution, was not progressive with time. The kills occur within the first 5 min., which seems to indicate that the HOCl gas was responsible. However, it is difficult to form a definite opinion because desiccation of the bacteria has been shown (Tables 2*a*, *b*) to slow up the absorption of gas, and because of the possibility of the re-evaporation of HOCl gas from the drying droplets.

Free HOCl gas was produced by passing CO_2 through the hypochlorite solution, the residual fluid being titrated to determine the exact amount of gas evolved. This method was adopted in confirmation of the second series of experiments (p. 563) because we were not altogether convinced that all the HOCl present was in the gaseous form (a small quantity of $CaCl_2$ was also present). In the present tests there was no possibility of germicidal mist entering the chamber as the gas was passed through a tube arranged to trap any drops caused by the bubbling process.

Table 2*a* shows the results obtained in these tests against *E. coli*, where, following our usual practice, the introduction of the bacterial mist immediately followed the germicide. The results being very good, we suspected that the still moist bacilli absorbed a lethal dose of the gas in the first few seconds; indeed, the killing rate seems to indicate this. It will be seen that in the $(4\cdot6\times10^6)^{-1}$ mist practically all the kill was registered on the first (5 min.) plate, while, when the concentration was halved there was presumably no kill in half an hour. By reversing the order of introducing the two mists it was thought that this point could be more clearly demonstrated; therefore, another set of tests was performed wherein the bacteria were nebulized 5 min. before the HOCl gas was produced, thus allowing good time for drying of the bacteria. It can be seen from the results of these tests given in Table 2*b* that a considerable slowing down of the killing rate occurred. We had anticipated that no kill at all would take place. It is highly probable that the bacterial mist was never completely desiccated, owing to the hygroscopic nature of the broth in which the bacilli were suspended.

Table 2*a*. *The effect of* HOCl *gas on nebulized broth emulsions of* E. coli

Vol. HOCl gas used ml.	Equiv. conc. 1 % NaOCl $(y\times10^6)^{-1}$	Percentage survivors after			
		5 min.	15 min.	30 min.	Mean
1·16	$y=8\cdot2$	85	96	88	90
1·99	4·6	1·8	0·9	0	0·9
5·0	1·8	0	0	0	0

Table 2*b*

1·22	7·6	37	25	25	28·7
2·81	3·3	8·7	7·5	0	5·4
3·69	2·5	4·0	0	0	1·3

Stability of hypochlorite solutions. In order to obtain some idea of the comparative rate of loss of HOCl from hypochlorite solutions, various test solutions were placed in Petri dishes exposed to the air, and samples were withdrawn at suitable intervals and titrated for hypochlorite content. The surface exposed to atmospheric action is small compared to that exposed when the solution is dispersed as a mist, and, therefore, any changes found can be assumed to represent more rapid changes in a mist. Fig. 2 presents the type of behaviour found. A weakly alkaline, 0·8 %, solution of NaOCl does not change materially in 3 hr.; the addition of 10 % glycerol causes a gradual initial loss of HOCl which increases in speed, finally becoming very rapid. Acidified hypochlorite solutions lose HOCl rapidly without any initial lag, the loss being quicker in the presence of glycerol.

It would appear that some interaction occurs between glycerol and hypochlorous acid. In the case of a weakly alkaline solution the action is delayed until some HOCl is liberated by CO_2 absorbed from the air; it does not appear to be due primarily to acidity of the glycerol, since the loss of HOCl is accelerated by glycerol in the presence of acetic acid. The failure of glycerol to give the improved results (see Table 6) expected in the biological experiments is probably due to the interaction between it and the hypochlorous acid in reducing the amount of hypochlorite available.

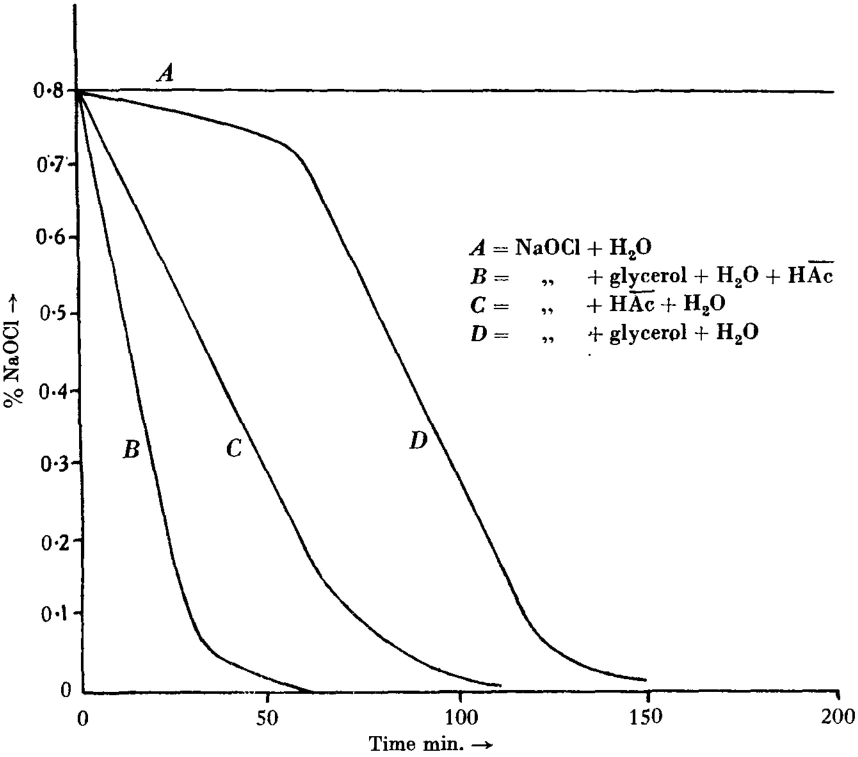

Fig. 2. Decrease n available HOCl in hypochlorite solutions.

(3) *The effect of chlorine gas*

Chlorine gas was collected in suitably calibrated tubes which were sealed at each end until required for use, when they were cut and the gas expelled into the test chamber by a current of air. Again, the chlorine concentration, in most instances, was adjusted to be equivalent to the available chlorine in the concentrations of hypochlorite previously employed. Table 3 illustrates the results obtained against *E. coli*.

It can be seen that the equivalent concentrations of chlorine gas are much higher than those of HOCl in the previous experiment (Table 2) and very much higher than those of hypochlorite as a coarse mist (Table 1*c*). In the lower concentrations possible very slight effects are noted, but these are not progressive as are those of the higher concentrations due, probably, to absorption by the bacterial mist reducing the concentration below the lethal point. The highest dose of chlorine contained at least six times more gas than that available in the $(5\times10^6)^{-1}$ concentrations of hypochlorite solutions.

Table 3. *The effect of chlorine gas on nebulized broth emulsions of* E. coli

Vol. Cl_2 used ml.	Equiv. conc. of 1 % NaOCl sol. $(y\times10^6)^{-1}$	Percentage survivors after 5 min.	15 min.	30 min.	Mean
0·2	$y=20$	88	68	83	80
0·8	5	66	45	43	51
2·0	2	63	32	15	37
5·0	0·8	46	30	15	30

(4) *The effect of salt solution*

Because we had previously found that mists of certain inorganic salts, e.g. $ZnCl_2$, $CaCl_2$, exerted a lethal effect on bacteria in the air, it was decided to determine how far the germicidal action of the hypochlorite solution under investigation was due to its high NaCl content (16·5 %). A 17·3 % NaCl solution in water was prepared, the additional 0·8 % salt being added to compensate for the NaCl available by decomposition of the NaOCl. The mists of such a solution were shown to have some effects, but not so great as when hypochlorite was present (Table 4). Tests were done with the mist produced by the "Aerograph" gun and the "Atmozon" nebulizer, the latter giving inferior results, probably, again due to more rapid evaporation of water from the smaller droplets. However, the amount of NaCl dispersed into the air by the "Atmozon" was uncertain owing to the distillation effect referred to earlier.

Table 4. *The effect of mists of sodium chloride solution on nebulized broth emulsions of* E. coli

Conc. of 17·3 % NaCl sol. $(y\times10^6)^{-1}$	Percentage survivors after 5 min.	15 min.	30 min.	Mean	
$y=10$	86	79	67	77	"Atmozon"
5	108	100	83	97	
20	69	80	48	65	"Aerograph"
5	100	38	27	55	
2·5	38	20	19	26	

(5) *The use of alkaline hypochlorite solutions*

In order to determine the effect of hypochlorite solution in conditions where it was unlikely that free HOCl could be present, either as a gas or in solution, 1 % of NaOH was added to the proprietary solution. The results were, as expected, much poorer than those obtained with hypochlorite alone, as will be seen from Table 5, the tests being performed with mists from the "Aerograph" gun.

Table 5. *The effect of mists of alkaline hypochlorite solutions on nebulized broth emulsions of* E. coli

Conc. of 1 % NaOCl $(y\times10^6)^{-1}$	Percentage survivors after 5 min.	15 min.	30 min.	Mean
$y=20$	99	68	80	82·3
13	76	57	37	57
5	70	47	21	46

Two additional experiments with 5 % NaOCl solution in an "Atmozon" nebulizer were carried out against the "F" coccus and gave results as follows:

Table 5*a*. *Comparison of the effects of neutral and alkaline hypochlorite solutions on nebulized broth emulsions of the "F" coccus*

Solution of hypochlorite	Conc. of 5 % NaOCl $(y \times 10^6)^{-1}$	Percentage survivors after 5 min.	15 min.	30 min.	Mean
Neutral	$y=52$	27	2·0	0	9·7
Alkaline	51	87	85	63	78·2

Here again the findings with the neutral solution are strikingly superior.

(6) *The addition of glycerol to hypochlorite solution*

It was thought that if the action of hypochlorite took place in a liquid phase, the kill would be improved if glycerol were added to slow up the evaporation of water from the droplets. The results obtained against *E. coli* by mists of 1 % NaOCl solution containing 10 % of glycerol are set out in Table 6.

Table 6. *The effect of the addition of* 10 % *glycerol to the hypochlorite solution* (E. coli)

Mist conc. of 1 % NaOCl sol. $(y \times 10^6)^{-1}$	Percentage survivors after 5 min.	15 min.	30 min.	Mean	
$y=25·6$	110	89	77	92	"Atmozon"
13·3	100	60	25	61·6	
2·2	62	17	0·4	26·4	
20	64	21	27	37·3	"Aerograph"
13	57	35	17	36	
5	50	36	20	35·3	

It should be noted that in the case of the three "Atmozon" tests, fresh samples of the hypochlorite solution were used in the nebulizer each time, the residues being titrated separately for HOCl content. The figures shown in the mist concentration column represent the true concentrations as calculated from the titration result and not from the weight of solution atomized as in Table 1. Comparison of Tables 1 and 6 reveals two main points of interest: (1) the fine ("Atmozon") mist of the glycerol solution apparently shows improved results over those of the plain solution, (2) the coarse mist of the glycerol mixture produced by the "Aerograph" is less effective than that of the plain solution. The explanation of this difference seems to be that it is due to retarded evaporation in the presence of glycerol. In the case of the fine mist longer retention of water and the active principle is an advantage, but since precipitation is hastened in the case of the large particles, with consequent rapid decrease in concentration, this constitutes a disadvantage. Furthermore, provided that each droplet in the finer mists contains a lethal dose of germicide, such droplets are more active and more numerous weight for weight of germicide used than those in a coarse mist. On the other hand, rapid loss of fluid from large droplets causes decrease in size but increase in mobility, resulting in more rapid contact with the suspended bacteria, but this effect will be slower than in the case of smaller droplets, due to their greater initial size; the presence of the glycerol will accentuate this.

(7) *The use of hypochlorite in* CO_2 *free air*

The object of carrying out tests with hypochlorite mists in air depleted of CO_2 was finally to establish whether the effectiveness of the solution was essentially due to the action of CO_2 in liberating HOCl as a gas. The atmosphere of the chambers was freed from CO_2 by placing within them a dish of 40 % NaOH solution, and allowing this to act for 72 hr., the chambers being first loaded with agar plates sufficient for three tests and then sealed, so that apart from the few seconds required to introduce the bacterial and germicide mists no CO_2 could enter. The precaution was taken of working the atomizers with CO_2-free air. No airing of the chambers was done, but time was allowed between tests for the settling out of the mists. The mists were tested in order of increasing concentrations so that residual mists from a previous test would have but a negligible effect on the concentration of the ensuing one. The mists were produced by the "Aerograph gun", the result of the tests being shown in Table 7.

Table 7. *The effects of mists of hypochlorite solution in air free from* CO_2 (E. coli)

Mist conc. $(y \times 10^6)^{-1}$	Percentage survivors after 5 min.	15 min.	30 min.	Mean
$y = 20$	40	18	8	22·0
13	25	4·6	1·9	10·5
5	2·5	0·37	0	0·96

When taken as a whole and compared with the results in Table 1*c*, *d*, the figures in Table 7 do not appear to be very different. This finding may be taken to indicate that CO_2 plays but little part in the reaction.

(8) *The effect of the concentration of* NaOCl *and* NaCl *in the solutions atomized*

In order to determine the effect of particle size of the mists, a few tests were performed with mists of the 1 % NaOCl solution produced by the "Aerograph" gun and passed through a selector centrifuge (described in our previous paper) arranged to pass only particles of 1 μ radius or less. The results given by these selected mists were better than those given by the "Atmozon" since the distillation effect was avoided, but poorer than those given by the "Aerograph" alone. Owing to the smaller size of the droplets from the selector they dry more quickly than the average droplets in the gross output, and at the same time there may have been considerable evaporation during their passage through the centrifuge.

To overcome these difficulties dilutions of the hypochlorite solution were dispersed by the "Aerograph" gun, it being arranged that the same amounts of NaOCl were present in the chamber as in previous tests. Calculations showed that the extra water used was not sufficient appreciably to affect the humidity. Thus we may assume as a first approximation that, if the hypochlorite be diluted with an equal volume of water and the weight of hypochlorite atomized be kept the same as in comparative experiments with the neat 1 % solution, the number of droplets in the air will be double, their average weight halved and their average radius approximately 0·8 of its former value; at the same time the mobility of the droplets will be considerably increased and their drying rate accelerated. Table 8 shows the results obtained with dilutions of 1/2, 1/4, 1/5, 1/10 and 1/20 of the stock 1 % preparation. The condensed results of all the tests expressed in terms of $(1 \times 10^6)^{-1}$ mist are graphically illustrated in Fig. 3.

Table 8. *Comparison of the effects of mists of dilutions of* 1 % *hypochlorite on atomized* E. coli

Dilution of 1 % hypochlorite solution	Mist concentration $(y \times 10^6)^{-1}$ as 1 % NaOCl $y = 60$	40	20	13	5
Nil	—	—	60·2	8·2	1·62
1/2	—	—	10·6	2·08	0
1/4	—	0	0	0	0
1/5	0·07	0	0	—	—
1/10	—	5·8	0·19	0	0
1/20	—	74·3	2·65	0	0

It is evident from the table that the effect of dilution is remarkable. A mist concentration of $(20\times 10^6)^{-1}$ of the 1/5 dilution (equivalent to $(100\times 10^6)^{-1}$ of the original solution) gave only 0·92 % survivors over the half-hour period, a result approximately the same as that obtained for the mean of four tests expressed in terms of $(1\times 10^6)^{-1}$ mists (Table 10). The existence of this sharp maximum shows that in order to obtain the best results a compromise between particle size, mobility and rate of drying is necessary, but the significance of these results together with those of other tests will be considered later (p. 572). On the other hand, it was found that a 17·3 % NaCl solution (Table 4) when diluted 1/5 in water prior to atomization did not give improved results.

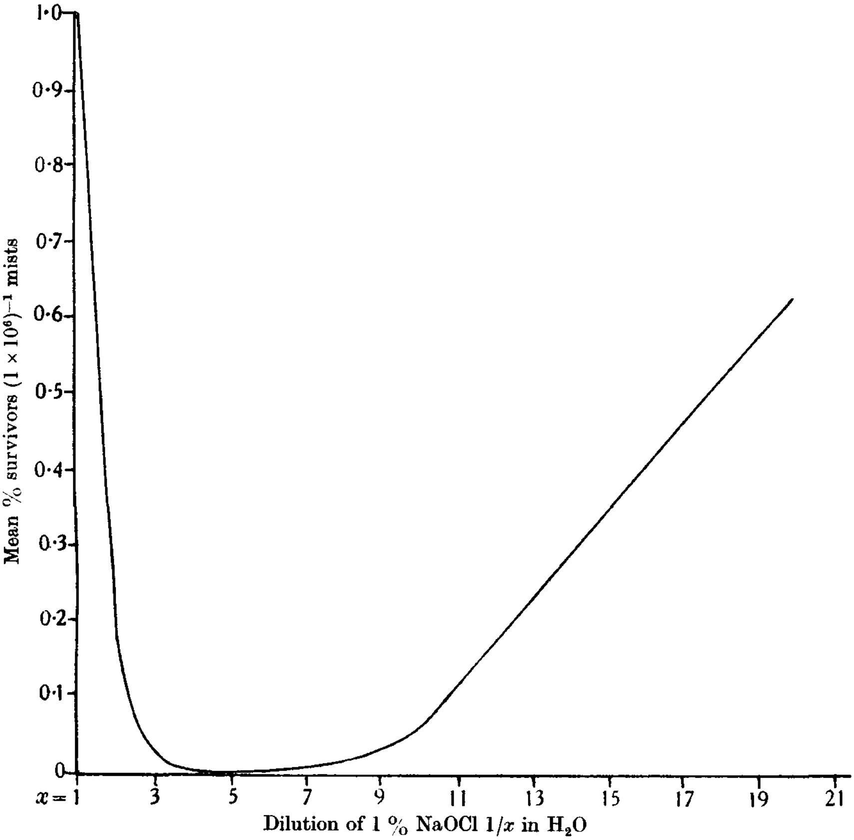

Fig. 3. Efficiency curve of dilutions of 1 % NaOCl.

Examination of the mists by means of the ultramicroscope showed that the particles in mists from the diluted solution had approximately the radius expected from the degree of dilution. The initial loss of water was too rapid to be detected and further drying of the remaining residues was too slight for detection by this means.

Obviously, if a compromise between droplet size and rate of drying is to be reached in order to obtain the best results, then the amount of NaCl present will play an important part in these two considerations. The extent of the influence of NaCl was demonstrated by utilizing a solution of NaOCl which we prepared containing only 0·79 % NaCl and 0·05 % Na_2CO_3. A $(20\times 10^6)^{-1}$ mist of this preparation gave only 0·54 % survivors of *E. coli* in 5 min., subsequent and more concentrated mists giving complete sterility. This solution, therefore, is more effective than the proprietary mixture previously employed. However, a 1/5 dilution of our preparation was slightly less effective than the proprietary solution similarly diluted and used under exactly similar conditions, thus supporting our contention that if the particle size is too small then the rate of drying becomes too rapid.

It seemed of interest to investigate the effect of reducing the NaOCl content without altering the NaCl concentration. The proprietory solution was diluted 1/5 with 17 % NaCl solution, and gave a mean survival rate of 0·3 % against 0·99 % obtained for the neat solution. This is about the order expected from the increased number of droplets, as owing to the extra NaCl added there will be no increase in mobility of the particles from the diluted solution as there was when distilled water was the diluent.

The effect of diluting the hypochlorite solution with watery glycerol has also been tested to ascertain whether, by slowing down the evaporation rate, the kills would be improved. A dilution of the original solution of 1/5 was made in 2 % watery glycerol so that the relative amount of glycerol to hypochlorite was the same as that previously used (Table 6). The results expressed as the mean percentage survivors of *E. coli* over the half-hour period are shown in the following table:

Mist concentration $(y \times 10^6)^{-1}$ *as* 1 % NaOCl

$y = 40$	20	13
1·04	0·08	0

Comparison of these results with those for the corresponding dilution in Table 8 shows the glycerol mixture to be somewhat inferior. This was also confirmed by tests on both solutions when mists of $(100 \times 10^6)^{-1}$ were employed, the plain dilution giving complete sterility in 30 min. where the glycerol dilution showed 0·42 % survivors. The mean percentage survivors over the 30 min. period for these two were 0·92 and 2·1 % respectively. Again this difference may be assigned to the slight interaction between hypochlorite and glycerol and/or the damping effect of the glycerol. Further comments on the glycerol mixtures will be made in the section headed "The persistence of mists".

(9) *Factors influencing the effective mist concentration*

The distillation effect to which reference has already been made several times has been demonstrated by us (1940, p. 295) with aqueous salt solutions and other less volatile substances. We have attributed the difference in results obtained with the "Atmozon" and "Aerograph" mists of hypochlorite solutions partly to this cause, the mist concentration calculated in the former case from the weight differences being grossly in error because of the loss of volatile solvent during atomization. Estimations of the effective concentration in the air were made by aspirating samples from the chamber through a wool filter (this method has been described in our previous paper and found to give reliable results with NH_4Cl smokes) and comparing the figures so obtained with those calculated from the weight loss of the "Atmozon" nebulizer, or the volume of solution used in the "Aerograph" gun.

In the case of the "Atmozon" apparatus the mean value obtained for the mist in the air was only 10·8 % of the weight loss. Thus, considering the fineness of the mist (with minimum loss due to settling out) there must be a very large error due to distillation of water. When the "Aerograph" gun is employed in conjunction with the neat germicide, on an average 24·2 % of the weight atomized was recovered in the filter. With this instrument there can be no distillation effect, but, in view of the large particle size of some of the mist, a considerable proportion could be expected to be lost by settling out. When the "Aerograph" was used with a 1/20 dilution of the germicide 33·6 % of the output was recovered. However, even here, in spite of the reduced size of the residual nucleus, there must still be a considerable loss by particles falling out of the air during the sampling period.

It is of interest to examine the previous results given in Table 1*a*, *b*, and to compare them with those of the 1/20 dilution when all are corrected for the amount of mist present according to chemical estimation. The following table shows that the percentage of survivors on the basis of an actual $(1\times 10^6)^{-1}$ mist tallies more closely under these conditions.

	Mean result
"Atmozon"	0·448 % survivors
"Aerograph"	0·331 „
"Aerograph", 1/20 sol.	0·331 „

It should be noted that the estimations only account for those particles which are small enough to persist in the air and do not include any HOCl present in the air as vapour.

THE PERSISTENCE OF MISTS OF SODIUM HYPOCHLORITE SOLUTIONS

Persistence of germicidal aerosols is considered by us to be one of the characteristics essential for real efficiency. The commercial product we have used for our tests appeared likely to possess this property by reason of its high salt content, but the relative volatility of the active constituent (HOCl) suggested that the persistent residue would probably be inactive (cf. NaCl experiments, Table 3). Tests carried out to determine the active persistence were of two kinds, viz.: (1) Lethal mists were introduced into the test chamber and equal volumes of bacterial emulsion were sprayed into control and test chambers, plates being exposed in each at the usual intervals. After the half-hour plate had had its 3 min. exposure, i.e. at 33 min., second inocula were introduced into each chamber and a further series of plates exposed at corresponding intervals. (2) Lethal mists were introduced into the test chamber and allowed an "ageing" period of 15 or 30 min. before the bacterial mists were blown in. *E. coli* in broth emulsion was the test organism in both series of tests, the germicidal mists being introduced by the "Aerograph" gun.

The falling off in germicidal action on the second inoculum was particularly marked in the case of the undiluted germicide and the 1/5 dilution in 2 % glycerol. On the other hand, the glycerol diluted solution showed a better persistence in the "ageing" type of test. Tables 9 and 9*a* demonstrate clearly the effects referred to above. The decrease in activity of the mists as they become older seems to add further corroboration to the arguments against the gas theory. The older the mist the greater the proportion of HOCl gas liberated and available (assuming none to be lost by diffusion, and we are satisfied that this does not occur in our sealed chambers), as already stated.

Table 9. *The effects of a second inoculum of* E. coli *into once used mists of* (*a*) *the* 1 % NaOCl *solution*, (*b*) 1/5 *dilution in* H_2O, (*c*) 1/5 *dilution in* 1 % *glycerol*

Mist. conc. of 1 % NaOCl $(y\times 10^6)^{-1}$	Percentage survivors of 1st inoculum after			Percentage survivors of 2nd inoculum after		
	5 min.	15 min.	30 min.	5 min.	15 min.	30 min.
(*a*) $y=$ 5	14·8	11·1	4·6	90·5	66·6	74·1
1	0·78	0	0	60·8	40·0	33·3
(*b*) 40	38·1	20·8	9·05	47·4	58	38
20	18·6	2·2	0	50	7	3·7
(*c*) 40	0	0	0	46·5	15·5	5·8
20	0	0	0	2	0	0

Table 9*a*. *The effect on* E. coli *of "aged" mists of* (*a*) 1 % NaOCl *solution*, (*b*) 1/5 *dilution in* H_2O, (*c*) 1/5 *dilution in* 2 % *glycerol*

Mist. conc. of 1 % NaOCl $(y\times 10^6)^{-1}$	Age of mist min.	Percentage survivors after		
		5 min.	15 min.	30 min.
(*a*) $y=$ 5	15	57·2	30	13·3
(*b*) 40	15	70·0	2·8	0
40	30	66	8·0	1·0
(*c*) 20	15	0·18	0	0
20	30	0·52	0	0

We should like to point out here that some of the results just set out are not in keeping with those recorded earlier in this communication. For instance, the watery dilution of 1/5 gave poorer results than those recorded in Table 8. We drew attention in our first paper (Twort *et al.* 1940) to the difficulties of repetition of results in this type of work despite every effort made to control the experimental conditions and keep them constant. These differences illustrate the need for the controlling of every test and the basing of conclusions on numerous results. There elapsed a period of about 3 months between the tests recorded in Table 8 and those in Tables 9 and 9*a* so that variations in the sensitivity of the culture may have occurred, due to the use of different batches of media, etc., though naturally we endeavour to keep to a standard in this respect also.

A further source of error affecting droplet size of both bacterial and germicide mists is that of wear or partial blocking of the "air-brush" nozzle. The diameter of the "Aerograph" nozzle is 0·007 in. so it can be appreciated how easily partial blocking may occur; we have had to replace worn nozzles and needle valves. Both these factors would materially influence the particle size.

CONSIDERATION OF THE EXPERIMENTAL RESULTS

So far we have done little more than enumerate the various aspects of the problem before us and tabulate our experimental results. For the purpose of quick comparison the figures for all the tests mentioned have been brought together in Tables 10 and 10*a*, and condensed by the method of expressing in terms of $(1 \times 10^6)^{-1}$ mist the mean survivor figure to the nearest decimal point in each test. A single figure, being the mean of three or four tests, thus gives an approximate summation of each set or series of experiments.

Table 10. *The mean survival rate of* E. coli *over* 30 *min. in mists of hypochlorite solutions and constituents thereof*

Table ref. no.	Germicide	Mean survivors	
		"Atmozon"	"Aerograph"
(1)	1 % NaOCl	7·0	0·99
	+1 % CO_2	8·84	0·92
	+1 % $\mathrm{H\overline{Ac}}$	2·71	0·21
(5)	+1 % NaOH	7·38	5·9
(6)	+10 % glycerol	4·86	4·25
(4)	17·3 % NaCl sol.	13·56	19·27
(7)	1 % NaOCl − CO_2	—	0·7
(2)	HOCl sol.	—	1·97
(2*a*)	HOCl gas	3·7	
(2*b*)	HOCl gas	1·96	
(3)	Cl_2 gas	17·15	

Table 10*a*. *The mean survival rate of* E. coli *over* 30 *min. in mists of diluted hypochlorite solutions* (*Table* 8) *expressed as* $(1 \times 10^6)^{-1}$ *of* 1 % NaOCl

Diluents	Dilutions					
	0	1/2	1/4	1/5	1/10	1/20
Water	1·32	0·23	0	0	0·06	0·664
2 % glycerol	—	—	—	0·02	—	—

The figures shown in Tables 10 and 10*a* can only be used as a broad basis of comparison since the rate of kill is not clearly demonstrated, reference to individual tests being necessary for final adjudication in this respect. Further, the number of survivors is, of course, not always strictly inversely proportional to the concentration of germicidal mist employed.

If we consider first the figures of Table 10 in relation to the atomizers used, it is clear that in each series the "Atmozon" figures were considerably higher, except when glycerol was present. Even so, there may be a slight difference in favour of the "Aerograph" figure. The sodium chloride experiments can be disregarded because the salt in the hypochlorite solution is of no practical value as a bactericide in comparison with the other constituents. For the superiority of the large droplet mists ("Aerograph"), there appear to be two explanations: (1) their rate of evaporation is slower, thus allowing longer time for contact to take place while they are still moist, (2) the small droplets do not contain a lethal dose of germicide whereas the larger do. However, in the glycerol experiments, where evaporation was slowed down (p. 567), the improvement in performance of small droplets is so marked (the bacterial survival rate being approximately halved) that the second explanation does not seem very probable, particularly in the light of the finding that glycerol-containing solutions rapidly lose HOCl (p. 565). It would appear, therefore, that to give the best results, the size order of mist droplets of the 1 % sodium hypochlorite solution should be from 0·4–1 μ radius.

It follows as a natural conclusion from the foregoing arguments that hypochlorous acid in the gaseous state was not responsible for the kills obtained; otherwise it could be expected that rapidly evaporating small droplets would be more efficacious than larger drops. To this may be added the evidence of the experiments with diluted solutions. It was found that a distinct maximum effect is given with dilutions in the region of 1/5, higher and lower dilutions giving poorer results (Fig. 3). If the hypothesis that the effect is due to HOCl gas is correct, then no such maximum would be expected since the smaller the particles the greater the total surface exposed to the action of CO_2, with consequent more complete and rapid evolution of HOCl gas; whereas a particulate mechanism would lead one to expect the existence of a maximum effect at a specific dilution. Again, in our estimations of the effective mist concentrations, only suspended droplets were collected, neither the rapidly falling particles nor the vapour being included. The close agreement of the mean figures for the percentage survivors when different means of atomization are used (due allowance being made for effective mist concentration) is strong support for the conclusion that it is the hypochlorite in the mist form which acts upon the suspended bacteria. Further evidence against the gas hypothesis is afforded by the acetic acid experiments. Here it will be seen that the performance of the small particles is improved nearly threefold, and that of the larger ones nearly fivefold, but if the free gas were acting this improvement should at least be reversed, as again more rapid loss of HOCl would occur with the smaller particles. It may also be adduced from these last-mentioned tests that the small droplets, in view of their increased efficiency, must contain a lethal dose of active agent, in spite of some loss of HOCl from them, or at any rate that there are sufficient contacts between germicide and bacterial drops to prove effective.

Thus it may be stated that under the conditions of our tests the hypochlorous acid in solution and not the molecularly dispersed gas was the form in which the germicide being considered was lethal. Additional weight is given to this reasoning when the results of the alkaline solutions are scrutinized, these in both instances being the poorest of the whole series, because no free or insufficient free acid was present in the mist particles. Also, in this connexion, it will be noted that the adverse effect of NaOH on large particles is shown to a greater degree than on small particles, probably as a result of the former having a proportionately smaller surface area available for the absorption of CO_2.

Superficially, the experiments on the effect of changing the concentration of CO_2 are contrary to our explanation of the most effective mechanism. It is probable, however, that the rate of absorption of CO_2 by the droplets is slow compared with their rate of loss of water, the droplets, therefore, becoming dry before any appreciable amount of HOCl can be liberated in them. Since the bactericidal action of hypochlorite is unimpaired by the absence of CO_2, it is presumed that sufficient HOCl is provided by hydrolysis, except when a considerable amount of NaOH is present.

From an examination of the results relative to tests in which no mists were utilized it will be seen that hypochlorous acid gas is effective, and rapidly so when in sufficient concentration. The mechanism of its rapid action appears to be due to its high rate of absorption by the moist bacteria. When low concentrations are present none or very few organisms absorb a lethal dose before equilibrium is established between the air and the bacteria, but with high concentrations this rapidity of absorption is demonstrated by the early and very considerable kills. The presence of moisture in the organisms seems to be essential, as shown by the results in Tables 2*a*, *b*. Disregarding the $(8{\cdot}2 \times 10^6)^{-1}$ result, which is probably a bad one (cf. Tables 2 and 2*b*) it will be observed that when the bacteria are partially dried (Table 2*b*) the kills are generally less satisfactory. This is corroborated by the results shown in Table 2, in which tests the HOCl gas was liberated from an atomized watery solution of the gas. These results were the best obtained by the use of HOCl gas, possibly because water from the germicide mist in slightly raising the humidity of the air slowed down evaporation of water from the bacilli (but more probably because the $CaCl_2$ present caused retention of some HOCl in the mist). It has already been mentioned that hypochlorite mist equivalent to $(100 \times 10^6)^{-1}$ of 1 % NaOCl, obtained from the proprietary product diluted five times with water, allowed of only 0·92 % survivors. The amount of HOCl gas required to give a similar survival rate was equivalent to $(4{\cdot}6 \times 10^6)^{-1}$ of 1 % NaOCl or twenty-two times the amount available from the diluted solution. The superiority of suitable mists over the vapour of HOCl is, therefore, obvious.

Chlorine gas, while not without effect in high concentration was, with the exception of NaCl, the least effective substance tested in this series of experiments. It would appear that the most effective agent is hypochlorous acid in solution or nascent chlorine resulting therefrom.

EXAMINATION OF THE POSSIBILITIES OF THE PRACTICAL APPLICATION OF SODIUM HYPOCHLORITE MISTS FOR AERIAL DISINFECTION

The simplest and at the same time most effective method of utilizing the information gained from the experiments so far recorded appeared to be that of atomizing the plain proprietary solution diluted 1/5 with water and dispersed as a mist from the "Aerograph" air-brush, or similarly working nebulizer. The evidence so far advanced in favour of this germicide would not, however, justify its practical application without investigating its capabilities in relation to "droplet infection". We have shown in our previous paper that the lethal effect of many germicides is greatly impaired when saliva is contained in the bacterial particles, as compared to their action on mists of broth suspensions of organisms. It is also most important that any germicide to be used in the mist form in the presence of man should not be toxic or irritant in any way, and preferably odourless, invisible and non-corrosive. Thus all these points should be considered before advocating the general use of this method of air disinfection. We have made a few tentative tests in these directions, and record them here as guidance for those interested in this problem.

The action of hypochlorite on salivary flora

Experiments on the action of mists of sodium hypochlorite solutions on three atomized human salivas (diluted with an equal volume of water) were carried out with precisely the same technique as that employed for the broth emulsions of *E. coli*. Standard blood agar was used as a culture medium, and the germicide mists were produced by the "Aerograph" gun. For the purpose of obtaining full comparison the tests have been performed with mists of the neat proprietary solution, and 1/5 dilutions thereof in water and 2 % watery glycerol. The results of these tests are recorded in Tables 11, 11*a* and 11*b* respectively. As we had anticipated, considerably stronger mist concentrations were required when saliva was used instead of broth emulsions of *E. coli* as the air-infecting agent. It can be seen from comparison of the tables that from 4–8 times the coli survival rate occurs with the salivary flora in mists of the neat product. When mists of the 1/5 dilution are employed the survival rate of the salivary organisms is decreased to at least 1/5th and sometimes to 1/15th of that of the

Table 11. *The effect of mists of* 1 % *hypochlorite on atomized salivas*

Conc. of 1 % NaOCl sol. $(y \times 10^6)^{-1}$	Percentage survivors after				
	5 min.	15 min.	30 min.	Mean	
$y=20$	107	89	105	100	Saliva "A"
13	97	77	91	88·4	
5	69	25	37	43·6	
1	11·3	13	8·4	10·9	
13	75	45	53	56	Saliva "B"
5	72	47	50	56·3	
1	1·11	0·61	0	0·57	
13	70	62	75	69	Saliva "H"
5	21	12	13	15·3	
1	1·43	0	0	0·48	

Table 11*a*. 1 % NaOCl *diluted* 1/5

Mist conc. $(y\times 10^6)^{-1}$ equivalent to 1 % NaOCl	Percentage survivors after				
	5 min.	15 min.	30 min.	Mean	
$y=20$	27·8	4·37	0	10·72	Saliva "A"
10	17·6	1·0	0	6·2	
5	1·58	1·0	0	0·86	
20	26·2	6·35	8·42	13·66	Saliva "B"
10	18·75	5·55	5·83	10·04	
5	1·75	0·88	0	0·88	
20	46·5	10·4	10·0	22·3	Saliva "H"
10	7·55	2·91	3·33	4·59	
5	10·5	1·7	1·74	4·65	

Table 11*b*. *The effect of mists of* 1/5 *dilution in* 2 % *watery glycerol of* 1 % *hypochlorite on atomized salivas*

Mist conc. $(y\times 10^6)^{-1}$ equivalent to 1 % NaOCl	Percentage survivors after				
	5 min.	15 min.	30 min.	Mean	
$y=20$	31·4	11·4	4·0	15·6	Saliva "A"
10	3·33	2·29	1·0	2·21	
5	1·51	1·11	0	0·87	
20	20·9	5·33	3·85	10·03	Saliva "B"
10	14·0	4·5	1·82	6·77	
5	5·47	0·91	0	2·13	

1 % solution; an improvement corresponding approximately to that found in the *E. coli* experiments. It is difficult to give an accurate estimate of the increased efficiency in the latter case because the mist concentrations of the diluted solutions covered a much lower range than those of the neat solution. On the whole there seems little to choose between the watery and glycerol dilutions in the saliva experiments (Table 12). Here the results of each set or series of tests have been expressed in terms of $(1\times 10^6)^{-1}$ mist, and for reasons of strict comparison we have only included tests covering the same range of mist concentrations.

Table 12. *Comparison of survival rate of atomized salivary flora with* E. coli *treated with mists of* 1 % NaOCl *and* 0·2 % NaOCl (*expressed in terms of* $(1\times 10^6)^{-1}$ *mist*)

	Percentage survivors of			
Solution	Saliva "A"	Saliva "B"	Saliva "H"	*E. coli*
1 % NaOCl	7·76	7·78	4·18	0·99
0·2 % NaOCl in H_2O	0·443	0·633	0·821	0
0·2 % NaOCl in 2 % glycerol	0·402	0·536	—	<0·19

The action of mists of 1 % NaOCl *on some organisms other than* E. coli

In addition to tests already recorded, a few have been performed on three other organisms, viz. *M. phlei*, *B. lactis aerogenes* and *S. enteritidis* (Liverpool "virus" strain) atomized from broth suspensions. We had previously found that these organisms were more resistant than *E. coli* to phenolic germicides, and as Table 13 shows they are more resistant to hypochlorite.

Table 13. *Survival rates of* M. phlei, B. lactis aerogenes *and* S. enteritidis (*Liverpool*) *in mists of* 1 % NaOCl

Mist conc. of 1 % NaOCl $(y \times 10^6)^{-1}$	Mean percentage survivors over 30 min. of		
	M. phlei	*B. lact. aer.*	*S. ent.* (L.)
$y = 20$	100	44	100
13	71	67	62
5	68	4·8	37
1	35	0	0·07

No air tests have been done with these bacilli or *E. coli* suspended in albuminous fluids, but presumably their resistance under such conditions would be increased to an extent similar to that found when using phenolic germicides.

Whilst *E. coli* is an easy organism with which to work because its cultural requirements are not exacting it is, nevertheless, not the ideal test organism for germicidal aerosols. The ease with which it is killed by agents not effective against salivary flora and its rapid loss of viability on desiccation, added to the fact that it is not representative of the type of organism which these aerosols are intended to combat, should preclude its use for any but initial tests. Obviously, if the value of a substance for air disinfection is in doubt, but a test is required, then *E. coli* could be employed; a poor or negative result settling the question provided the experimental conditions were otherwise correct. We have largely used this bacillus in these tests for the reasons stated in the beginning of this paper.

DISCUSSION

The experiments were carried out in view of certain claims which are being made for the use of a sodium hypochlorite solution as a source of hypochlorous acid gas for aerial disinfection. In the course of our previous investigations we had, after a few tests, dismissed this substance as being of very little use, which indeed was correct in comparison with certain phenolic substances we were using at the time. We were not, in those early days, alive to the importance of particle size, nor was the part played by evaporation fully appreciated. We believe we have shown now that in the case of NaOCl, as with phenolic bodies, these are the two fundamentals which govern aerial disinfection.

The claim that NaOCl sprayed into the air is bactericidal by virtue of the HOCl gas liberated has not been substantiated by the two nebulization methods we have employed. We have, nevertheless, shown that HOCl gas in the air has some lethal action on the suspended bacteria, but that about twenty times more of the molecularly dispersed gas is required than when it is in solution in a suitable medium. This is in contradistinction to phenolic compounds, the vapours of which are not appreciably bactericidal to organisms suspended in the air.

The proprietary solution which we have used for the tests was found to be most effective when diluted about five times, its activity being increased 5–15 times by this procedure. Complete sterilization of the organisms in atomized human salivas was obtained in 15–30 min. by mists in $(5 \times 10^6)^{-1}$ concentration.

We have so far not been able to investigate the toxicity of hypochlorite mists to animals, that is to say, the chronic effects of constant inhalation. Human tolerance to the odour of the mists has not been seriously assessed by us, but some of our test subjects have objected to the odour and Trillat (1938) abandoned the use of hypochlorite on this account. Masterman (1938) states that "in no case was the slightest discomfort or annoyance caused to any of the occupants" where the volume concentrations of the gases V/V in air varied from $(1 \times 10^6)^{-1}$ to $(10 \times 10^6)^{-1}$, these figures representing mist concentrations of the neat product of $(2{\cdot}7 \times 10^6)^{-1}$ to $(27 \times 10^6)^{-1}$. The concentration of mists of the 1 % solution which would be effective against air-borne pathogens appears to be in the region of $(1 \times 10^6)^{-1}$ which is much higher than that recommended by Masterman. It is probable that at such a concentration the odour would become objectionable and possibly the mist irritant, and it is certain that visibility would be impaired owing to the denseness of the mist. Another objection we foresee is the deposition of quantities of salt where mists are continuously employed; and, in fact, this deposition has been noticed in our experimental chambers. The NaCl would prove corrosive to certain metals, and generally increase the appearance of dampness during humid weather. The bleaching and rotting effect of free chlorine on fabrics is also a contingency for serious consideration.

The foregoing objections would largely be overcome by the use of a more dilute solution which, as we have shown, is more effective and should prove cheaper. Probably a further reduction in the NaCl content would prove beneficial; at any rate it would reduce the objection to the dissemination of so much salt. We have not yet attempted to experiment in this direction as initially our interest was centred in elucidating the action of NaOCl on bacteria suspended in the air. It may be that objection will be raised to the increased volume of water atomized into the air by the use of a diluted solution, but if it be assumed that the normal relative humidity of the air is 60 %, the total volume of water employed in producing a mist equivalent to $(5 \times 10^6)^{-1}$ of 1 % NaOCl would only raise this figure to 62 %. The water would be carried away as vapour by the air changes and would not be deposited as would a certain proportion of the salt in the concentrated product.

The utility of 2 % watery glycerol as a diluent for NaOCl is doubtful and it seems that more investigations will be necessary before final judgment on its advantages or otherwise can be established.

Although our studies up to the present have been almost entirely devoted towards the decontamination of air of pathogenic bacteria by means of germicidal aerosols, we have, nevertheless, for our own satisfaction superficially examined the effectiveness to the same end of ultra-violet light and continuous filtration of circulating air.

In our ultra-violet light experiments we used a mercury vapour lamp as the source of light and the "F" coccus, "Liverpool virus" and saliva as bacterial contaminants of the air. A Wells's centrifuge (1936) was used for collection of the bacteria from the air. Briefly, good kills were obtained of atomized broth emulsions of the "F" coccus, considerably less of broth emulsions of the more resistant "Liverpool virus" and still less of the bacteria present in atomized saliva. The results, as far as the relative resistance of the different types of bacterial aerosols utilized are concerned, run more or less parallel with those obtained when utilizing germicidal aerosols consisting either of phenolic substances or of hypochlorite solutions. Fig. 4, in the form of a graph illustrates the effect of ultra-violet light on the "F" coccus in the air.

As regards the continuous filtration of air as a means of removing bacteria, we had formed the opinion that, in view of the average size of most bacteria-containing particles floating about in the air, and that some of the apparatus on the market is claimed to be capable of removing the majority of the much finer particles of tobacco smoke (on an average $0\cdot5\,\mu$ or less in radius), this method should be even more efficacious in removing bacteria both on account of their greater average size and, as regards those of importance at any rate, of their greater moisture content. All we wish to say about this method at present is that in our tests, utilizing cotton wool, filtration has removed the bacteria at 80 % of the theoretical rate. This is illustrated in Fig. 5.

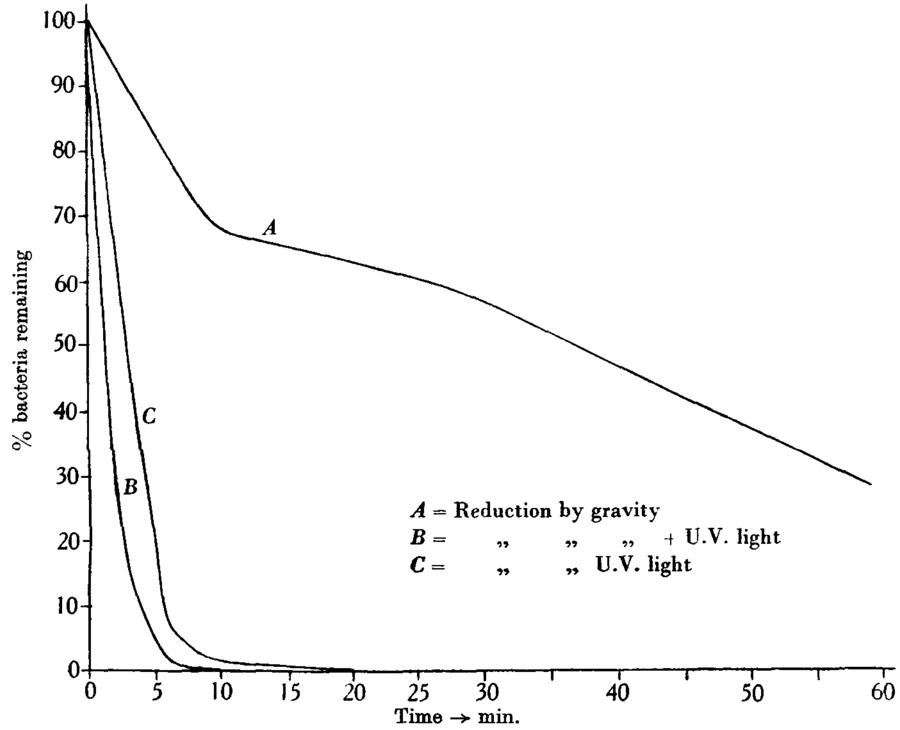

Fig. 4. Effect of ultra-violet light on the viable bacterial count of air ("F" coccus).

Some tentative experiments on the protection of animals from infection by air-borne bacteria have been performed with ultra-violet light, and complete protection was obtained under conditions where the controls succumbed to the

"Liverpool virus". Animal experiments on the efficacy of continuous filtration as a protective measure have not yet been carried out.

In connexion with our experiments on air disinfection there are two points to which we would like to draw attention. In the first place our animal experiments have been performed with bacteria (the "Liverpool virus" and Danysz bacillus) which are essentially intestinal and not lung pathogens, and secondly our animal tolerance tests of the germicides we have examined have not included the crucial testing over long periods of time for any chronic effects of irritation on the respiratory passages and tissues. In the latter connexion it is more than likely that the upper respiratory passages would suffer earlier than the lining of the lung alveoli, and that similarly any beneficial therapeutic effects would also be more easily registered in the throat and trachea than in the lung. We may mention in support of this idea some experiments performed on mice with magnesium oxide smoke. It was found that the whole lung of mice exposed to inhalation of the smoke contained on an average only one-sixth the amount of magnesium collected from the larynx and trachea, the figures being: lung 0·000002 g., larynx and trachea 0·000012 g. of MgO. In control animals there was possibly a trace in the larynx and trachea, but less than 0·000001 g., while no trace was demonstrable in the lung. This smoke, of

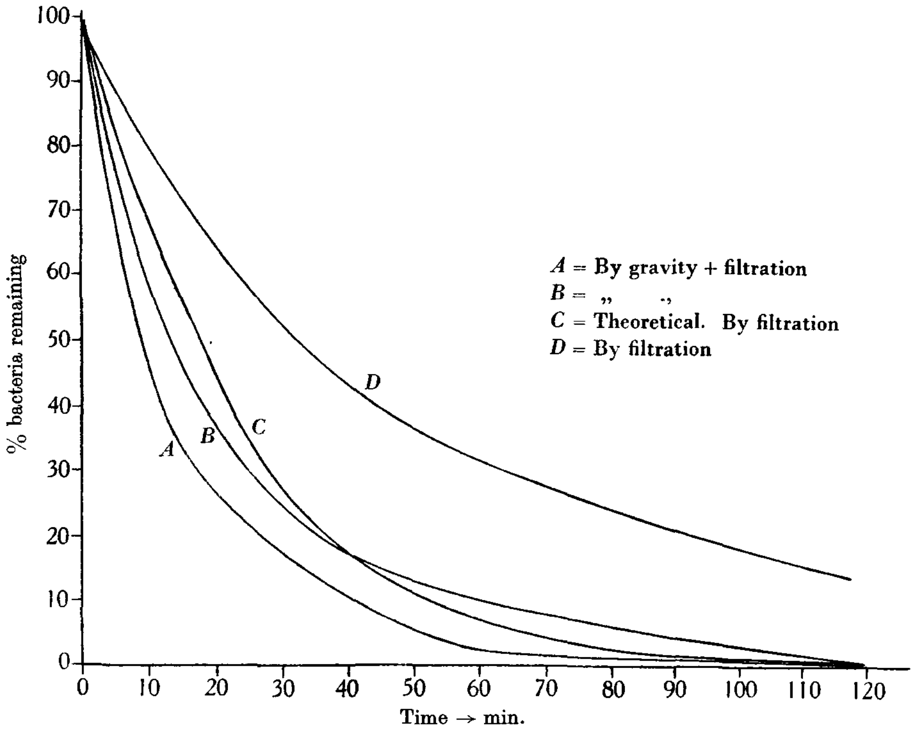

Fig. 5. Rate of removal of bacteria by filtration (air flow 20 l./min.).

an average particle size of 0·5 μ radius, being dry would, presumably, penetrate better than moist bacterial particles; on the other hand, some coagulation of the smoke soon occurred owing to its denseness on generation.

SUMMARY

A commercial preparation of sodium hypochlorite dispersed in the form of a mist has been tested for germicidal action on bacteria suspended in the air.

The relative merits of acid, neutral and alkaline solutions have been discussed.

A maximum of efficiency dependent on dilution has been shown to exist, and experiments with water and glycerol as diluents have been performed.

The value of hypochlorous acid gas and chlorine as aerial bactericides has been tested, and experimental evidence advanced to show that while these are to some extent effective as gases, the acid, at any rate, is more lethal in solution as a mist.

Further evidence of the importance in aerial disinfection of size and evaporation rate of mist particles has been obtained.

Objections have been raised to the proprietary use of the preparation on account of the liberation of chlorine, and its high salt content, but the necessity for the presence of a certain amount of salt has been shown.

Decrease in germicidal activity of the mists when required to sterilize atomized salivas has been indicated.

The active persistence of sodium hypochlorite mists has been shown to be short.

A few notes on the use of ultra-violet light and filtration methods for combating aerial infections have been added.

It was found that the greater part of a magnesium oxide smoke inhaled by mice was retained in the upper respiratory passages.

GENERAL CONCLUSIONS

The inference to be drawn from the investigations is that there are definite possibilities for the use of hypochlorite mists in combating aerial infections, but that the odour, irritant effects, opacity of the mist and corrosiveness on metals, etc. will limit the sphere of utility. The first three properties constitute objections against the use of continuous mists for controlling human air-borne disease.

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