GERMICIDAL MISTS AND VAPOURS IN AIR DISINFECTION

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THE ACTIVE LIFE OF GERMICIDAL AEROSOLS

During the last year further investigations of germicidal mists, especially those produced by heat-volatilization of phenols, have been carried out. We are primarily interested in continuous disinfection of the air in the presence of man, and all our original experimental results appeared to show that a small quantity of the higher phenols in the mist form was more potent to bacteria than when in the vapour form. Thus, persistence as a mist long enough to ensure a duration of lethal effectiveness covering the time taken for at least one air turn-over was considered to be a desirable characteristic of a germicide to be used for our purpose.

In our previous publications we have, unfortunately, used the terms persistence, stability and durability in a rather loose and irregular manner when referring to characteristics of our mists. It may be an advantage to give some precision to our meaning of the above terms, and we propose to define them as follows. Persistence of a mist for a given time implies that, within this time, some, at least, of the constituents remain in the particulate state. Stability implies that the composition of the particles does not change, although their size may alter. Durability refers to active life as a germicide. Thus, a mist may persist for an almost indefinite period, excluding gravitational effects, etc., and may rapidly become stable, but its durability of lethal effectiveness to bacteria may be very short, generally due to evaporation of the active principle. An example of such a mist is a saline solution of 1 % sodium hypochlorite, in which after a short time only an inactive nucleus of NaCl remains. On the other hand, a glycol solution of hexyl resorcinol, while presumably not persisting so long and taking a little longer to become stable, nevertheless, has a much longer active life. There are, of course, mists which have a considerably shorter persistence, a transitory stability and a relatively short active life, an example being a watery solution of resorcinol, while mists of some of the glycols have little persistence, stability or durability.

The behaviour of the germicide as regards durability of lethal effectiveness is of particular importance, although it seems that to obtain a durable action one has to sacrifice speed of action, and in practice a compromise has to be made according to the conditions prevailing and the objective aimed at. As will be seen later, recent evidence points to the vapour state rather than to the mist state of the germicide as being the active one, which is contrary to the indications obtained from earlier experiments wherein direct contact between germicide and bacterial particles was visualized as playing the chief part in the process. This, however, does not alter the fact that in practice the higher phenols, at least, must be dispersed as a mist in order to obtain maximum disinfection effect of the air; and durability of lethal effectiveness can best be obtained for a reasonable time by using germicides with a relatively low vapour pressure, ensuring a slow decay of the operative particles. The reason for this is not quite clear at present, but it may be that it has some connexion with local saturation of the air immediately surrounding the germicide particle.

In making durability examinations our technique varied in three main ways: (1) The heat-volatilized germicide rapidly recondensed to a varying degree according to the substance under test, etc., and after the desired interval of time the bacteria are nebulized, and the plates of agar later exposed. At the termination of the test the air in the experimental chamber is swept out by the aid of a fan-blower, and the whole process repeated with another time interval between inoculation of germicide and bacterial mist. With this technique one has to be careful that there is no residual germicide left over from one test to another. (2) Here the first test, the shortest time interval, is carried out as before, but at its termination the chambers are kept closed. After a further period of time a second series of plates is inserted and a second amount of bacteria nebulized and so on, but there is no further addition of germicide. There is thus no question of building up of germicide, but there is a possibility of building up of bacteria in the air, especially, of course, in the control chamber. It is for this reason that when there is no airing of the chambers between tests there may be a tendency for the apparent number of bacteria surviving contact with the germicide mist gradually to become less. This may occur with a series of tests carried out within whatever time is necessary for gravity to deplete more or less wholly the air of the control chamber of the particular organism in suspension. (3) Here the technique is similar to that used in method 2, but the chambers are aired so that building up of bacteria in the control chamber as well as of germicide in the test chamber is avoided. It will be noted that the experimental conditions prevailing during the first test of each series are similar, only varying from the second test onwards. For the general technique employed in air disinfection experiments and the manner of recording results see our previous publications.

The results of the examination of several aerosols as regards durability of lethal effectiveness have been given elsewhere (Twort, Baker, Finn & Powell, 1940; Twort & Baker, 1942), some additional tests of the same kind being recorded here. In Table 1 is shown the percentage of survivors of atomized *C. xerosis* emulsified in sterile, neat saliva, after (*a*) 5–8, (*b*) 15–18 and (*c*) 30–33 min.

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contact with an aerosol concentration of the phenol of 1 mg./m.^{3} of air. Technique 1 was used, a fresh amount of germicide being volatilized for each age period, with the chambers aired between tests. It will be noted that the resorcinol figures are much superior to those previously recorded elsewhere. The present figures relate to the mean of four experiments carried out with resorcinol obtained from two sources, each individual experiment giving closely parallel results and, therefore, considered to be reliable. The durability of hexyl resorcinol far surpasses that of any of the other aerosols, confirming our previous findings with mists generated mechanically. There is probably not a great deal of difference in the durability of the remaining nine aerosols.

Table 1. *The durability of heat-volatilized phenolic germicides* 1 *mg./m.*^{3} *Percentage of survivors of* C. xerosis *emulsified in saliva*

	Age (min.)						
	0	5	10	15	30	60	120
Germicide		(*a*) 5–8 min. contact					
Resorcinol	6·5	21·0	35·0	50·0	56·0	—	—
Hydroquinone	14·0	57·0	71·0	57·0	86·0	—	—
Pyrogallol	34·0	—	—	55·0	100·0	100·0	—
Pentachlorphenol	11·0	—	—	59·0	47·0	100·0	—
Benzyl cresol	4·0	4·3	5·3	65·0	75·0	97·0	—
Benzyl phenol	1·5	4·2	10·0	45·0	62·0	70·0	—
Hexyl resorcinol	14·0	—	—	37·0	17·0	22·0	39·0
Eugenol carbinol	20·0	12·0	15·0	33·0	44·0	53·0	—
Salicylic acid	22·0	32·0	55·0	55·0	100·0	—	—
(Benzoic acid)	48·0	17·0	77·0	62·0	100·0	—	—
		(*b*) 15–18 min. contact					
Resorcinol	0·0	0·5	1·9	11·9	17·5	—	—
Hydroquinone	1·0	6·7	8·2	13·0	37·0	—	—
Pyrogallol	19·0	—	—	25·0	50·0	63·0	—
Pentachlorphenol	0·0	—	—	36·0	38·0	73·0	—
Benzyl cresol	1·0	0·9	0·9	23·0	56·0	92·0	—
Benzyl phenol	0·0	0·0	1·6	19·0	26·0	69·0	—
Hexyl resorcinol	4·0	—	—	2·8	2·0	0·7	4·3
Eugenol carbinol	1·1	0·0	1·8	6·4	9·8	43·0	—
Salicylic acid	5·9	35·0	27·0	44·0	85·0	—	—
(Benzoic acid)	44·0	5·0	60·0	61·0	100·0	—	—
		(*c*) 30–33 min. contact					
Resorcinol	0·0	0·1	0·9	2·6	7·0	—	—
Hydroquinone	0·0	2·5	1·1	2·6	12·0	—	—
Pyrogallol	3·5	—	—	3·0	12·0	58·0	—
Pentachlorphenol	0·0	—	—	3·7	11·0	77·0	—
Benzyl cresol	0·0	0·4	0·7	6·5	18·0	33·0	—
Benzyl phenol	0·0	0·0	2·2	4·5	6·8	32·0	—
Hexyl resorcinol	0·0	—	—	0·0	0·0	0·0	1·7
Eugenol carbinol	0·5	0·0	0·7	0·2	5·7	31·0	—
Salicylic acid	0·9	9·0	10·0	22·0	83·0	—	—
(Benzoic acid)	30·0	9·8	56·0	45·0	100·0	—	—

ATTEMPTS TO PROLONG THE ACTIVE LIFE

Our standard germicide solutions were made up in methylated spirit, the solution being diluted with water or spirit for convenience in measuring out small amounts for heating. An endeavour was made to increase the active life of germicidal aerosols by the addition of relatively non-volatile substances, but without much success. Solutions containing 50 % resorcinol were prepared in 2, 5, 7 and 10 % glycerol, 0·5 and 5 % sorbitol, 10 % glucose, 5 % transformer oil, and 10 % ethylene glycol and carbitol. None was appreciably superior to the ordinary spirit-water solution when heat-volatilized, and if the quantity of adjunct was further increased there was a noticeable damping in the rate of kill. Several different mist concentrations of resorcinol and some of the other phenols were tested in this way with up to 50 % glycerol, again without greatly improving durability except at the expense of rate of kill.

An analysis of sixty pairs of results regarding active life showed the ratio of superior kill to be 32 : 18 in favour of glycerol containing aerosols, ten results being similar. Differences, when present, were, however, usually small, and of doubtful significance.

In another series of experiments, broth instead of saliva was used as emulsifying agent for *C. xerosis*, and in others the flora of normal saliva constituted the test

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organism. In general, the trend of the results followed expectations, the more sensitive the test organism to a particular germicide the longer was evidence of lethal effectiveness forthcoming. Thus, presumably, contact between germicide and bacteria may take place without our being aware of the fact if the latter happens to be relatively resistant to the former. From these observations it was deduced that the germicide may be detrimentally affecting the organism long after insufficient of the former remains to prevent subsequent demonstration of growth on the agar plates. Whether or no this detrimental effect would suffice materially to interfere with cross-infection is a matter for future experience to show. It has been observed with certain substances, notably with those of high vapour pressure, that in the borderline concentrations between effective kills and no definite results the bacterial colonies are frequently very small compared with those on control plates, prolonged incubation up to 48 hr., however, tending to conceal this effect. This has been shown not to be due to germicide mist having fallen on the medium, even in relatively high concentrations, and demonstrates probable damage to the organisms while in contact with mists or vapours while still suspended in the air (see slit-sampler experiments later).

Table 2. *Building up of germicide. Fresh mist every* 15 *min. Percentage survivors of* C. xerosis *after* 5–8 *min. contact*

Age of 1st germicide mist min.	(*a*) Saliva		(*b*) Broth	
	Resorcinol 1 mg.	Hexyl resorcinol 1 mg.	Resorcinol 1 mg.	Hexyl resorcinol 0·2 mg.
(1) 10– 13	38·0	25·0	28·0	11·0
(2) 25– 28	20·0	16·0	26·0	3·2
(3) 40– 43	11·7	9·7	27·0	1·9
(4) 55– 58	10·8	9·2	29·0	1·5
(5) 70– 73	9·5	9·4	34·0	1·6
(6) 85– 88	6·0	7·2	30·0	0·7
(7) 100–103	6·0	6·1	26·0	0·4
(8) 115–118	5·7	6·3	27·0	0·7
(9) 130–133	5·9	4·2	20·0	0·2
Mean	12·6	10·3	27·4	2·4
(9*a*) 140–143	0·0	0·1	0·9	0·0
(9*b*) 155–158	0·0	0·0	0·0	0·0

BUILDING UP OF GERMICIDE

The experiments embracing this aspect of our problem consisted in atomizing 1 mg. of germicide per m.^{3} of the test chamber every 15 min., and 5 min. later inoculating the air with the test organism. Agar plates were exposed 5 min. later, for 3 min., i.e. when each additional germicide mist was 10–13 min. old and had been in contact with the bacteria for 5–8 min. At the termination of the experiment two further plates were exposed at 15–18 and 30–33 min. to cover our usual time periods. It was a crude attempt to simulate continuous infection plus continuous disinfection, such as would operate in practice.

Nine consecutive doses of germicide and bacteria were utilized, emulsions of *C. xerosis* in neat saliva serving as test organism in the (*a*) experiments, and emulsified in broth in the (*b*) experiments, the results of both being shown in Table 2. The figures represent the mean of two complete sets of experiments. Owing to the high lethal effectiveness of hexyl resorcinol to broth emulsions of this bacterium the amount of germicide volatilized was here reduced to 0·2 mg./m.^{3} The colony counts on the control plates had mostly doubled by the time the third injection of bacteria had been made into the chamber, but henceforth there was little increase, coagulation, falling out by gravity, etc., eventually compensating for each fresh addition.

The cumulative effect is not so marked as might at first have been expected. In the case of resorcinol persistence of the mist is too short and, as will be seen later, when the amount used is not large vapours of this phenol in the absence of mist do not give much evidence of kill on the 5 min. plate. As regards hexyl resorcinol, although the mist may persist we know that, contrary to many other germicidal aerosols, the number of bacteria surviving is not usually inversely proportional to the mist concentration unless it is lower than 1 mg./m.^{3} of air, and, therefore, evidence of an increased effect due to building up might not be demonstrable. Vapour concentration may in all cases have been practically at a maximum. Once again is seen the striking difference in sensitivity of the test organism to these phenols according to the agent in which the organism is emulsified. There is little difference in the mean percentage number of survivors when saliva is the vehicle, but something like a fiftyfold difference when broth is utilized. We have, meanwhile, been unable to find a satisfactory explanation for this phenomenon, it occurring with all other organisms so far subjected to test.

RESIDUAL GERMICIDE IN TEST CHAMBERS

Provided the concentration of germicidal mist being used is not high and the experimental chambers are well aired with the help of a fan-blower between each test, the amount of residual germicide left on the floor and

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walls from one test is not sufficient materially to affect the results of subsequent tests. When using our customary concentration of 1 mg. of phenol per m.^{3} of air the interior of the chamber is hosed down from time to time with water, perhaps every month, and no trouble is experienced due to residues. If the experiments entailed the use of 10 times this concentration of substances with relatively low vapour pressures the chambers had to be washed out between each test, while with a concentration of say 100 mg. per m.^{3} of air a bactericidal effect might be demonstrable for days after completion of the experiment, and the most thorough hosing with water had to be resorted to in order to remove the last trace of germicide.

In Table 3 is given the percentage of survivors of the test organism after 5, 15 and 30 min. contact, when the germicidal mist concentration was 10 mg. and when it was 100 mg./m.^{3} of air, fresh bacterial mist only (technique 2) being added at intervals to find out duration of lethal effectiveness of the strong concentrations of germicide. By comparison, if instead of keeping the experimental chambers closed during the whole series of tests, they are aired between each test and a fresh 100 mg. of germicide volatilized to deal with each fresh bacterial mist (technique 1) the percentage of survivors was found to be definitely less. It may be mentioned that in clearing the chambers the fan-blower is often used for 5–15 min., and then the chamber is sealed up or left open according to desire. This fan blows approximately 3 m.^{3}/min. through the chambers, taking 2 min. for one air turn-over. When utilizing technique 1, 5 days after inoculation of the test chamber with 100 mg./m.^{3} of resorcinol very few *C. xerosis*, emulsified in neat saliva, were able to survive for much longer than half an hour, while within 3 days there was only an occasional survivor after being 15 min. in the air of the chamber. When dealing with a single dose of the germicide, with consequently very little possibility of residue being present, we have seen in Table 3 that durability was much more transitory.

Table 3. *Percentage of survivors of* C. xerosis *emulsified in saliva to a mist of* (*a*) 10 *mg. and* (*b*) 100 *mg. of germicide base per m.^{3} of air*

Age of mist	Resorcinol (min.)			Hexyl resorcinol (min.)			Benzyl phenol (min.)		
hr.	5	15	30	5	15	30	5	15	30
				(*a*)					
0	0·8	0	0	2·3	0	0	0	0	0
0·5	4·2	0	0	2·5	0	0	0	0	0
1	79	34	1·8	3·5	0·4	0	2·8	0·5	0
2	73	95	33	6·2	0·4	0	35	17	1·7
4	—	—	—	8·2	2·2	0	45	45	17
6	—	—	—	17	1·1	0	90	60	31
24	—	—	—	59	50	46	100	100	100
				(*b*)					
0	0	0	0	—	—	—	0	0	0
0·5	1·2	0	0	—	—	—	0	0	0
1	34	1·4	0	4	0	0	0	0	0
2	100	46	4	2·5	0	0	0	0	0
4	—	—	—	1·1	0	0	0·4	0	0
6	—	—	—	0·9	0	0	0·4	0	0
24	68	97	30	30	0	0	100	68	75
120	—	—	—	65	59	48	—	—	—

Although in the experiments being discussed evidence of kills was still being obtained it was decided at the end of the fifth day thoroughly to wash out the chambers with water, and to leave them airing overnight. Despite this cleansing process there still appeared to be sufficient germicide hanging about to cause death of an occasional test organism, judged by the slightly fewer number of colonies on our test plates compared with the control plates. It is usual for the air of the control chamber to be seeded first, and it was deemed advisable to alternate the seeding of the chambers to eliminate a possible source of error. This was done on the following day, with consequently a further 24 hr. for airing. The test chamber was found then to be seemingly quite clear of the last trace of germicide.

It appeared probable from these results that vapours rather than mists might be at work. In three of the four last experiments, after the use of the blower there was a 5–15 min. wait before atomizing the test organism. This gave time for the germicide to evaporate anew from the floor and walls. Although, provided the solid or liquid germicide is present on the surrounds, the atmosphere must, to some extent at least, become impregnated with vapours, it was, nevertheless, not anticipated that such a marked kill would be obtained so long after all the original mist had presumably been swept from the air. We did not expect vapours of the phenols we were using to be so bactericidal as their vapour pressures were not high, and we could see no reason for supersaturation followed by condensation on the atomized bacteria (see later). Airing of the experimental chambers seemed to have little effect, and therefore the possibility of the air of the room in which the chambers are erected being saturated with vapour had to be visualized, but on further consideration and calculation it was decided that this factor was negligible.

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EVAPORATION AT ROOM TEMPERATURES

The direct application of the agent as a paint on ordinary surfaces followed as a natural sequence to our experiments in which the germicide was presumably evaporating into the air from mist deposits on the surround of the chamber. It may be an advantage to repeat here the approximate dimensions of the experimental chambers: diameter 1·45 m.; height 1·83 m., area of floor 1·65 m.^{2}; total area of surround 11·64 m.^{2} and capacity 3·03 m.^{3} Some evaporation tests indicated that the rate of evaporation of resorcinol from the floor was of the order of 3 mg./m.^{2}/hr. if saturation of the contained air is not reached. The temperature of the chambers was thermostatically controlled at 20° C. and the relative humidity between 65 and 70 % (see later).

In a tentative experiment 30 ml. of a 10 % watery solution of resorcinol, equivalent to 1000 mg. of the phenol per m.^{3} of space, was painted on the floor (0·55 m.^{2}/m.^{3}), the chamber being closed while evaporation of the water was taking place. As condensation of the water on the observation windows occurred when the relative humidity of the outside air was too high to allow of total accommodation of the 30 ml. of water used in the paint it was sometimes necessary to ventilate the chambers for a short time until the film of moisture on the windows had disappeared, the chambers being then closed. 6, 8 and 24 hr. after painting the floor an emulsion of *C. xerosis* in saliva was sprayed into the chambers. Our findings were not unexpected in view of the results obtained with germicides volatilized from the hot plate. In the last (24 hr.) test the 30 min. plate was sterile while only 5 % of the normal number of colonies was present on the 15 min. plate. The two earlier tests gave practically similar results, but in an experiment with benzyl cresol there appeared to be a definite fall away in effect 24 hr. after painting.

In seeking to verify that the kill was accomplished by the vapours of the phenols it occurred to us that possibly the lowering of temperature of the bacterial particle by the mechanical process of nebulizing and the rapid evaporation of water might be sufficient to cause condensation of the germicide on the particle. In order to investigate this question it was necessary to test the effect of the germicide after any initial cooling of the bacterial particle had been neutralized by the surrounding atmosphere.

A 10 % solution of resorcinol was painted on the floor in an amount, as before, equivalent to 1000 mg. of the germicide per m.^{3} of chamber capacity, and an emulsion of *C. xerosis* in saliva, containing double the usual number of organisms, was at once atomized into the test and control chambers, the floor of the latter being painted with pure spirit. It was found that there was no bactericidal effect until a lapse of half an hour had been given for the phenol to evaporate, three-quarters of an hour being required in order to obtain a really clear-cut picture. Further, seeing that it is presumably only a matter of seconds before a temperature equilibrium between bacterial particle and surrounding medium is established, germicide condensation on the cooled bacterium was apparently not an essential factor in the lethal process. In other words, it is to be assumed that first stage, and probably also second stage evaporation of water, etc., from the bacterial particle has taken place long before sufficient of the phenol to be lethal to the bacteria has gained access to the atmosphere of the chamber when starting from zero time after painting.

Having obtained some data as regards the action of resorcinol it was now decided to carry out similar experiments with several of the other phenols with the bactericidal activity of which we had familiarized ourselves, as well as with some of our solvents. We hoped by this means to gain more information regarding the relation of vapour pressure, etc., to lethal effectiveness on air-borne bacteria. As before, the floor of the chamber was painted with a methylated spirit solution of the germicides in an amount sufficient to give 1000 mg. of the base per m.^{3} of air space. An emulsion of *C. xerosis* in sterile saliva again served as the test organism.

The results of this series of tests are shown in Table 4. A discussion of them will be left over for a moment, but it may be remarked in passing that, naturally, if a certain amount of time elapses between application of the 'paint' and exposure of the test organism their survival in the air is curtailed. For instance, the percentage of survivors to the propylene glycol 24 hr. after application was, after 5 and 15 min. exposure of the test organism, only 15 and 0·3 respectively.

Some of the paints were tested for durability of activity. Without treating the floor further with germicide, the next and following days a fresh inoculum of bacterial mist was atomized into the chamber, the original one having, of course, during the preceding 24 hr. fallen out. When the chambers were kept closed durability was good, and as will be seen from the results given by resorcinol and hexyl resorcinol in Table 4A, a kill was still being obtained when the experiment was terminated. Actually there was no obvious falling off in effect, which indicated incidentally that leakage from the chamber was not great. However, later experiments of a similar nature, with the ports of the chamber left open, gave equally good kills over many days.

Scrutiny of our results led us to suspect that a variable factor which we had not located was operating, and our suspicions were easily verified. The trouble was found to be due to the arrangement of the tubular heaters of the room, they being so disposed that the heat radiating from those nearest to the chambers passed directly under the floor of the chambers thereby raising the temperature of the floor to a maximum of sometimes 8° C. above that of the walls and the contained air. Thus, during the summer when the heaters were usually not operating all was well, but during the colder days there might be an appreciable variation in the temperature of the floor so that the amount of germicide which evaporated in a given time from our paints was not constant.

The fault was rectified so that the temperature of the floor remained constant at about 20° C. in all weathers. We wished to ascertain how far the paints could be diluted before evidence of a failing in effect was observed, in the first place utilizing 100 instead of 1000 mg. of germicide base per m.^{3} of air space. Two tests with resorcinol and one with pyrocatechol were carried out,

the 5, 15, 30, 45 and 60 min. plates giving the following respective percentage of survivors:

Resorcinol (1) 83·0, 14·3, 13·3, 0 and 0.
Resorcinol (2) 83·0, 100·0, 3·9, 0 and 0.
Pyrocatechol 92·0, 34·0, 4·0, 0 and 0.

The resorcinol figures are seen to be better than those shown in Table 4, while those appertaining to pyrocatechol are very much worse.

An endeavour was made to increase the rate of kill by speeding up the evaporation of the 100 mg./m.^{3} of the germicide with the aid of-gentle fanning. The results of these experiments are illustrated in Table 5, and it will be seen that the figures of both resorcinol and pyrocatechol are much improved. Granted that the data shown in Table 4 may be unreliable, it is, nevertheless, worth while comparing them with those in Table 5. Those referring to ordinary phenol are relatively very poor, but were considerably improved by utilizing double the amount of germicide base in a subsequent test. The figures for benzyl cresol are also worse, while those of hexyl resorcinol, resorcinol, hydroquinone and eugenol carbinol are definitely better than the previous ones for the 1000 mg. paint.

A 10 mg./m.^{3} paint of ten of the germicides was next tested, and in all cases the kill was markedly delayed, despite the use of a fan.

Table 4. *The effect of phenolic and glycol 'paints'* (1000 *mg./0·55 m.^{2}/m.^{3}*) *on air-borne* C. xerosis *emulsified in saliva*

'Paint'	Approx. b.p.	P.C.	Percentage of survivors (min.) 5	15	30	45	60
Thymol	232	30	22·0	5·6	0	0	0
Chlor-thymol	—	—	4·7	0	0	0	0
Phenol	181	1	0·7	0	0	0	0
p-Chlor-*m*-cresol	230	35	3·3	0·1	0	0	0
Pyrocatechol	245	—	0·6	0	0	0	0
p-Chlor-*m*-xylenol	255	65	53·0	9·6	0	0	0
Amyl-*m*-cresol	265	250	62·0	4·5	0·4	0	0
Resorcinol	276	0·3	100	92·0	29·0	3·2	2·3
Benzyl cresol	320	105	47·0	15·0	0	0	0
Benzyl cresol, low fraction	—	—	45·0	20·0	0	0	0
Benzyl cresol, high fraction	—	—	88·0	50·0	9·0	10·0	4·3
Hydroquinone	285	—	75·0	46·0	13·2	11·7	0
Orcinol	288	0·5	18·0	0·5	0	0	0
Pyrogallol	293/730 mm.	—	41·0	1·0	0	0	0
Benzyl phenol	321	62	100	37·0	1·6	0	0
Hexyl resorcinol	330	50	100	100	78·0	36·0	4·7
Eugenol carbinol	Dec.	—	75·0	51·0	44·0	30·0	6·2
Phloroglucinol	Dec.	0·3	35·0	6·0	2·8	2·1	3·0
Cellosolve	135	—	53·0	19·0	4·0	1·2	0
Ethylene glycol	197	—	81·0	47·0	3·0	0	0
Carbitol	202	—	78·0	2·4	0	0	0
Propylene glycol	216	—	100	14·0	0	0	0
Diethylene glycol	244	—	43·0	7·8	4·6	4·7	0

Temperature of painted surface 20–28° C. No fanning. P.C. = phenol coefficient

Table 4A. *The effect of aged resorcinol 'paints'* (1000 *mg./0·55 m.^{2}/m.^{3}*) *on air-borne* C. xerosis *emulsified in saliva*

Age days	Min. contact 5	15	30	45	60	90
			Resorcinol			
0	100	92·0	29·0	3·2	2·3	0
1	60·0	5·6	1·0	—	—	—
2	56·0	12·5	0	—	—	—
3	100	15·0	0	—	—	—
6	44·0	1·2	0	—	—	—
8	56·0	6·2	0	—	—	—
			Hexyl resorcinol			
0	100	100	78·0	36·0	4·7	0
1	50·0	36·0	0·9	—	—	—
2	73·0	57·0	1·9	—	—	—
5	55·0	67·0	6·2	—	—	—

Temperature of painted surface 20–28° C. No fanning.

ACTIVE LIFE OF 'PAINTS' WHEN CHAMBERS WERE OPEN

In the surface-evaporation experiments so far carried out the chambers had mostly been kept closed. It was now decided to find out how long the 'paints' would continue to act with the ports of the chambers left permanently open, except during the actual test in the presence of organisms. Evaporation into a confined space should, of course, be slower and durability of lethal effectiveness longer than when there is free access

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of the vapours to the outside air. Either condition might prevail in practice, although more often the latter than the former. This series of experiments served also for the titration of the germicide for the minimum amount noted that the 1000 mg. on the whole appeared to increase slightly in activity as time went on, and was at least as efficacious on the 14th day as it was on the first. It will also be noted that initially the 10 mg. killed

Table 5. *The effect of bactericidal 'paints'* (100 *mg./0·55m.²/m.³*) *on an aerosol of* C. xerosis *emulsified in saliva*

Agent	Percentage of survivors (min.)					
	5	10	15	30	45	60
Phenol	50·0	30·0	11·4	2·2	—	—
p-Chlor-*m*-cresol	7·2	3·0	2·6	1·2	—	—
Pyrocatechol	1·3	0·3	0	0	0	0
p-Chlor-*m*-xylenol	45·0	21·0	13·6	2·6	0	0
Amyl-*m*-cresol	68·0	6·3	2·2	1·0	0	0
Resorcinol	30·0	0·5	0	0	0	0
Benzyl cresol	48·0	40·0	37·0	15·0	7·4	0
Benzyl cresol, low fraction	48·0	50·0	33·0	8·6	3·8	0
Benzyl cresol, high fraction	100·0	100·0	84·0	49·0	29·0	12·0
Hydroquinone	39·0	4·2	1·0	0	0	0
Orcinol	26·0	0·9	0	0	0	0
Pyrogallol	66·0	10·0	4·8	0	0	0
Benzyl phenol	100·0	80·0	28·0	2·5	0	0
Hexyl resorcinol	58·0	28·0	3·0	0	0	0
Eugenol carbinol	57·0	30·0	30·0	4·4	—	0

Temperature of floor = 20° C. Slight fanning.

Table 6. *Titration of resorcinol 'paints'. Ports of chamber open*

Amount mg.	Age days	Percentage of survivors (min.)						
		5	15	30	45	60	90	120
1000	0	100·0	92·0	29·0	3·2	2·3	0	0
	1	56·0	16·0	0	—	—	—	—
	2	79·0	35·0	0	—	—	—	—
	3	51·0	40·0	0	—	—	—	—
	6	55·0	1·0	0	—	—	—	—
	8	42·0	2·2	0	—	—	—	—
	10	25·0	1·5	0	—	—	—	—
	14	18·0	1·2	0	—	—	—	—
100	0	100·0	100·0	1·9	0	0	—	—
	1	57·0	3·7	0	0	0	—	—
	5	55·0	4·1	2·4	0	0	—	—
	6	48·0	12·5	1·6	0	0	—	—
	7	68·0	52·0	11·0	6·6	0·1	—	—
10	0	90·0	10·0	0	0	0	—	—
	1	83·0	60·0	27·0	16·0	9·5	—	—
1	0	63·0	60·0	21·0	24·0	9·0	—	—
	1	100·0	100·0	80·0	70·0	18·0	—	—
0·1	0	94·0	55·0	28·0	5·3	2·0	—	—
	1	73·0	58·0	80·0	84·0	92·0	—	—

Temperature of floor 20–28° C.

which would give evidence of killing after different contact times.

The amount of germicide painted varied from 0·1 to 1000 mg. per m.³ of chamber space, the area painted being again 0·55 m.²/m.³, and the test organism again *C. xerosis* emulsified in neat, sterile saliva. The results given by resorcinol are shown in Table 6. It will be more quickly than did the 100 mg., and the latter more quickly than did the 1000 mg. Actually, the 10 mg. figures are the mean of two tests, the repeat test confirming the results of the original one. Because there may have been a variation in floor temperature while this series of tests was being carried out much reliance cannot be placed on the results. However, it can at least

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be said that even with the ports of the chamber kept open for a fortnight after treatment of the floor with 1000 mg. of the germicide, the air of the chambers on closing the ports becomes unsuitable for survival of the test organism.

THE BUFFERING OF MISTS BY VAPOURS

It is obvious that the presence of vapours of a germicide in an atmosphere should enhance the persistence of mist particles of the same germicide. In order to investigate this point, which, incidentally, is of some practical importance, the chambers were not washed out at the termination (14th day) of the first experiment cited in Table 6, but instead the residual vapours were supplemented by heat-volatilization of a small quantity of the germicide. The difference of durability of lethal effectiveness of the mist on a salivary emulsion of *C. xerosis*, in the presence and absence of the floor paint, is shown in Table 7, the mist consisting of 1 mg. of resorcinol per m.^{3} of chamber space. The results of a later experiment where the floor paint was 100 mg./m.^{3} are also shown. The presence of vapours increases lethal effectiveness of the mist. When the mist is 15–30 min. old it has no demonstrable effect, the mist having by this time presumably mostly passed to the vapour state, the air not being continuously fully saturated.

Table 7. *The lethal effectiveness of resorcinol mists in the presence and absence of vapours*

	Percentage of survivors		
	1000 mg. paint (min.)		
Age min.	5	15	30
0	0·6	0	0
5	2·3	0	0
10	8·6	0·4	0
15	12·5	1·7	0
30	34·0	1·2	0
60	38·0	3·8	0
Vap. only	18·0	1·2	0
	100 mg. paint (min.)		
	5	15	30
0	2·0	0	0
5	11·9	0	0
10	21·0	0·8	0
15	38·0	0·7	0
30	27·0	0·9	0
60	85·0	6·0	0
Vap. only	53·0	3·9	0
	No paint (min.)		
	5	15	30
0	6·5	0	0
5	21·0	0·5	0·1
10	35·0	1·9	0·9
15	50·0	12·0	2·6
30	56·0	18·0	7·0
60	—	—	—
Vap. only	—	—	—

TESTS IN OTHER ROOMS, ETC.

These experiments were dictated by the desirability of ascertaining primarily whether or no the results obtainable in ordinary rooms were comparable to those recorded when using our experimental, lead-lined, twin chambers. If bactericidal activity of the germicide depends upon its being in the vapour phase, then the question of the formation of a monomolecular film and adsorption would assume additional importance.

The first tests were carried out in our animal room where resorcinol was being volatilized from a hot plate on one side of the central partition, at the rate of about 100 mg./m.^{3}/hr. A broth emulsion of the '*F*' coccus, a saprophytic, gram-positive, white *Micrococcus*, was used as test organism. It may be well to recall that in all tests we have carried out with resorcinol aerosols they proved to be less potent to bacteria emulsified in broth than when emulsified in saliva. The number of organisms collected on control and test plates is shown in Table 8,

Table 8. *Number of survivors ('F' coccus) to a resorcinol aerosol, in animal rooms*

	(*a*)		(*b*)	
Min.	Control	Test	Control	Test
2– 4	370	480	610	130
5– 8	320	285	50	0
15–18	185	145	5	0
30–33	60	11	12	0
	(*c*)		(*d*)	
	Control	Test	Control	Test
2– 4	670	100	815	625
5– 8	160	60	720	415
15–18	0	0	240	10
30–33	1	0	160	0

series (*a*) before volatilization, (*b*) 2 hr. after starting to volatilize, (*c*) 4 hr. after, and (*d*) 2 hr. after the hot plate had been switched off, a fresh mist of organisms being, of course, utilized in each series.

It is evident from these figures, as well as from others obtained from similar experiments, that there is some leakage. This was to be expected because the partition between the two rooms was not gas-tight, but whether there is also passage of the resorcinol vapours through the partitioning materials themselves (wood, glass and fibre board) is a question which remains to be investigated. The difference in the number of organisms collected in the two rooms does not give a true picture of the efficacy of the resorcinol because there is at all times evidently a certain amount of the germicide present in the air of the control room. It, however, seems that the germicide does not operate so effectively in some rooms as in others. In two ordinary rooms with painted walls and distempered ceilings we were unable to get such striking kills as those obtained in our lead-lined chambers. The difference was more marked with the higher phenols than with substances such as ordinary phenol and propylene glycol, of substantially higher vapour pressure and used in much greater amounts. Altogether some fifty experiments of a variable nature

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were carried out in this connexion, involving mechanical and heat generation of the germicidal aerosol.

There was not available a second room to serve as a suitable control for the test rooms referred to above, and in order, if possible, to obtain more precise data as to the effect of the surround we selected two identical three-ply cabinets which several years previously had been coated inside with 'japan' black. Having done half a dozen control tests in the absence of germicide to find the fall rate of the organisms and to see that both cabinets gave similar figures, we proceeded to examine the efficacy of resorcinol and pyrocatechol aerosols under the new conditions. The results compared favourably with those provided by the twin, lead-lined chambers. Next the interiors of the cabinets were given two coats of ordinary white lead paint, made up with linseed oil and thinned down with turpentine substitute. The doors were left open for 3 days, and then the contained air tested for bactericidal qualities to *C. xerosis* emulsified in neat saliva. Our findings were illuminating. The 5 min. plates were sterile, as were the 15 min. plates up to at least the 5th week, the doors of the cabinets remaining open except during actual performance of the test. At the 8th week there was still some evidence of kill on the 30 min. plate, and when the doors were closed for 3 days only three out of some 500–1000 organisms were able to survive for 5 min. At the time of writing, 14 weeks since the cabinets were painted, the air of the interior continues to be unsuitable for the survival of the test organism for long, so that we have had to abandon, temporarily, any further attempt to investigate the effect of the lead paint on adsorption of the germicide from the air.

The problem was attacked from another angle, viz. by lining our experimental twin chambers with filter-paper sheet. The lining was, unexpectedly, found to have no effect on the 'catch' of bacteria on our plates nor on the percentage number of survivors in the test chamber proper inoculated with either heat-volatilized or mechanically atomized germicide. Thus there was, apparently, no greater adsorption of the germicide by the paper than by the lead lining. The experiments are being continued.

FURTHER EXPERIMENTS WITH PROPYLENE GLYCOL

In confirming our findings that propylene glycol is capable of destroying air-borne bacteria Robertson and his co-workers (Robertson, Bigg, Puck & Miller, 1942) appear to have had success with concentrations weaker than we found originally to be definitely effective. We took up the matter again and have recently carried out a comprehensive series of experiments comprising mechanical atomization, heating on a metallic plate at different temperatures, painting on surfaces round about room temperature and evaporation from Petri dishes. Durability of action was also tested, the test organism (*C. xerosis*) being emulsified in both broth and sterile, neat saliva. In some instances comparable experiments with ordinary phenol were undertaken.

Our general conclusions are that near the boiling temperature and possibly beyond the results with the hot plate are as good as those obtained by mechanical atomization. On painting surfaces or evaporating from Petri dishes at room temperature naturally some time has to elapse before the rate of kill approaches that obtained by mechanical or hot-plate atomization. A strong phenol paint (1000 mg./0·55 m.^{2}/m.^{3}) gave a quicker kill than did a similar glycol paint; on the other hand, the glycol appeared to be superior to the phenol when a smaller quantity (100 mg./m.^{3}) was evaporated from the hot plate. With the latter technique a 95 % or better kill during the 5th to the 8th minute was sometimes obtained with a concentration of 100 mg./m.^{3}, but with half this concentration the kill had almost always dropped to 50 %. The playing of a fan in the neighbourhood of the hot plate seemed neither to accelerate nor inhibit rate of kill, nor was there, apparently, any difference in sensitivity of the test organism whether emulsified in broth or saliva. We were unable to demonstrate that humidity of the air within limits (R.H. 35–70 %) affected much our results with propylene glycol.

THE EFFECT OF INORGANIC SALTS

Besides NaOCl (Twort *et al.* 1940) and iodine, we had previously found that zinc and calcium chloride, mechanically atomized, had apparently some bactericidal effect on air-borne bacteria (1940, p. 307), but on repetition of these tests we were unable to obtain conclusive results. It was thought that the kills in the first series of tests might have been brought about by dehydration of the bacteria, but, unfortunately, we have no records of the then prevailing relative humidity.

In view of the action apparently exerted by vapour pressure in influencing rate of kill by phenolic germicides, it was decided to test some further inorganic germicides in this connexion. We selected for this purpose: mercuric chloride, boric acid and copper sulphate. On mechanical atomization (aerograph brush) of 10 and 1 mg. of the substances per m.^{3} of air the only definite evidence of effect within half an hour, on *C. xerosis* emulsified in sterile saliva, was obtained with mercuric chloride, the volatile salt. Here all the plates were sterile with the exception of the 5 min. plate belonging to the weaker concentrations, and the weakest gave a 98 % kill. Subsequently it was found that 0·1 mg./m.^{3} sufficed to kill 80 % or more of the test organism within 10 min., and 95 % or more within 20 min. The use of broth instead of saliva as emulsifying agent did not appear to affect the sensitivity of *C. xerosis* to this particular germicide.

When substituting a hot plate for the 'aerograph' the kill was, on the whole, not quite so rapid, all evaporations being made from glass. Mercurous chloride, as might be expected from its dissociation on heating, proved to be less efficacious than the mercuric salt. The general indications from the results of these experiments is that death of the organism followed more from contact with vapours than with mist particles of the substances used.

GROWTH INHIBITION ON PLATES BY COLLECTED GERMICIDE MISTS

The slit-sampler designed by Bourdillon and his colleagues (Bourdillon, Lidwell & Thomas, 1941) was advocated as a useful piece of apparatus for ascertaining the rate of kill of bacteria in the air during the first few minutes of an experiment, but before utilizing the sam-

pler for this purpose it was considered necessary, in view of the fact that both bacterial and germicide particles would be collected on the medium at one and the same time, to find out the amount of germicide it would be safe to employ. Accordingly, preliminary tests were performed in which germicide mists were collected on plates of agar which were subsequently seeded in the control chamber with a bacterial mist. After incubation, evidence of bacteriostatic action was sought, together with that of diffusion of the germicide beyond the boundaries of the medium covered by the slit.

In the first series of experiments the mist concentrations were 1 mg. per m.^{3} of air. The gear giving a revolution of the agar plate in 3 min. was used, the volume of air passing the slit being 10 l./min. The theoretical amount of germicide collected by the agar could not be greater than 0·03 mg., and presumably would in most cases be appreciably less. Three samples were taken of each mist, i.e. between the 5th–8th, 15th–18th and 30th–33rd minutes after generation. The plates, together with a control, were then seeded with the '*F*' coccus by exposing them for 3 min. to an atomized broth emulsion 5 min. after generation. As germicides we selected: resorcinol—a very water-soluble, quick killer, with a low Rideal-Walker value (0·3); benzyl cresol—a practically non-water-soluble, quick killer, with a high R.-W. value (150); hexyl resorcinol—a practically non-water-soluble, slow killer, with a high R.-W. value (50); and NaOCl ('Chloros') as a mechanically atomized mist to compare with the heat-volatilized phenols.

None of the plates differed from the controls as regards size or number of bacterial colonies, with the exception of the one exposed to hexyl resorcinol mist between the 5th to the 8th minutes. Here the slit zone of the plate showed no macroscopic evidence of growth, while diffusion into the central and peripheral agar had clearly retarded growth, the colonies being much smaller than those of the control. Attention may be drawn to the fact that after 15 min. insufficient of this phenol was collected to give more than evidence of bacteriostatic action, although initial mist concentrations of it are known to have a duration of lethal effectiveness far beyond this time.

A mist concentration of 10 mg./m.^{3} was used in the next series of tests, 'Milton 2' being substituted for 'Chloros' and the plate speed being regulated to one revolution per 6 min. Again resorcinol and NaOCl gave no evidence of bacteriostatic action. In the case of benzyl cresol there was a reduction of about 50 % in the number of colonies on the 0–6 min. plate, and those present were very small. On the 6½–12½ and the 13–19 min. plates there was perhaps a slight reduction in number, but on the 30–36 min. plate there was even distribution and colony size was similar to that of the controls. The 30–36 min. plate was not exposed to hexyl resorcinol, but the slit zone of the other three plates showed no colonies, those in the area beyond the boundaries of the slit being also small and sparse, although becoming larger and more numerous as the experiment proceeded.

The concentration of the germicide was then increased to 100 mg./m.^{3}, mists of resorcinol, benzyl cresol and ordinary phenol being used. The appearance of the resorcinol plates was again similar to that of the controls. The ordinary phenol caused some diminution in the size of the colonies, especially marked on the plates covering 45–66 min. In the case of benzyl cresol there was a noticeable deposition of the germicide on the agar when the plates were removed from the sampler. The film was clearly visible to the unaided eye 66 min. after generation of the mist, and in the slit zone of agar no colonies were seen on any of the plates. As regards diffusion into the medium beyond the boundaries of the slit, while the control plate had about 300 colonies over this area, the benzyl cresol plates up to 12½ min. were apparently sterile, between 13 and 19 min. there were fifty-two colonies, between 30 and 36 min. 100 colonies, mostly small, as they were between 45 and 51 min., but were nearly normal in size by the hour.

An attempt was made to obtain a positive result with resorcinol by using 1000 mg./m.^{3}, but the most that was obtained was a decrease in colony size, and as in the previous phenol experiment, most evident on the 45–66 min. plates. It is not, at the moment, quite clear to us why the earliest plates were not those to show the smallest colonies, for the number was not greatly in excess of the number on the last plates.

In general then there is evidence of bacteriostatic action on the agar plates when using 1000 mg./m.^{3} of resorcinol, 100 mg./m.^{3} of phenol, 10 mg./m.^{3} of benzyl cresol and 1 mg./m.^{3} of hexyl resorcinol, the last two being possibly sufficiently potent to be bactericidal. Diffusion into the medium outside the boundaries of the slit is relatively most marked in the first two germicides, probably due to their greater water solubility. Thus, for slit-sampler tests proper it is deemed safe to use a mist concentration of 1 mg./m.^{3} of the first three germicides, including NaOCl, but hexyl resorcinol should be used in a weaker concentration.

ANIMAL EXPERIMENTS

Although we had long ago come to the conclusion that the inhalation by man of phenolic bodies of the type and at the concentration in the air we would consider using for air disinfection purposes was very unlikely to cause any inconvenience whatever, we, nevertheless, deemed it advisable to collect further evidence in this connexion. Inhalation might do harm where injection and ingestion in relatively large amounts were well tolerated. We are, at the moment, particularly interested in tolerance to resorcinol because it is this phenol which we have recommended should first be put on trial in field tests.

The results of some injection and ingestion experiments with a variety of phenols have been described previously (Twort *et al.* 1940, p. 324). On intraperitoneal injection of resorcinol the M.L.D. for the average 20 gm. mouse was found to be about 5 mg. On the other hand, each mouse (100 used) consumed from the drinking water, on an average, as much as 80 mg. of resorcinol per week for 25 weeks, without showing any evidence of intoxication. It is true that the rise in body weight of the average animal did not quite keep pace with that of the controls, but this was presumably due to the quantity of fluid taken being relatively small, especially towards the end of the experiment, the concentration of resorcinol in the drinking water being gradually increased and making the fluid unpalatable. The survival rate was good, and on microscopical examination of the internal

organs no evidence was found of intoxication of the organs of the host nor of the several different parasites habitually present in this variety of animal kept in captivity (Twort & Twort, 1932).

Tolerance to the inhalation of resorcinol over a considerable period of the life span of the animal was the next aspect of the subject investigated. As usual 100 animals were treated with the substance under test, a second 100 serving as controls, each group consisting of fifty males and fifty females. The animals were, as far as possible, matched, and the massed body weight of each sex group was approximately equal. The animal house was divided into approximately two equal parts with a partition of wood and fibre board, an observation window and a door being inserted. The chief data to be collected as evidence of effect of the phenol were: body weight, death-rate, condition of the internal organs and the presence of parasites.

It had been hoped that the duration of the experiment might have reached at least one year, but this was, unfortunately, not possible, and at the end of the 43rd week the whole of the animals surviving had to be killed. This, however, in our experience, represents at least half the life span of mice kept under war-time conditions. The capacity of the room was approximately 30 m.^{3}, or if allowance is made for space occupied by cupboards, etc., about 24 m.^{3} Almost exactly 2500 g. of resorcinol were utilized during the 43 weeks, equal to an average of 345 mg./hr., or 12–14 mg./m.^{3}/hr. The hot plates were operated for 5 days of each week, a solution dripping machine operating for only 4 hr./day during the first 35 weeks, and a hot-plate capsule machine with half the output rate for 8 hr. during the last 10 weeks. Thus, during the actual operation of the machines the rate at which the resorcinol mist was being generated was approximately 100 mg./m.^{3}/hr., and 50 mg./m.^{3}/hr. respectively. If air turn-over of the test room is reckoned as one per hour, which is very unlikely except on windy days as the window and door are kept closed, then on an average, for 5 days a week, during 6 hr., the mist concentration should have been 75–90 mg./m.^{3}, excluding gravitational and adsorption effects. It is probably conservative to estimate that our animals were exposed for a quarter of the day to at least 100 times as much of the germicide as recommended for use in practice, and during the remaining three-quarters of the day were in an atmosphere heavily charged with vapours.

It has not, meanwhile, been possible to complete the post-mortem examination of the organs, but the carcases have been preserved in the hope that at a later date they may be studied histologically. During life there was no evidence whatsoever that the continual inhalation of resorcinol vapours and mists had the slightest detrimental effect on the general health of the animals. At the commencement of the experiment the body weight of the average animal of each of the four groups was 23·5 g. At the termination of the experiment there were forty-five male and forty-one female controls, and forty male and forty-one female resorcinol animals alive, with an average body weight of 31·5, 28·7, 33·6 and 29·8 g. respectively. If the massed body weight and the average body weight of the surviving controls is considered as 100, the percentage figures of the resorcinol animals were:

Massed body weight			Average body weight		
Males	Females	Total	Males	Females	Total
99·2	103·9	101·3	106·6	103·9	105·2

differences which, in our opinion, have no significance, and which seem to indicate that when we come to examine the internal organs little of interest will be found either histologically or as regards prevalence of parasites.

DISCUSSION

The question to which we have been trying to find an answer is: Does death of the bacterium result from contact with mist and smoke particles or from contact with vapours? Are mists apparently so active by virtue of the large surface offered for evaporation or are vapours apparently active by virtue of recondensation into mist particles? On starting our investigations on air disinfection we had anticipated that the phenols selected for test would be operative in the vapour phase, but it was very soon found that the best results were obtained by distributing the germicide in mist form. Thus, we inclined to the view of Bechhold (1935), although our colleagues, Finn & Powell (1940, p. 290), were unable to reconcile the rate of kill we obtained on the basis of direct contact between germicide and bacterial particles as sole mechanism of the reaction. The position now is that while our laboratory tests seem to show that the actual kill is accomplished by the vapours, in practice it is necessary to disperse small amounts of lowly volatile germicides as mists in order to utilize them at maximum efficiency. This may be done mechanically, or by heat-volatilization followed by condensation, according to convenience. With agents of high vapour pressure it apparently does not matter if there is no condensation, because mists again become vapours in a few seconds. The latter agents do not need a very large evaporating surface in order to fill the atmosphere with the requisite number of lethal units.

Perhaps the most important characteristic of the germicide, from the point of view of lethal effectiveness on air-borne bacteria, is the vapour pressure. Phenol coefficient (test-tube) and water solubility have also to be kept in mind, as have other probably less important features such as hygroscopicity, diffusion coefficient, etc. Superficially the test-tube phenol coefficient would seem not to come much into the picture, but as a rule among a series of phenols this particular characteristic rises with decrease of vapour pressure which is often accompanied by decrease of water solubility, complications tending to obscure the mechanism underlying the disinfection process. Further, rate of kill in relation to durability of action had to be considered when assessing values.

We have always assumed that the actual mechanism whereby phenolic germicides kill bacteria in the air and in the test-tube is similar, although plainly the air and test-tube phenol coefficients show no parallelism. If it be conceded that this conception is not unreasonable, then vapour and phenol coefficient would, *ceteris paribus*, have a similar value regarding rate of kill of air-borne bacteria, if vapours solely are lethal. For example, hexyl

resorcinol with a test-tube phenol coefficient about 150 times that of resorcinol should kill as quickly as resorcinol if the vapour pressure of the latter is not more than 150 times that of the hexyl derivative. Actually, when we compare the effect of these two phenols as paints on the floor of our experimental chambers the resorcinol comes the more quickly into action. This may be due to the influence of other factors, and also to the difference in vapour pressure being greater than 1:150. On this theory it should be possible when using paints to counterbalance the phenol coefficient of phenols having more or less similar outside characteristics, by manipulating the temperature of the atmosphere in which they were operating on bacteria, so that rate and durability of activity were, at least, of the same order. However, both known and unknown factors would render proof of this a difficult matter.

As from a paint of resorcinol the surrounding air apparently becomes impregnated with a lethal dose of vapours quicker than from a paint of hexyl resorcinol, so is a similar state of affairs to be expected when mists are substituted for paints. Hence we see why increasing the mist concentration of our phenols does not lead to a proportionate improvement in kill beyond a certain point, at least, not to be compared with that taking place in the test-tube. For example, a mist concentration of 0·2 mg./m.^{3} of hexyl resorcinol operates nearly as efficiently as a mist 15 times this concentration, while within these concentration limits the number of survivors to resorcinol is approximately inversely as the concentration. Did we substitute test organisms of greater or less sensitivity than those mostly used, the difference in the two phenols would presumably still remain (assuming that specificity of lethal effectiveness was avoided), although mist concentration limits would alter.

The apparent difference in the degree of lethal effectiveness of our germicides in the air of our lead-lined test chambers and in the air of habitable rooms appears to become less striking as the total amount of germicide utilized is increased. With germicides of relatively high vapour pressure, which have to be used in quantity to give a demonstrable effect, the percentage of the total which does not come into play is too small materially to influence the final result; on the other hand, with highly active, low vapour pressure germicides removal of a similar amount might represent the total utilized, leaving none to exert lethal action on the test organism.

The formation of a monomolecular film may be the cause of the trouble, but this would operate in favour of the larger space with its relatively smaller area of surround. In any case it would seem reasonable to assume that the germicide is to a greater or lesser extent adsorbed by the bacterium before the former reaches the surround, although one can visualize that re-evaporation from the bacterium might take place before a lethal dose was at any one time adsorbed, and consequently the bacterium is able to survive. It may be that when a bacterial particle is in close proximity to a germicide particle re-evaporation lags behind adsorption with consequentially the more rapid accumulation by the bacterium of a lethal dose, but while this may be an explanation for the necessity of nebulizing the low vapour pressure germicides it does not explain the hypothetical effect of an adsorptive surround, except in so far as decay of the particle may be hastened.

When selecting a germicide for use in continuous disinfection of the air the question of the quantity required to give promise of success has from most points of view to be given serious consideration. In this connexion may be mentioned the prominence given recently in the medical press of this country to propylene glycol, as a result of researches carried out in America by Robertson and others. Although we had shown some time ago (Twort *et al.* 1940) that on heat volatilizing this glycol it had an efficiency as an air disinfectant somewhat similar to that of ordinary phenol, we were much more impressed by the performance of some of the higher phenols. In order to attain our arbitrary standard of kill (90–95 % within 10 min.), it was necessary to use 100 parts of glycol compared with 1 part of the best of the higher phenols, and although the former is presumably much less toxic than the latter for higher organisms we are not prepared to say that the host-parasite tolerance ratio is in favour of the glycol. Moreover, 100 mg./m.^{3} of any vapour foreign to ordinary air is a heavy dose to be continually inhaled. Robertson contends that our results with propylene glycol were due more to the spirit used as a diluent than to the glycol itself. Naturally, we carried out suitable controls as we habitually do with all solvents we use, and although we may not always mention the fact when the solvent proves apparently to be inert in the concentration tested, we do not understand why our findings should be doubted seeing that they have been verified by our critics.

The position of our arbitrary standard of kill in relation to the requirements of a reasonable control of cross-infection is at the moment quite speculative. Basically, it would appear that the more exacting the requirements the more would one favour the use of germicides with a high vapour pressure (glycol); on the other hand, if it turned out that our standard was too severe one would favour a germicide with a very low vapour pressure (hexyl resorcinol). At the moment we recommend for trial a germicide with an intermediate vapour pressure (resorcinol), with the proviso that it be distributed in the air as mist particles and not as a vapour, thereby ensuring that according to our arbitrary standard the prophylactic agent is operating with maximum efficiency.

Although in this discussion we have ignored any part played by direct contact of bacterial and germicide particles we are not disposed to believe that this never occurs. While our former results may perhaps all be explained as due to the action of vapours, many of them were only obtained by the use of mists in a particular condition. A few to which we refer are:

HOCl gas in solution appeared to be 20 times more effective than the free gas.

Better results were obtained with a coarse than with a fine mist of hypochlorite solutions in saline-water.

Better results were likewise obtained with coarse mists of solutions of resorcinol mechanically generated, but with fine mists of hexyl resorcinol.

The ratio of hexyl resorcinol to propylene glycol of 1:10 provides a mixture for mechanical nebulization of a greater persistence and also of a relatively greater bactericidal potency to air-borne organisms than ratios above or below this.

The more this mixture is diluted with water the less does it become effectively operative as a mist, the number of survivors not being inversely proportional to the dilution.

Such results as these would seem to indicate either that there is direct contact between germicide and bacterial particles or that the former provides a nucleus in the neighbourhood of which there is maintained a concentration of vapour sufficient to lead to death of the bacterium. In either case persistence of the germicide base as a mist is of importance, and only ceases to be so where the atmosphere as a whole has reached a degree of saturation of vapours compatible with lethal effect at a maximum. On the other hand, bodies atomized as very fine mists have a larger total surface area for evaporation, to the advantage of lowly volatile compounds, but to the disadvantage of the more volatile.

CONCLUSIONS

1. In air disinfection, while actual collision between germicide and bacterial particle may be of rare occurrence it is, nevertheless, necessary when using the higher phenols in small amounts to disperse them as particles (droplets) in order to obtain a demonstrable bactericidal effect within a reasonable time.

2. When the vapour pressure of the germicide is high, decay of small particles is rapid, and we have been unable to observe that mode of dispersion results in any difference in degree of activity.

3. We have so far obtained no evidence of a specificity of lethal effectiveness of germicides in the air to the several test organisms used. If the potency in the air of ordinary phenol is taken as 1, that of the propylene glycol is about 1 while that of some of the higher phenols is over 100, there being little parallelism between the test-tube and air phenol coefficient.

4. Among some inorganic materials tested, mercuric chloride was actively bactericidal on air-borne bacteria, while non-volatile materials such as copper sulphate and boric acid had no definite demonstrable effect.

5. Surface evaporation of higher phenols at room and other relatively low temperatures from large areas showed that rate and durability of kill of the test organisms was a function of volatility, as had previously been shown when utilizing smaller surfaces and a higher temperature or by mechanical atomization.

6. Rapidity of action and durability of action appear to be incompatible. Thus, a large quantity of a germicide with a high vapour pressure kills rapidly, but a small quantity will have no appreciable effect, even in unlimited time. On the other hand, a large quantity of a germicide with a low vapour pressure, while taking longer to kill, will have a more durable action, and actually will act in a much smaller quantity.

7. The efficiency of an aerial disinfectant is gauged by the amount required to give a 95 % kill of the test organism within 10 min. (actually the 5th to the 8th minute). As regards removal of living organisms, this is approximately equivalent to 21–22 air turn-overs per hour. The question of loss of virulence of the 5 % survivors has not, meanwhile, been satisfactorily answered from either the disinfection or ventilation angle.

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