FURTHER RESEARCHES ON BACTERICIDAL MISTS AND SMOKES

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In our original paper on germicidal aerosols (Twort, Baker, Finn & Powell, 1940) it was shown that the most satisfactory results were obtained with a solution of 10% hexyl resorcinol in propylene glycol together with a little alkali and wetting agent, the mixture being mechanically atomized into mist particles of appropriate size. Although it was confirmed by other workers that a high degree of lethal effectiveness on air-borne bacteria was achieved by the above procedure, objections were raised to its use in practice at the moment on the grounds that:

(1) The germicide base was difficult to obtain.

(2) The solvent was only manufactured in America, and not in bulk there.

(3) The mechanical apparatus advocated involved the use of metals now required for the making of war weapons.

We endeavoured to overcome these objections, and as a result of further research were led to suggest the use of balsamic smokes and the like (Twort & Baker, 1940) as a means of depleting the air of pathogenic bacteria. It was thought at one time that all forms of apparatus for generating the smokes could be dispensed with by smouldering candles, etc. of the crude substances we were using, but it was soon found that as regards balsams such smokes did not work. It appears that in the case of balsams and balsam-charcoal mixtures employed in candle form combustion of the active bodies occurs at the glowing tip of the candle, but when the mixes are heated on a hot-plate distillation of the active principles takes place, the charcoal in the mix not glowing till a later stage. On the other hand, such substances as cardboard and wood smouldered on a hot plate or as a strip provide active smokes, the activity presumably being due to compounds formed during partial combustion.

The knowledge that humidity of the air was playing a far greater part in influencing our results than we had originally thought likely (Baker & Twort, 1941) induced us to revert to the heating of pure phenolic substances, a procedure which, owing to the poorness of the 'kills' previously obtained (Twort *et al.* 1940, p. 319), due to conditions of low humidity, we had temporarily discarded as of little value. It was soon found that a hot plate was apparently as useful as a mechanical atomizer for making a mist when humidity conditions were satisfactory, and the former procedure for the time being became our choice for experimental purposes. The simplicity of the technique renders

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it very rapid and amenable to variation, but it is not suggested that under all circumstances it would be the method of choice in practice. Nor, of course, do we wish to imply that a final decision as regards experimental procedure has been reached. We know too little about the optimum particle size of either our mists or 'smokes' to be in a position to claim exceptional merits for heat as against mechanical generation of our bactericidal aerosols. More will be said about this question in later pages.

The general experimental technique employed will be found described in our previous papers. Briefly, the heat-volatilization method consists in placing a measured quantity of the phenol on an electric hot plate. Mechanically produced mists are formed in an 'Atmozon' type nebulizer with known jet size, and at a predetermined air pressure. We often now record our results somewhat differently. At the moment we are more concerned with the 'kill' obtained within the first 10 min. (actually from the 5th to the 8th minutes) of the experiment, and rarely make more than two further plate exposures, viz. at the 15th and 30th minutes. When, for purposes of comparison or brevity, it is wished to condense the results obtained with several different mist concentrations of a single germicide (i.e. of germicide base) the number of survivors compared with 100 on the control plates exposed at the three time periods is found. The mean percentage number of survivors in each mist concentration is then reduced to a single figure, estimated to be applicable to a mist concentration of 1 g. of base in 1000 $m.^3$ of air. The reduction is simple, and is based on our finding that, within limits, the number of bacteria surviving is, on an average, inversely proportional to the concentration of germicide-base in the air. Thus a mean of 5 % survivors when the germicide concentration is 1 in 5000 is equivalent to 0·33 % when the concentration is 1 in 300, equal to 1 % when the concentration is 1 in 1000 $m.^3$ As will be understood, mists of phenols of which the operative particles retain their bactericidal activity for a long time tend to give a better final figure when the concentration is low than when it is high, especially when rate of kill is slow, the reverse being the case where persistence is short and rate of kill rapid.

The following are some of the germicide bases and solvents recently utilized in our heat-volatilization tests:

Germicide bases

Resorcinol	Pentachlorphenol	Para-chlor-meta-cresol
Phenoresorcinol	Benzyl phenol	Cinnamic acid
Pyrocatechol	Benzyl cresol	Benzyl cinnamate
Hydroquinone	Amyl-meta-cresol	Phloroglucinol
Hexyl resorcinol	Para-chlor-meta-xylenol	Vanillin

Solvents

Water	Diethylene glycol	Glycerol
Methylated spirit	Butylene glycol	Pine oil
Propylene glycol	Carbitol	Benzyl alcohol
Ethylene glycol	Pinacol	Cyclohexanol

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Test organisms

The flora of normal saliva
Str. agalactiae
B. lactis aerogenes
C. xerosis
N. catarrhalis
M. phlei
M. tuberculosis piscium
A Gram-positive, saprophytic, white *Micrococcus* ('F' coccus)
A Gram-negative, saprophytic streptobacillus

For technical reasons the salivas were diluted with equal quantities of water, while the agar growths of the remaining organisms were either emulsified in broth, or in water plus sterile saliva. In half a dozen tests where the saliva was not diluted the number of test organisms able to survive the effect of the germicides was more than double that found when the saliva was diluted. The flora of the saliva, for obvious reasons superior for our purpose to broth emulsions of laboratory cultures, was used in our primary tests for ascertaining the merits of various substances which suggested themselves as likely to be of use as bactericidal aerosols. Unless the results of such tests proved to be moderately satisfactory, the substance under examination was, at least temporarily, discarded. In an ideal experiment an animal would be employed as donor and recipient of the micro-organism concerned, the use of man not being feasible, but in our laboratories we have had to be satisfied with a piece of apparatus as donor of, say, normal saliva, and a Petri dish or an animal as recipient.

Table 1. *The mean percentage number of survivors over the half-hour period of the salivary flora*

	1 g. of germicide base per m.³ of air		
Germicide	300	1000	5000
Benzyl phenol	0·19	0·69	10·59
Benzyl cresol	2·67	1·36	32·00
Pentachlorphenol	1·09	2·10	7·59
Resorcinol	0·21	2·20	26·90
Hexyl resorcinol	2·60	2·51	14·93
Phenoresorcinol	—	3·72	—
Cinnamic acid	5·55	6·36	22·00
Pyrocatechol	1·43	7·93	60·00
Hydroquinone	3·09	8·40	100·00
Para-chlor-meta-xylenol	21·00	45·00	55·00
Benzyl cinnamate	50·00	45·00	80·00
Vanillin	—	62·00	100·00
Para-chlor-meta-cresol	44·00	100·00	100·00
Phloroglucinol	46·00	100·00	100·00
Amyl-meta-cresol	50·00	100·00	100·00

In Table 1 is given the mean percentage number of survivors over the half-hour period, obtained when heat-volatilizing the phenols diluted in water and/or methylated spirit, in the presence of the flora of saliva A as test organism. The figures given are those of three different mist concentrations, viz. 1 g. of base in 300, 1000 and 5000 m.³ of air, the phenols being listed in

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order of potency of the middle concentration (see later). This is a small number of experimental results on which to assess bactericidal potency of a substance to air-borne organisms, but it should suffice to warrant the provisional discarding of some of the substances. Actually several other mist concentrations of some of the germicides were tested, the results of which confirmed, very broadly speaking, those given in the table.

The absence of correlation of Rideal-Walker values and our figures is here striking. For instance, amyl-meta-cresol with the highest Rideal-Walker value (250) compares very unfavourably as a bactericidal aerosol with most of the remaining phenols, while resorcinol with a Rideal-Walker value much below that of phenol itself is remarkably potent in mist form.

On these figures we rejected, for the time being, the last nine substances, as probably of relatively little use, although nos. 7–9 inclusive gave quite good results in the 1 in 300 mist concentration. On the other hand, nos. 1–3 inclusive we had eventually to exclude on account of their irritant effect on mucous membranes, and inhalation of mists of no. 6 was not altogether pleasant. This left us with resorcinol and hexyl resorcinol for further examination, which was carried out especially on various other test organisms, benzyl phenol and benzyl cresol serving as controls.

Table 2. *The mean percentage number of survivors over the half-hour period of broth emulsions of six bacteria*

Mist concentration of germicide base 1 g. per 1000 m.^{3} of air.

Test organism	Hexyl resorcinol	Benzyl phenol	Benzyl cresol	Resorcinol	Average
1. 'F' coccus	0·41	0·55	0·09	4·50	1·39
2. *Str. agalactiae*	0·19	3·33	3·33	63·18	17·51
3. *C. xerosis*	0·49	0·03	0·87	16·27	4·42
4. *N. catarrhalis*	0·37	3·73	3·27	6·97	3·59
5. *M. phlei*	1·26	4·25	5·38	10·66	5·39
6. *M. tub. piscium*	5·35	21·33	7·53	12·78	11·75
Average	1·35	5·54	3·41	19·06	—

In Table 2 is shown the mean percentage number of survivors of atomized broth emulsions of six different bacteria exposed to heat-generated mists of the four chosen germicides. Each set of figures represents the results obtained with three separate mist concentrations of germicide, reduced as already explained to a concentration of 1 g. of base in 1000 m.^{3} of air.

It will be seen that easily the best average figure is given by hexyl resorcinol, and easily the worst by resorcinol. Such results were, in a general sense, in conformity with previous findings when using broth emulsions of bacteria as test organisms. It was expected that among this group of organisms the acid-fast representatives would be the most difficult to kill. In the case of resorcinol, however, both *Str. agalactiae* and *C. xerosis* proved surprisingly resistant.

Enlightenment on this and other points was gained from the results of a further group of experiments in which equal parts of sterile saliva and water

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were substituted for broth in making up the bacterial emulsions. The comparative figures obtained are shown in Table 3.

Table 3. *The mean percentage number of survivors over the half-hour period of water-saliva emulsions of six bacteria*

Mist concentration of germicide base 1 g. per 1000 m.^{3} of air.

Test organism	Hexyl resorcinol	Benzyl phenol	Benzyl cresol	Resorcinol	Average
1. 'F' micrococcus	0·55	0·10	0·33	0·47	0·36
2. *Str. agalactiae*	5·80	2·92	2·88	4·52	4·03
3. *C. xerosis*	1·16	1·14	0·47	3·98	1·69
4. *N. catarrhalis*	1·15	0·02	1·27	1·14	0·90
5. *M. phlei*	6·86	7·08	4·17	26·91	11·26
6. *M. tub. piscium*	11·54	8·15	7·39	7·68	8·69
Average	4·51	3·24	2·75	7·45	—

Here all four germicides gave the poorest kills of the acid-fast bacilli. Confirmation is again obtained of the damping effect of saliva on the bactericidal potency of hexyl resorcinol, and the enhancing effect on the potency of resorcinol except in the case of the acid-fast bacilli, the differences being here within the experimental error. However, contrary to what was found when using broth emulsions, benzyl cresol gave the best average figure, followed by benzyl phenol. The reader may be reminded that in experiments of this nature there is likely to be a very great experimental error, and without a comprehensive knowledge of the results of kindred tests it may be difficult to evaluate correctly the significance of many of the differences shown. Some of our results may perhaps advantageously be examined in more detail.

While the lethal effectiveness of our mists to bacteria is conveniently assessed in the manner above described, in practice we would prefer, meanwhile, to aim at a time lag considerably shorter than that of 30 min. In Table 4 is shown the percentage number of survivors of our six test organisms from the 5th to the 8th minute of the experiment only, when the concentrations of germicide mist were 1 g. in (*a*) 300, (*b*) 1000 and (*c*) 5000 m.^{3} of air.

Table 4. *Percentage of survivors from 5th to 8th minute in experiments recorded in Table* 2

	Hexyl resorcinol			Benzyl phenol			Benzyl cresol			Resorcinol			Average		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
1	0	0·7	13	0·5	0·2	15	0	0·3	1·6	1·7	10	72	0·6	2·7	25
2	0	1·5	11	0	0	100	0	0	100	46	57	100	12	15	78
3	0	0·2	19	0	0	0·2	0	0	29	11	75	82	2·8	19	33
4	0	1·5	4	0	12	100	0	0	55	1·9	30	67	0·5	11	57
5	1	2·7	6·5	0	1·2	100	0	0	73	10	30	100	2·8	8·5	70
6	8·8	16	15	0	100	100	0·3	5	100	11	16	100	5	34	79
Av.	1·6	3·8	11	0·1	19	69	0·1	0·9	60	14	36	87	—	—	—

The resorcinol figures are easily the poorest. Hexyl resorcinol is outstanding where the experimental conditions are severe, i.e. the concentration of germicide mist is low (*c.* = 1 g. in 5000 m.^{3} of air), the time lag is relatively

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short, and the test organism (acid-fast bacilli) is relatively resistant. Where water-saliva was the emulsifying fluid for the bacteria the corresponding figures are seen to be quite different as shown in Table 5.

Table 5. *Percentage of survivors from* 5*th to* 8*th minute in experiments recorded in Table* 3

	Hexyl resorcinol			Benzyl phenol			Benzyl cresol			Resorcinol			Average		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
1	0·4	0·4	8	0	0·8	0·6	0·6	1	1	0	1	4·2	0·3	0·8	3·5
2	1·5	9·4	60	0·4	4·8	100	0·2	0·5	82	2·1	14	49	1·1	7·2	73
3	0·9	6·2	5	0·3	0·5	32	0·5	0·4	6·2	0	2·6	65	0·4	2·4	27
4	0	7·6	10	0	0	1	1·2	0·8	15	0	0	45	0·3	2·1	18
5	7·1	13	25	0·2	1	100	0·3	2·8	60	1·6	22	95	2·3	9·7	70
6	21	25	41	1·2	9·5	100	0·7	1·3	100	0·5	3·8	100	5·9	9·9	85
Av.	5·2	10	25	0·4	2·8	56	0·6	1·1	44	0·7	7·2	60	—	—	—

The resorcinol figures are a great improvement on those of the corresponding broth emulsion experiments, and those of hexyl resorcinol are worse. The average figures for four of the six test organisms are here also better, those belonging to the two acid-fast bacilli being mostly of the same order as before.

It was of interest to compare the survival rate when the germicide mist concentration was 1 g. in 300 m.^{3} of air, after a plate exposure lag of (*a*) 5 min., with the rate when (*x*) approximately one-third (1 in 1000) and (*y*) one-seventeenth (1 in 5000) the amount of mist was distributed in an equivalent volume of air, after plate exposure lags of 15 and 30 min. respectively; all plates, as usual, remaining uncovered for 3 min. The average figures are shown in Table 6, i.e. the average of each organism tested against the four germicides, and the average of each germicide tested against the six organisms.

Table 6. *Comparison of plate exposure lag and concentration of germicide*

	Broth			Saliva		
	a	*x*	*y*	*a*	*x*	*y*
1. 'F' *Micrococcus*	0·6	0	2·5	0·3	0·1	1·4
2. *Str. agalactiae*	12·0	0·4	75·0	1·1	1·3	42·0
3. *C. xerosis*	2·8	0	12·0	0·4	0·1	15·0
4. *N. catarrhalis*	0·5	0·4	13·0	0·3	1·1	3·0
5. *M. phlei*	2·8	8·6	68·0	2·3	1·7	45·0
6. *M. tub. piscium*	5·0	21·0	65·0	5·9	1·1	75·0
Hexyl resorcinol	1·6	0·2	0·3	5·1	1·4	0·3
Benzyl phenol	0·1	14·0	50·0	0·4	0·3	50·0
Benzyl cresol	0·1	0·2	52·0	0·6	0·4	36·0
Resorcinol	14·0	5·3	54·0	0·7	1·4	34·0

a, *x*, *y*, see text.

These figures show perhaps more plainly than any of the others the importance of time (columns *y*) for the action of hexyl resorcinol, and mist concentration for the action of the three remaining germicides. The exceedingly poor kill by resorcinol, at the 5 min. interval, of *Str. agalactiae* emulsified in

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broth (Table 4) makes the relevant figures somewhat exaggerated. It may be mentioned that when the mist concentration of the germicides was 1 g. in 1000 m.^{3} of air the plates exposed after a lag of 30 min. instead of 15 min. were almost always sterile.

From a general survey of the whole of our experimental results the indications are that the decreasing order of sensitivity of our test organisms to the four selected germicidal mists generated by heat volatilization is somewhat as follows: 'F' coccus, *N. catarrhalis*, *C. xerosis*, flora of saliva A, *Str. agalactiae*, *M. phlei* and *M. tuberculosis piscium*. Thus it may be that, by the disinfection procedures we have studied, the spread of cerebrospinal meningitis may prove to be more easily controlled than the spread of air-borne streptococcal infection and tuberculosis. It may be worth while pointing out that during our investigations we have found, on the whole, emulsions of *Str. agalactiae* in sterile watery saliva give results approximately parallel to those obtained when using saliva A. It is sometimes more convenient to use the former as test organism, and there is the advantage that variations in the flora and other constituents of the saliva of different individuals, and even in a single individual, likely to introduce fallacies, are avoided. The fact that the organisms have been grown on an artificial medium and not in their natural environment has, however, to be retained. Broth emulsions of *Str. agalactiae*, as will be seen from the tables, give, of course, no parallelism.

In all the experiments so far described in this paper care has been taken to ensure that the relative humidity of the air of our test chambers was high enough to allow the germicidal aerosol used to exert its maximum effect. Mostly the humidities have been within the limits of from 70 to 80 %, but such a range is not considered conducive to the comfort of the average individual, and, consequently, it was necessary to investigate the possibilities of there being a critical humidity range. We had previously found that, when atomized, resorcinol was more sensitive to changes in humidity than was hexyl resorcinol, and that broth emulsions were more sensitive than saliva emulsions or the flora of the saliva itself. It may be mentioned here that the presence of excess moisture apparently had no effect on the number of colonies on the control plates, either absolutely or relatively.

A broth emulsion of *C. xerosis* was chosen as test organism for the hot-plate experiments detailed in Table 7, group (*a*), and a 50/50 water-saliva emulsion for those in group (*b*). The figures represent the average result at three different mist concentrations, reduced to the standard concentration of 1 g. in 1000 m.^{3} of air. The series of experiments at the 40 and 50 humidity range were duplicated, the average results of the two series being shown.

Some of the figures are irregular, but from these and other experimental results many of which have been recorded in previous publications, it appears that the optimum percentage relative humidity is reached at about 60, no notable improvement in 'kill' being registered above this. On the other hand, in the neighbourhood of 50 % the efficiency of the germicide mist may fall off

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markedly, while below 30 % lethal effect of even relatively concentrated mists was nil or almost nil.

It is especially interesting to find that while an increase in moisture content of the air enhances the bactericidal potency of aerosols, according to Wells (1935) an increase of 25 grains of moisture per lb. of dry air may cause a loss of 90 % in bactericidal power of ultra-violet light, compared with its power when operating in an atmosphere with an absolute humidity below 50 grains per lb. These two methods of aerial disinfection will thus presumably not operate successfully in parallel, but in practice might be installed in series, to be used according to the humidity conditions prevailing in the particular atmosphere to be treated.

Table 7. *The effect of relative humidity* (R.H.). *Mean percentage number of survivors of* C. xerosis

Mist concentration of germicide base 1 g. per 1000 $m.^3$ of air

Approx. R.H.	Hexyl resorcinol	Benzyl phenol	Benzyl cresol	Resorcinol
		(a) *Broth emulsion*		
20	16·00	100	100	100
40	3·00	—	—	—
50	0·37	—	—	—
60	0·32	—	—	—
70	0·75	—	—	—
80	0·49	0·03	0·87	16·27
		(b) 50/50 water-saliva emulsion		
20	100	100	100	100
30	43	—	—	13·83
40	15·80	—	—	10·43
50	14·92	—	—	8·8
60	2·97	—	—	0·46
70	5·10	—	—	0·54
80	1·16	1·14	0·47	3·98

Having ascertained experimentally the possibilities regarding the utility of germicides for disinfection of air, it remained to examine how our laboratory procedures could be adapted to practice. The problem is not quite so simple as it might superficially appear, being complicated by the necessity of incorporating a second substance (usually a solvent) in the germicidal mixture. The ideal solvent should have characteristics similar to those which we consider essential for the ideal germicide base, except that it need not have necessarily a high lethal effectiveness to air-borne bacteria. There are, however, further features regarding the solvent which are of importance, either when atomization is mechanical or by heat. Hygroscopicity, miscibility with water, viscosity, boiling point etc. have to receive due consideration as they may affect the quantity of the germicide base in the mist being generated, as well as the quality after generation. We have previously drawn attention to the fallacies which may be introduced by hygroscopicity and distillation when mechanical atomizers are used (Twort *et al.* 1940, p. 295), while when heat volatilization is the procedure chosen, as a rule the nearer the boiling-point

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of the solvent is to that of the germicide base itself the better, unless a constant boiling mixture of the two can be obtained.

Our search for the most suitable solvents led us to the provisional conclusion that the neat solid or liquid phenols so far tested give results as good as are given by water or spirit solutions. Polyhydric alcohols suggested themselves as practical solvents, although the possibility of their having a damping effect on the bactericidal activity of the phenols was foreseen. Among these alcohols propylene glycol was previously selected as probably the most serviceable for making up solutions of hexyl resorcinol intended for mechanical atomization. This glycol is, however, at the moment not easily procurable, and because the humidity was low in many of our previous experiments we have instituted further researches in an endeavour to find a suitable substitute.

Table 8. *A comparison of solvents (hot plate). The mean percentage number of survivors over the half-hour period of the flora of the saliva*

Solvent	Base/solvent, W/V	Hexyl resorcinol	Benzyl phenol	Benzyl cresol	Resorcinol
Water	0·02–0·1	1·86	1·40	1·78	4·82
Methylated spirit	10	4·75	1·14	5·56	2·76
Propylene glycol	10	12·12	2·58	0·86	2·04
Propylene glycol	50	10·92	—	—	2·75
Ethylene glycol	50	3·32	—	—	3·38
Diethylene glycol	50	4·87	—	—	4·65
Butylene glycol	50	10·47	—	—	2·95
Carbitol	50	6·09	—	—	1·34
Pinacol	50	4·90	—	—	3·22
Cyclohexanol	50	2·40	—	—	3·62
Benzyl alcohol	50	4·12	10·91	4·96	6·12
Glycerol	50	1·76	2·48	3·82	5·09

The mixtures shown in Table 8 relate to heat-volatilized, mostly 50/50 W/V solutions of the phenols in the solvents, the idea being, except in the case of water, to use the minimum amount possible of the solvent compatible with germicidal efficiency of the phenolic base. Three different concentrations of mist were used with each solvent, and the mean percentage number of survivors of the salivary flora over the half-hour period, reduced as before to represent survivors at a concentration of 1 g. of base in 1000 $m.^3$ of air, compared with the number found when using water and methylated spirit as solvents. The number of survivors where the actual mist concentration was 1 in 1000 was of the same order as in the table, but the figures tended, on the whole, to be smaller.

It is curious to find that propylene glycol is apparently such a poor solvent for hexyl resorcinol, but not for the other three phenols when the mixtures are heat-volatilized, whereas it was this particular solvent which was selected as the best, tested specifically for making up mixtures of hexyl resorcinol to be mechanically atomized. On the other hand, water and glycerol gave the best results with hexyl resorcinol, and nearly the worst with resorcinol, the reverse having been found previously when the mixtures were mechanically atomized.

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The strengths of these solutions were, however, not always similar, and this may be a very important factor in the difference obtained, lethal effectiveness often not being directly proportional to the strength of solution used.

It is difficult to sum up exactly the value of these results, for when due allowance is made for experimental error, chance hardly seems to account for some of the differences observed. It would seem that, as a rule, the solvent plays a more specific part in mechanical atomization than it does in heat volatilization. Thus a good solvent in the former case may be a bad one in the latter, and in both methods of generating the mists a good solvent for one phenol may be relatively a bad one for another. How near these concepts were to the truth it remained for further experiments to show.

It was considered advisable in the first place to carry out another series of tests with the mechanical atomizer, as near as possible comparable to those involving heat volatilization. Preliminary tests were necessary to find out the size of jet and air pressure best suited to resorcinol mixtures, and the adjustments in both required when substituting hexyl resorcinol. The 'Atmozon'

Table 9. *The effect of size of jet and air pressure. The mean percentage number of survivors over the half-hour period of a water-saliva emulsion of* C. xerosis, *when propylene glycol was the germicide solvent*

Pressure	Jets	Hexyl resorcinol			Resorcinol		
lb./in.²	diam. in.	0·020	0·031	0·062	0·020	0·031	0·062
2–3		20·93	6·17	7·41	30·23	3·14	1·44
4·5		28·06	4·45	8·02	—	—	—
5·5		—	2·39	—	—	—	—
10		—	5·68	—	5·55	2·41	—
15		4·52	—	—	—	—	—
25		6·23	—	—	3·65	—	—

type of nebulizer was used, the diameter of air and liquid holes being strictly the same in each particular jet. A slightly larger liquid hole than air hole will materially alter the quantity and quality of the mist generated at a given pressure, and such a combination is probably never an advantage in practice. Propylene glycol was selected as solvent, and *C. xerosis*, emulsified in water-saliva, as test organism. The mean percentage number of survivors over the half-hour period, as the experimental technique varied, is shown in Table 9, the figures representing the average survivors in three to five different concentrations of germicide mist.

Seeing that, in our experience, a high-pressure and a small jet are factors leading to the production of small mist particles, the above results were, in the light of previous observations, in many ways unexpected. Probably the only information of value gained here is that a small jet in conjunction with a low air pressure is an unsatisfactory combination for use with the two phenolic mixtures under test. With the compressors available we could not go further in this direction, so we selected for use a jet diameter of 0·031 in. and an air pressure of 10 lb., and proceeded to examine mixtures containing other

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solvents. The results compared with those given by mixtures containing propylene glycol are shown in Table 10.

As far as hexyl resorcinol is concerned the results are in conformity with previous findings. Resorcinol had only been tested when dissolved in propylene glycol, and then solely as a 10 % solution, whereas in the present series all mixtures were made up with 25 % W/V of the germicide base. For technical reasons the 50 % W/V mixtures used in the hot-plate experiments could not be utilized so that a strict comparison with the results of mechanical atomization cannot be made. However, it seems clear that our solvents influenced the lethal effectiveness of the germicide base to a greater extent when the mixture was mechanically atomized than when it was heat-volatilized, so that a wider choice of solvents is likely to be found suitable for use in the latter procedure.

Under some circumstances it may be an advantage for the germicidal mixture to impart a certain degree of odour to the atmosphere, and there are, of course, many substances which suggest themselves for this purpose. Among aromatics we have tested we rather favour balsams, especially, perhaps, balsam of Peru. This substance imparts, according to all our test subjects, a pleasant odour to the atmosphere, tending to mask the slight pungency detectable when using some phenolic mists, and has the advantage of being emollient to the respiratory mucous membranes, thereby reducing the tendency to coughing, itself a factor in the dissemination of disease. The balsams, in addition, are, as we know, themselves useful as germicidal aerosols, more especially when employing heat, but here a disadvantage is that a non-volatile residue may cause a certain amount of inconvenience from the technical point of view.

Table 10. *Comparison of solvents. Mechanical atomization. Survivors of emulsions as in Table* 9

	Hexyl resorcinol	Resorcinol
Propylene glycol	5·68	2·41
Cyclohexanol	12·37	13·53
Glycerol	66·22	36·60
Ethylene glycol	6·74	5·56
Carbitol	13·77	2·28

We have recently carried out a series of experiments with mixtures of phenols and balsams, with and without the addition of charcoal and wood flour. The results of related previous experiments could not be relied upon because the conditions as to the relative humidity prevailing at the time they were performed were unknown, though probably low. Sometimes the mixes were made up into candles which were subsequently smouldered erect or inverted, or cardboard was soaked with the balsams, and then smouldered. At other times the mixes were heated on a hot plate or each ingredient heated separately. The present results confirmed that a hot plate was superior to a candle, but in some tests not much superior to smouldered cardboard soaked in

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the phenolic mix. While there was no evidence that the presence of the balsams, etc., enhanced the bactericidal effect of the phenols, there was, on the other hand, no definite evidence of interference.

INTERFERENCE

The question of the possible interference of smokes, especially tobacco smoke, with the activity of the phenolic aerosols has continued to occupy our attention. We had already found that there was a certain measure of interference with the activity of mechanically atomized propylene glycol solutions of hexyl resorcinol, and since that time have investigated the effect of several other types of smoke in addition to tobacco smoke. The smoke from smouldered cardboard seemed to interfere less than that from tobacco, while magnesium oxide and ammonium chloride 'smokes' had relatively little effect on the germicidal activity of the phenol.

The results of recent tests wherein four heat-volatilized phenols were used against the flora of the saliva proved to be difficult to evaluate. Here the mist concentrations of the phenols was 1 g. in 300, 1000 and 5000 $m.^3$ of air, that of the tobacco smoke being kept constant, 1 g. of tobacco being smouldered per 10 $m.^3$ of air. There was, apparently, a little interference, but only when the mist concentrations of the phenols were fairly high. As a matter of fact, at a concentration of 1 g. per 5000 $m.^3$ of air, in all four tests the 'kills' were better in the presence of smoke than in its absence, especially at the 30 min. period. Somewhat similar findings were met with in a parallel series of experiments in which 1 g. of balsam of Peru in 300 $m.^3$ of air was substituted for the tobacco smoke. The results were further reconfirmed by smouldering an incense-charcoal candle as source of secondary smoke. Experimental error or some other unknown factor may increase our difficulty in assessing the significance of these observations. In any case, presumably when two active aerosols are present the effect may be additive or subtractive, the resultant effect being dependent upon the ratio between the two substances, and on the total mist concentration.

A review of the whole range of our results relating to interference with, and stimulation of the reaction between germicide and bacterium particles enables us to gauge with more confidence than was previously possible the degree of influence of the factors concerned. Solvents may be useful for technical purposes, but their presence in heat-volatilized mixtures did nothing to enhance the bactericidal effect of the phenolic base, and appeared more likely to damp the reaction. Accessory mists and smokes appear as likely to do harm by interference as they are to do good by virtue of their inherent germicidal activity. Some smokes have, however, other virtues which may be useful. The presence of saliva in the bacterial particle has manifestly a damping effect on the activity of hexyl resorcinol and a stimulating effect on resorcinol. Water vapour has proved, in our experience, the sole general stimulator of the reaction, its effect being truly remarkable. Indeed, experiments have shown

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that when the relative humidity reaches a certain low limit a phenol which under other conditions may be actively germicidal may become almost inert towards the test organism. As an excess of water vapour will tend to lower electrical resistance and thereby aid neutralization of any charge which may happen to be possessed by the germicide and bacterial particles we may have here at least a partial explanation for the influence of humidity, if the two types of particle are of the same sign. Experiments with the ultra-microscope and in our test chambers are being devised in this connexion.

Hypochlorites

As a result of investigations on the use of hypochlorites for aerial disinfection we had come to the general conclusion that while such substances had definite possibilities in combating aerial infections, certain demerits would limit the sphere of utility (Baker, Finn & Twort, 1940, p. 581). Subsequently (Baker & Twort, 1941, p. 127), however, it was found that hypochlorites, like phenols, had a higher lethal effectiveness to air-borne bacteria when the relative humidity of the air was high than when it was low, but it is only quite recently that we have had an opportunity to examine satisfactorily the question of the real merits of hypochlorites.

We chose as starting material 'Chloros', a product of Imperial Chemical Industries, containing about 10% of sodium hypochlorite. This was kindly provided by Dr Bourdillon of the Medical Research Council laboratories. Two solutions containing 1 and 0·2% of hypochlorite were made up by adding 9 and 49 parts respectively of distilled water to 1 part of the original solution. The test organisms were those contained in normal saliva A, the prevailing percentage relative humidity being between 70 and 80. In the first series of experiments the hypochlorite solutions were mechanically atomized with the Aerograph 'paint brush', in the second with the Aerograph 'M.P. gun' and in the third with a cheap form of hand spray. Although unlikely to be of use the two solutions were, as further controls, also heated on the hot plate. The results are shown in Table 11.

Table 11. *The lethal effectiveness of sodium hypochlorite to the flora of normal saliva*

Mist concentration of germicide base 1 g. per 1000 m.^{3} of air.

Percentage of NaOCl in solution	Mean percentage number of survivors (half-hour)			
	Aerograph brush	Aerograph gun	Hand spray	Hot plate
1	1·85	13·67	17·96	42
0·2	2·75	—	17·24	65

Both dilutions of the hypochlorite seem to be of equal merit. It appears that under favourable experimental conditions sodium hypochlorite can, weight for weight, give as good a result as the best of the phenols. With both types of aerosols wastage has to be avoided by the choice of an atomizer

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which provides mists consisting of suitably sized operative particles. Apparently about seven-eighths of the mist generated by the hand sprayer takes no part in the disinfection process, even although it is to be presumed that hypochlorous acid readily diffuses into the air from the bulk of the solution having already fallen to the floor. It was difficult, however, with the hand sprayer to be accurate as to the quantity and quality of mist actually used, not only because of the large volume relative to the size of test chamber, emitted with each pump, but also owing to the personal factor in varying the pumping pressure. The results with the hot plate are more in conformity with expectations than were those of a series of experiments with the 'F' coccus and a different brand of hypochlorite solution, previously described. The larger amount of sodium chloride present in the original solutions ('Milton') may partly account for their greater lethal effectiveness when atomized as dilute solutions.

PERSISTENCE

The active life of germicidal aerosols is obviously of great importance, and we have previously recorded our observations on this point concerning mechanically atomized germicides. Recent tests have been made on similar lines with (*a*) heat produced mists of resorcinol and hexyl resorcinol, and (*b*) mechanically atomized sodium hypochlorite.

Table 12. *The active life of hexyl resorcinol, resorcinol and sodium hypochlorite mists. Percentage of survivors of* C. xerosis

Age of mist	Hexyl resorcinol			Resorcinol $(1000 \times 10^6)^{-1}$			Resorcinol $(100 \times 10^6)^{-1}$			Sodium hypochlorite		
min.	5	15	30	5	15	30	5	15	30	5	15	30
0	0·11	0	0	16	0·9	0	1·9	0	0	0	0	0
5	—	—	—	45	5·6	15	3·6	2·1	0	0·4	0	0
10	—	—	—	89	44	25	—	—	—	79	75	21
15	0·2	0	0	81	90	33	5·4	1·2	0·6	70	67	13
30	1·2	0	0	85	75	65	60	53	6·2	100	100	100
60	2·5	0	0	—	—	—	100	100	100	100	100	100
120	11·5	0	0	—	—	—	—	—	—	—	—	—
240	54	0·5	0	—	—	—	—	—	—	—	—	—

In Table 12 are given the results of tests in which the most suitable conditions were chosen for each germicide, thus: a mist concentration of 1 g. in 1000 m.^{3} of air for hexyl resorcinol and resorcinol; and 1 g. in 5 m.^{3} for 1% NaOCl ('Chloros'); against *C. xerosis* emulsified in broth for hexyl resorcinol and NaOCl, and the same organism in sterile, neat saliva for resorcinol. Subsequently, further tests were performed in which 1 g. of resorcinol in 100 m.^{3} of air was used. The mists were generated in the usual way, after the humidity of the air in the test chambers had been raised to between 60 and 70% R.H.; the test organisms being blown in after the intervals of mist ageing indicated in the table.

The survival rates of the organisms show the extremely good persistence of hexyl resorcinol, and the relatively poor persistence of resorcinol mists. As

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was to be expected the strong mist of the latter remained effective for longer than the weak one. These findings have an important bearing on the practical application of germicidal aerosols, in the following ways. When air changes are few, say two or three times an hour, hexyl resorcinol can be relied upon to continue its work, especially in view of the fact that the minimum effective mist concentration of this phenol is very low. Where air changes are rapid, however, this compound has little advantage over resorcinol. On the other hand, under any conditions of air change, it would be necessary continually to reinforce the concentration of resorcinol, due to the rapid decay of the particles by evaporation. This would tend to cause a building up of vapour, especially when air changes are few, and while such vapours would be germicidally ineffective they might affect the host. With frequent air changes this building up would not be of any appreciable dimensions.

The persistence of sodium hypochlorite as an effective mist is of the same order as that of resorcinol. The results with the 10 and 15 min. old mists appear to confirm our previous finding that HOCl gas exerts a slight lethal action (cf. percentage survivors after 30 min. contact) if given time for absorption. This action, however, disappears after an ageing period of about 30 min.

Discussion

Among the phenols tested hexyl resorcinol and resorcinol remain our choice for use in practice, in the presence of man. Benzyl phenol, benzyl cresol and pentachlorphenol are powerful bactericides of air-borne bacteria, but they compare unfavourably with the resorcinols, on the score of irritant effects on mucous membranes. The average individual lethal dose of these phenols for animals appears to be of the same order, but the important unknown quantity is the reaction of man and animals to their continuous inhalation, although there seems no reasonable grounds for thinking that they would prove in the slightest degree harmful in the concentrations one would contemplate using in practice. A concentration of 1 g. in 1000 or more m.^{3} of air should give a wide margin of safety, especially as probably only a fraction of the phenol inhaled would be retained and absorbed into the system. Only a very small portion of the ordinary therapeutic dose could possibly be available in the system, even of those inhaling the mists during the whole of the 24 hr. Continuous inhalation by man of air containing mists of the phenols discussed, in concentrations higher than 1 g. in 1000 m.^{3} of air should, we feel, not meanwhile be entertained.

If it is found that the arbitrary standard of 'kill' aimed at (95% within 10 min.) is unnecessarily severe, hexyl resorcinol should be preferable to resorcinol, while if practical tests show that the standard is not severe enough then something better than either of the phenols under consideration will have to be found. A water-soluble substance with a lower vapour pressure than that of resorcinol, and a reasonable test-tube phenol coefficient is of the kind visualized. The relatively transient life of resorcinol as an active mist particle,

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whether the mist be generated mechanically or by heat, means that the total kill by this phenol is not greatly improved by time, especially in low concentrations of mist. But although such a mist may have lost most of its activity within 5–10 min., the bacteria contacted during this time very speedily succumb. The benefits derived from the use of resorcinol should thus be most evident in situations where there is crowding. It will be appreciated that if either by design or chance the concentration of germicide in the air falls below a certain level it is then that such a phenol as hexyl resorcinol asserts its superiority over the less persistent mists of the other phenols tested.

During our investigations we might have expected, on occasions, to have encountered the phenomenon of a specificity of lethal effectiveness of individual germicides, but evidence of this has, so far, not been observed to our satisfaction. It might, of course, be a step forward could an aerosol be discovered which would act specifically against a certain type of pathogenic organism

When assessing the significance of the 'kill' obtained with our aerosols it is well to remember that collection of the bacteria from the air by the open Petri-dish method employed by us does not necessarily provide a representative sample of the bacteria in the air being examined. As gravity is solely relied upon, it is obvious that the plates exposed soon after introduction of the bacterial mist into the atmosphere will catch a relatively greater proportion of large particles. The large particles are more likely than the smaller ones to contain bacterial clumps, and are more likely to contain a greater surround of medium, as well as larger individual bacteria. All these factors should work, on an average, to the disadvantage of the germicide. On the other hand, particles which are small on generation are, by virtue of their more active Brownian motion, likely to contact quicker other particles, especially small ones, such as those of the germicide or of ordinary dust. While contact with dry dust may tend to aid survival of the bacterium, on the whole these last two factors probably work to the advantage of the germicide, although, perhaps, there is no record on our plates of what is happening.

Whether or no the colony counts of the plates exposed during the 5th to the 8th minutes of the experiments do justice to the germicidal aerosols under examination is a question which we are endeavouring to answer by further tests in which sufficient time will be allowed for the majority of the larger particles to settle out before introduction of the germicide mist, and exposure of the first plate. However, most of our conclusions regarding the relative merits of the different germicides, etc., are based on the average results given by the three plates exposed at intervals as the test proceeds, so that all but the smallest particles are probably taken into account. Although many of the very small particles may be floating about in the air long after exposure of our last plate, the bacteria forming part of such particles should, from all points of view, be very vulnerable, and few should be capable of causing infection in cases where our last plate is shown to be sterile.

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We are still in the dark regarding the mechanism whereby excess moisture in the air enhances the potency of bactericidal aerosols. Some of the possibilities discussed in a previous paper have recently been investigated experimentally, but without much success. The subject is worthy of study, and it would indeed be interesting if it turned out that one of the factors responsible for the increased spread of some epidemic diseases during certain seasons of the year was identical with that underlying increased potency of aerosols: a factor favouring survival of the micro-organism concerned in the active vegetative state.

Conclusions

1. Hexyl resorcinol and resorcinol were the two most suitable phenols tested as germicidal aerosols. Some others which were equally lethally effective to bacteria had certain prohibitive demerits.

2. A mist of hexyl resorcinol 'kills' more slowly, but will act in a weaker concentration and persists longer than does a mist of resorcinol. Therefore, where air changes are frequent and contacts are close resorcinol should presumably be the more useful in preventing cross-infection. Under reversed conditions hexyl resorcinol should prove the more useful.

3. Good results can be obtained by the use of either a hot plate or a suitable piece of mechanical apparatus for generating mists of both the above phenols.

4. A solution of sodium hypochlorite is a powerful germicide in aerosol form. The results obtained were disappointing when using a hand sprayer for generating the mist. The solution, as may be supposed, was useless for providing an efficient heat-volatilized aerosol.

5. Atomized emulsions of two acid-fast bacilli proved to be more resistant than *N. catarrhalis*, etc., to the phenolic germicidal aerosols tested, *Str. agalactiae* and *C. xerosis* occupying an intermediate position.

6. Attention to the choice of solvent is of more importance when the phenolic mixture is to be mechanically atomized than when it is to be subjected to heat. Except for technical reasons, their use is more likely to damp than enhance the reaction between germicide base and bacterium.

7. Interference with the action of the primary germicidal aerosol by accessory mists and smokes does not appear to be a matter of much consequence.

8. Bacteria suspended in water-saliva as compared with broth were rendered more sensitive to resorcinol, but the 'kill' by some other phenols was not much affected. Hexyl resorcinol was definitely adversely affected by the presence of saliva in the bacterial particle.

9. A water-saliva emulsion of *Str. agalactiae* had a sensitivity to the aerosols about equal to that of the flora of a normal saliva.

10. The lethal effectiveness to bacteria of germicidal aerosols appears to

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reach its peak at a percentage relative humidity of the atmosphere of approximately 60, no obvious improvement in 'kill' being observed above this.

11. At a percentage relative humidity of 50 the activity of the aerosol may decline markedly, while at about 30 and lower it may be nil or almost nil, even when using relatively strong mist concentrations of the germicide.

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