THE EFFECT OF HUMIDITY OF AIR ON THE DISINFECTION CAPACITY OF MECHANICALLY ATOMIZED AND HEAT-VOLATILIZED GERMICIDAL AEROSOLS

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During the course of our experiments on the lethal effectiveness of organic smokes to bacteria in the air (Baker & Twort, 1940, p. 587), we experienced, as time went on, more and more difficulty in obtaining satisfactory results, whereas in the early days of these investigations our findings were relatively consistent. In the first place, our charcoal-incense 'candles', when smouldered, failed to give the results expected, and in seeking an explanation for the failure it was thought possible that the temperature of smouldering was too high and that there was pyrolysis of the emitted smoke as it passed over the glowing point of the candle. Attempts were made to elucidate the matter by (1) smouldering the 'candle' inverted, and (2) heating the crushed 'candle' on a hot plate of regulated temperature. Both methods gave somewhat improved results, but these still remained very poor, and our next procedure was to examine the effect of the smokes obtained from incense supplied from different sources. The results were irregular, as they were with several other substances which were re-tested. Only Peru balsam continued to give good 'kills' for some time, but, finally, we were unable to reproduce our original results even with this product. While there seemed to be evidence that the smoke, generated by smouldering on a hot plate such substances as cardboard and the incense-charcoal mix, was somewhat more lethally effective than when generated by smouldering as a 'candle' or strip, the experimental results as a whole had, by this time, become consistently bad.

In seeking an explanation for this disconcerting state of affairs, the most obvious variation in our experimental conditions was a seasonal one, and in view of the fact that the room in which our experimental test chambers are located is thermostatically controlled, and the temperature variation is rarely more than 3° C., it seemed possible that the humidity of the air might be an important factor. Like other workers in the domain of aerial disinfection, we had kept in mind the probability of temperature and moisture being factors in influencing the efficacy of a germicidal aerosol, and, since our earliest experiments (Twort, Baker, Finn & Powell, 1940, p. 298), we had taken the precaution of recording temperature and relative humidity. Unfortunately, owing to stress of circumstances, during last year, when our team of workers was being disintegrated, no such records were kept, so that the data available for making an analysis of humidity in relation to lethal effectiveness of

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germicidal aerosol were not so large as they might have been. Throughout this paper we often refer to the percentage relative humidity, but as the temperature in our experiments is kept constant we might equally well speak of absolute humidity. It remains for future investigation wherein the temperature is varied to show whether the absolute humidity or the percentage relative humidity is chiefly responsible for the results to be described.

In our examination of the question we first collected all available data relating to experiments carried out with mechanically nebulized germicides which had been, as far as possible, exactly duplicated during the previous two years, with the only obvious variant of humidity. Scrutiny revealed that of the twenty-six pairs available the better 'kill' was obtained with the higher humidity on twenty-one occasions, equal to 80·8%. In this analysis each member of the pair represented, not the result of a single experiment, but the mean of four to six experiments performed during a single day, when the variation in humidity was slight. The number of pairs available when single experiments were considered was, of course, large, and only the data belonging to the first sixteen of our twenty-six exactly duplicated series of experiments were examined. Considering the results given by each experiment of one day compared with all of a second day the first 250 pairs gave a better 'kill' with the higher humidity on 184 occasions, equal to 73·6%, and it was not thought worth while to continue the analysis further.

The germicidal aerosols utilized in the experiments under review were all produced by the mechanical nebulization of relatively water-insoluble phenolic substances (i.e. benzyl cresol, normal hexyl resorcinol and parachlormeta-xylenol), the test organisms being the 'F' coccus, a white, gram-positive micrococcus, *E. coli* and the flora of normal salivas. Glycols were used as solvents for the phenols, with and without the addition of small quantities of such substances as alkalis, dyes and wetting agents. Having obtained indications that humidity might be playing a more important part in influencing our results than had been previously appreciated, we were naturally led to inquire whether this influence was not perhaps greater than some of the other variable factors involved, and might we not have to modify some of the conclusions drawn from early experiments?

An examination of the average results given by groups of five and six experiments with 'S^2' and '2 S^2' respectively (10% and 20% of normal hexyl resorcinol in propylene glycol, etc. (Twort *et al.* 1940, p. 310)) versus various normal salivas provided twenty-four pairs, in eighteen of which (75%) better 'kills' coincided with the higher humidity. Thus, the variation in humidity perhaps influenced our results more than the variation in the flora of the salivas. The data from experiments with solutions of hexyl resorcinol in glycols, with and without the addition of small quantities of alkali, etc., examined in a similar manner provided forty-four pairs when the 'F' coccus was the test organism, and here the higher humidity gave the better 'kill' in 77·3% of cases. These findings, again, throw some doubt on the benefit derived from the addition of alkalis and wetting agents to our germicide mixtures, but the number of samples was very small although the indications so far obtained all point in the same direction. Consideration of similar experimental results when salivas were used in place of the 'F' coccus revealed, however, only twelve out of twenty-one in favour of the higher humidity, while in seven out of ten pairs relating to experiments with 'Aéryl' (45% resorcinol in 7% glycerol-water) and various salivas, 'kills' were the better in the presence of the lower humidity. In the latter groups of experiments the variation in the relative humidity was only 4 degrees, some being identical, and thus provided

Table 1. *Influence of humidity on the lethal effectiveness in the air of mechanically nebulized phenolic germicides*

Germicide	Organism	Mist conc.	R.H.	*S*	Mist conc.	R.H.	*S*
S^2/NaOH	Coccus	1500	68	49/1·16	1500	48	79/1·86
		3000	67	100/NDK	3000	47	79/2·19
		2000	67	91/2·27	1200	48	84/2·85
	Saliva	1200	69	72/3·98	3000	44	70/NDK
		2000	66	59/2·28	2000	43	79/NDK
		2000	70	69/NDK	2000	39	100/NDK
10% HR/PG	Coccus	870	68	3·3/0·15	680	36	18/1·30
		550	66	0·14/0·008	400	37	59/6·27
		430	68	0·3/0·023	500	38	22/1·82
	Saliva	680	68	46/2·87	870	48	24/1·64
		1000	69	33/1·78	550	47	30/2·57
		431	70	36/3·28	550	46	21/1·88
R.G.G.	Coccus	200	62	70/11·93	222	43	41/12·47
		300	64	37/4·44	250	45	84/20·00
		230	64	49/7·24	430	48	44/4·65
	Saliva	170	55	10·5/2·35	126	33	39/20·67
		170	59	2·3/1·62	174	38	34/11·67
		265	61	16/1·96	142	42	30/13·00

Mist conc. = cubic metres of air containing 1 g. of germicide mixture.
R.H. = percentage relative humidity.
S = survival rates (see text).
NDK = no definite kill at the concentration of mist used.
S^2/NaOH = 10% hexyl resorcinol in propylene glycol plus a little soda and wetting agent.
R.G.G. = 45% resorcinol in 7% watery glycerol plus a trace of brilliant green dye.
HR/PG = hexyl resorcinol in propylene glycol.

no pairs for analysis; on the other hand, the fact that we were here dealing with a very water-soluble phenol must not be lost sight of.

Our next procedure was to examine the validity of the above observations by direct tests in artificially humidified air. For this purpose we utilized 10% hexyl resorcinol in propylene glycol, S^2/NaOH and R.G.G. (similar to 'Aéryl') for providing the germicidal mists, and the 'F' coccus and the flora of a normal saliva as test organisms. The results of these experiments are shown in Table 1. The survival rate (*S*), i.e. the percentage of survivors in the presence of the germicide compared with the survivors in the control chamber containing no germicide, is given in two ways. The figures on the left represent the actual percentage of survivors after the first 5 min. of the experiment.

Those on the right represent the mean of the percentages surviving at 5, 15 and 30 min., reduced to figures which it is presumed would appertain were the amount of solution utilized for atomization equivalent to 1 g. per 100 cu.m. of air (Twort *et al.* 1940, p. 303). The reduction is made in all tables with the exception of Table 7, so as to bring into line experiments at various germicidal concentrations, to facilitate comparison. In the case where the mean number of survivors is greater than 50 it is designated as no definite kill (NDK), and it may be well to point out that, under the circumstances, there can be no reduction to the common-mist concentration of 1 per 100.

The results are significant from several points of view, but, meanwhile, it will suffice to note the outstanding improvement in the 'kill' of the 'F' coccus by the HR/PG mixture on the one hand, and of the flora of the saliva by R.G.G. on the other hand, when the air in which the germicides were exerting their specific function was relatively moist.

We are, of course, at the moment primarily interested in the possible influence of humidity on the lethal effectiveness of our smokes, and having obtained indications from our experiments with mists of phenolic germicides that here we might have a variable factor of supreme importance, we proceeded to institute a similar series of investigations on smokes in artificially humidified air. We had already taken the precaution to recommence the recording of temperature and humidity in our twin-chamber room for several weeks, but when artificially humidifying a second humidigraph was also placed inside the test chamber itself. Humidification was accomplished by atomizing with an Aerograph 'M.P.' spray-gun and compressor a quantity of water sufficient to give the required rise in humidity, the test and control chamber being treated similarly.

An analysis of all the experimental results obtained when the smoke from Peru balsam was tested against the 'F' coccus and the flora of saliva gave the following findings, a + sign signifying that the better 'kill' corresponded with the higher humidity:

No. of pairs	+	Percentage	
109	77	70·6	Peru *v.* 'F' coccus
62	50	80·6	Peru *v.* saliva A

The percentages in favour of the higher humidity were of the same order as those found by analysis of our early experimental results with mechanically nebulized phenolic germicides.

If experiments solely with various smokes and bacteria, carried out on a single day when the humidity was artificially varied, were compared, the corresponding figures were 105, 78 and 76·2%. Some of these experiments included tests with wood flour, and incense against *E. coli*. When such experiments were excluded, for reasons which will be apparent later, the corresponding figures were 76, 64 and 84·2%. When, among these experiments, we compared only the control belonging to its own raised humidity counterpart, the figures became 25, 23 and 92%. The last is really the fairest way of making the comparison (as for example in Table 1), because during the course of the day other influences may be at work, such as the building up of germicide (Twort *et al.* 1940, p. 318), slight temperature variation, etc. Since the incorporation in our chambers of a special blower to ensure rapid removal of residual smoke, etc., there does not seem so great a tendency for the 'kill' to improve as the day progresses.

Sample results of three typical experiments on the effect of raising the humidity are given in Table 2, 50 mg. of crude material per 3 cu.m. of air being used in the Peru balsam tests and twice this amount of incense (equivalent to 1 g. per 60 and 30 million ml. of air respectively).

It will be noted that while in each pair the number of survivors in '*a*' is greater than in its '*b*' counterpart, where the humidity is the higher, the smallest number of survivors given by an *a* test is, with one exception at the 5 min. period, greater than the largest given by a *b* test in each of the three experiments. Within both *a* and *b* tests there are shown irregularities due to

Table 2. *The effect of humidity on the lethal effectiveness of balsamic smokes*

	Exp. 446 Peru *v.* coccus		Exp. 447 Incense *v.* coccus		Exp. 448 Peru *v.* saliva A	
	R.H.	*S*	R.H.	*S*	R.H.	*S*
a	51	27/22·82	35	20/48·00	40	34/44·00
b	70	24/14·13	50	1·2/3·60	59	2·1/1·82
a	50	24/14·97	36	24/42·77	38	34/35·00
b	66	5/3·43	53	2·5/6·85	63	5/3·67
a	46	22/23·72	38	9·3/25·85	31	14/17·65
b	62	8/4·60	63	3·9/11·70	53	2·8/3·23

Abbreviations and calculations of survival rate as in Table 1.

variable factors other than humidity. Incidentally, it will be seen that although we had formed the opinion that our smokes were definitely more effective against the flora of the normal saliva than against emulsions of laboratory stock cultures (Baker & Twort, 1940), only in half of the six comparative Peru experiments was the number of survivors of the former less than of the latter type of test organism. On all six occasions, however, the humidity was in favour of survival of the salivary types, i.e. it was considerably lower.

Results of some former experiments with fractions of Peru balsam (distilled for us by our colleague, S. R. Finn), carried out when the relative humidity was below 50%, compared with recent results when the humidity was artificially raised, are very illuminating (Tables 3 and 4).

Our procedure was varied when testing the fractions against the flora of the saliva, the concentration of smoke (i.e. the amount of material heated) being in the first test twice and in the second test ten times greater in our early experiments when the relative humidity was low than in recent experiments when the humidity was artificially raised.

The figures in both these tables show the striking effect of humidity on the number of test organisms able to survive in the presence of the smokes. Table 4 especially illustrates how greatly the amount of material required to disinfect a given volume of air depends upon the relative humidity prevailing. Incidentally, the results also show that fraction 1 provides a more potent germicidal smoke than the pure balsam, and fraction 3 a less potent one.

Table 3. *The effect of humidity on the 'kill' of the 'F' coccus by fractions of Peru balsam*

Fraction	mg.	R.H.	*S*	R.H.	*S*
1	50	46	38/44·22	56	1/0·55
	100	46	2/4·72	59	0/0
2	50	46	19/22·35	55	2·5/1·38
	100	45	14/23·10	55	0·66/0·73
3	50	45	47/67·55	54	47/33·68
	100	45	12/26·82	60	18/27·75
Residue	50	48	62/56·72	70	19/14·28
	100	48	37/59·62	64	0·6/1·43

Abbreviations, etc. as in Table 1.

Table 4. *The effect of humidity on the 'kill' of the flora of saliva by fractions of Peru balsam*

Fraction	mg.	R.H.	*S*	mg.	R.H.	*S*
1	50	37	14/21·10	25	62	4·4/0·99
	100	37	6/17·23	10	64	16·5/1·30
2	50	37	50/33·07	25	64	6·4/1·58
	100	37	47/133·33	10	61	42/3·25
3	50	37	23/47·67	25	63	45/10·47
	100	37	36/47·27	10	65	39/3·77
Residue	50	37	25/45·17	25	61	7·5/3·08
	100	37	14/18·17	10	65	25/2·44

Abbreviations, etc. as in Table 1.

Table 5. *The effect of incense smokes on the flora of the saliva*

		Recent tests		Previous tests	
Smoke	mg.	R.H.	*S*	R.H.	*S*
Incense	50	75	7/4·42	45	12·6/13·47
	100	64	0·7/1·62	46	21/47·00
Benzoin	50	64	13/8·68	34	69/NDK
	100	62	2·9/6·65	34	61/NDK
Myrrh	50	62	11/6·52	34	26/NDK
	100	65	4·4/9·22	34	39/NDK
Olibanum	50	66	12·5/9·00	34	27/66·67
	100	65	4·1/5·68	34	21/39·62
Styrax	50	68	5·2/3·72	34	39/NDK
	100	63	1/1·15	34	43/NDK

Abbreviations, etc. as in Table 1.

The relative merits of the smokes derived from some of the commoner substances used in the manufacture of incense had also been investigated some time previously. The humidity at the time was low, and the 'kills' being poor no information of value regarding the point in question was in consequence forthcoming. The tests were repeated recently under more favourable conditions, the results being shown in Table 5.

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Again, we have evidence of the striking effect of a moist air on the capacity of smokes to disinfect the air. It will be noted that the improvement in the experimental conditions has been instrumental in giving information regarding possible differences in the efficacy of the various smokes generated from the several constituents embodied in incense.

It is not unlikely that, as in the case of mechanically nebulized phenolic germicides, the influence of humidity on germicidal effectiveness will vary with different smokes and with the concentration in which they are being used, besides varying with different test organisms. For example, when the relative humidity is low, the smoke generated by heating wood flour on an electric plate does not seem to have its lethal effectiveness reduced to the same extent as does the smoke generated similarly from balsams. Among the eighty experiments considered in Table 6 there were thirty-one in which the humidity was below 45% and nineteen in which it was above 55%, the survival rate, as recorded in the table, being in the former above, and in the latter below 17% in every case. Against these fifty balsam samples we had only twenty-one comparable wood-flour samples, although sixteen of these related to tests wherein the humidity was below 45%. In ten out of these sixteen the survival rate was below 17%, findings which appear to have some significance. All the wood-flour experiments were carried out with the flora of the saliva as test organism, and in the sixteen experiments we are discussing the mean humidity was 34·8% with a mean survival rate of 17%. The latter figures compare favourably with the 73% survival rate found in the twenty low humidity saliva experiments with balsams, referred to in Table 6. It must be pointed out in connexion with this question that wood-flour smokes in the presence of a highly humidified air are very potent (mean survival rate over ½ hr. in above experiments in average humidity of 60% being 2·07), but we have not done a sufficient number of tests to warrant our considering them as more potent than balsamic smokes.

As regards possible differences in the degree to which humidity has an influence on our results according to type of test organism utilized, we may refer to the data given in Tables 2–4. In order to compare the effect of the Peru smokes on the flora of the saliva with that on the broth emulsion of the coccus, it is, of course, necessary to divide the 5 min. survival rate figures given in the last column of Table 4 by 2 and 10 respectively. The indications point on the whole to a greater coccus fragility to the smokes, but the relative humidity was higher, and the value of the comparison is thus doubtful.

We further examined this question by scrutinizing the results of eighty experiments with balsams against our broth emulsions of coccus, and normal saliva, where humidity records were available. In this analysis experiments where the humidity prevailing was above and below 50% were placed in separate groups, the mean relative humidity and the mean survival rates over the half-hour period of the two test organisms, calculated as in the other tables, being shown in Table 6. It would appear from this analysis, taken

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in conjunction with the figures given previously, that our former conclusion from early work on smokes, of the greater sensitivity of the flora of the saliva compared with broth emulsions of stock laboratory cultures, will probably have to be modified on account of the disturbing effect of humidity. Our early experimental results were, however, so very consistent; and as many of the tests were carried out on consecutive days it must have been a curious freak of chance for the humidity to have been materially raised on just those days when the test organism happened to be the flora of the saliva. The influence of humidity of air on the sensitivity to our smokes of nebulized broth emulsions of some other test organisms we have used—such as *E. coli*, *Str. agalactiae*, etc.—has not been investigated sufficiently to warrant discussion at this stage of our researches.

The marked influence of humidity on the bactericidal potency of balsamic smokes, and its feeble influence in some tests with mechanically nebulized phenolic germicides, led us to inquire whether or not there would be any effect on our results if we resorted to heat volatilization of the pure phenol. Previously we had formed the opinion that generation of our mists by the

Table 6

	No. of tests	Relative humidity	Survival rate
'F' coccus	23	45·5	25·00
	6	50	10·00
	12	60	8·33
Saliva	20	37·5	73·33
	4	50	46·67
	15	63·5	5·00

application of heat was not the best method for obtaining the maximum lethal effectiveness from our materials (Twort *et al.* 1940, p. 319). On referring to our charts to ascertain the humidity prevailing when the heat experiments concerned were carried out, it was found that mostly the relative humidity was low, ranging from 36 to 54%. In the tests with benzyl cresol and amylmetacresol it did not rise above 42%. On the whole the data we had in hand were not of much value, and it was decided to examine the question by carrying out further tests.

In the present investigation we utilized in the first place crystalline hexyl resorcinol dissolved for technical reasons 1/1000 in methylated spirit; and also a simple solution of 10% hexyl resorcinol in propylene glycol, with no addition of soda or wetting agent, so as not to introduce complications, 1 part of this solution being diluted with 99 parts of methylated spirit. The following results (Table 7) were obtained with the 'F' coccus, when heating on a hot plate 0·1 ml. of our final solutions which gave a concentration of germicide base in our test chamber of 1 g. in 30,000 million ml. of air. The results are shown, as in Tables 3–5, in the order in which the experiments were performed, and although the concentration of germicide base in the test atmosphere was

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extremely low it was sufficient to demonstrate the effect of raising the humidity on the 'kill' of the coccus.

In a further series of tests the concentration of germicide in the air was increased, and parallel experiments were carried out with resorcinol, the pure germicide bases being dissolved on the one hand in spirit, and on the other hand in the solvents used in the mechanically nebulized mists. Lethal

Table 7. *Influence of humidity on the lethal effectiveness on the 'F' coccus of heat volatilized hexyl resorcinol*

(1 g. in 30,000 cu.m. of air)

R.H.	Percentage survivors 5 min.	15 min.	30 min.	Mean over half-hour
		H.R. and P.G.		
24	140	83	47	NDK
62	32·5	1·67	0·77	11·65
55	35·2	2·18	0·87	12·75
55	18·77	0·89	0·77	6·81
31	89	81	64	NDK
30	90	70	73	NDK
		H.R.		
28	105	67	44	NDK
68	56	3·24	0·35	19·70
63	37·5	1·73	0	13·08
67	48·6	1·67	0·27	16·85
35	75	114	100	NDK
35	100	67	111	NDK

Abbreviations, etc. as in Table 1.

Table 8. *Influence of humidity on the lethal effectiveness in the air of heat volatilized pure phenolic germicides in methylated spirit*

Germicide	Organism	Mist conc.	R.H.	*S*	R.H.	*S*
Hexyl resorcinol	Coccus	1200 as S²	77	15/0·44	38	100/NDK
		960	68	6/0·25	39	86/6·40
		600	69	8/0·5	40	98/10·9
	Saliva	1200	72	24/1·12	40	28/1·39
		960	66	35/1·78	40	57/4·63
		600	66	44/3·14	40	27/3·33
Resorcinol	Coccus	300 as R.G.G.	74	20/2·46	36	75/NDK
		150	68	5/1·36	36	61/26·67
		100	78	17/5·53	37	28/15·98
	Saliva	300	74	17/2·25	34	35/16·67
		150	68	0·8/0·25	35	12/4·87
		100	68	2/0·9	36	6/3·42

Abbreviations, etc. as in Table 1.

effectiveness of the bases in spirit on both the 'F' coccus and on the flora of the saliva are shown in Table 8, it being noted that the mist concentrations are not given in terms of the bases, but in terms of the mixtures in which they are usually used for mechanical atomization. This is done in order to facilitate comparison with the results shown in Table 1, as well as those obtained by heat volatilization of similar mixtures shown in Table 9. In forming an

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opinion as to the relative merits of the different procedures, due allowance must be made for possible errors in the concentrations of mists given, especially those mechanically generated. The figures are based on the weight of original material used which, as will be appreciated, may differ, perhaps to a large extent, from the actual weight present as a mist in the air during the experiment.

We usually prefer to base our conclusions on the average results of as many experiments as possible and to deal with a single set of figures in forming our

Table 9. *Influence of humidity on the lethal effectiveness in the air of heat volatilized phenolic germicides (in glycol and glycerol-water)*

Germicide	Organism	Mist conc.	R.H.	*S*	R.H.	*S*
10% hexyl resorcinol in propylene glycol	Coccus	1200	73	55/1·60	34	48/3·750
		960	67	22/0·80	34	77/NDK
		600	70	5/0·32	34	56/NDK
	Saliva	1200	76	38/2·64	38	56/NDK
		960	64	41/2·70	38	15/1·68
		600	70	23/1·68	38	31/4·10
R.G.G.	Coccus	300	70	3/0·32	40	35/4·77
		150	64	3/0·69	43	14/3·43
		100	77	3/0·98	43	10/3·33
	Saliva	300	75	4/0·47	40	17/2·74
		150	74	4/0·79	40	4/1·40
		100	68	6/2·61	42	2/1·0

Abbreviations, etc. as in Table 1.

Table 10. *Mean of the survival rates over the half-hour period shown in Tables* 1, 8 *and* 9

	R.H.	Table 1	Table 8	Table 9
		'F' coccus		
Hexyl resorcinol (10%)	High	0·06	0·40	0·91
	Low	3·13	8·6	3·75
Resorcinol (45 %)	High	7·87	3·12	0·66
	Low	12·37	21·32	3·84
		Saliva		
Hexyl resorcinol (10 %)	High	2·64	2·01	2·34
	Low	2·03	3·12	2·89
Resorcinol (45 %)	High	1·98	1·13	1·29
	Low	15·11	8·32	1·71

Table 1 = mechanical atomization.
Table 8 = heat volatilization of methylated spirit solution.
Table 9 = heat volatilization of polyhydric alcohol solution.

final opinion. To this end we have abstracted from Tables 1, 8 and 9 the mean percentage survival rate over the half-hour period, except those appertaining to S^2/NaOH, and divided them into groups of three where the humidity was high and three where it was low. The average survival rates of each group are shown in Table 10.

The disinfection of atmospheres of variable humidity was undertaken with several further aerosols, in all of which tests substantial benefit was derived from an increase of moisture. Some results are shown in Table 11.

Table 11. *Influence of humidity on the lethal effectiveness in the air of further germicidal aerosols*

Germicide	Organism	Mist conc.	R.H.	S	R.H.	S	Mist generation
Peru (F. 1)	Coccus	100	42	NDK	65	0	Mechanical
	Saliva	100	36·5	NDK	64	1·021	,,
10% BC/EG	Coccus	300	36	NDK	70	0·377	,,
	Saliva	300	32	56	75	10	,,
'Milton'	Coccus	20	28	NDK	72	1·390	,,
,,	,,	10	27	NDK	65	3·663	Heat
20% 'Milton'	,,	100	28	NDK	68	1·127	,,
Benzyl cresol	,,	1500	28	NDK	65	0·337	,,

BC/EG = benzyl cresol in ethylene glycol.
F. 1 = first distillate of Peru balsam.
'Milton' = a proprietary product containing about 1% hypochlorite.
Other abbreviations and calculations as in Table 1, S representing the right-hand column only and being the average survival rate in several experiments.

Discussion

There are five main aspects of these results which require examination:

(1) The influence of humidity.

(2) The utility of the solvents.

(3) The relative efficacy of heating and mechanical atomization.

(4) Comparative value of smokes from crude materials and pure chemical compounds.

(5) The sensitivity of the flora of the saliva compared with that of broth emulsions of stock laboratory cultures.

(1) The influence of humidity is clearly shown in all the results tabulated, with the possible exception of those depicting the action of hexyl resorcinol on the flora of the saliva. It is not clear to us at the moment why this exception should occur, and we cannot very well implicate the hydroscopic glycol because the heat volatilized phenol in methylated spirit does not seem to react appreciably to the presence of increased moisture, and also the coccus experiments fall into line with all the remainder. Our analysis of a few early results with S^2 and $2S^2$ had given indications that, possibly, raising the humidity was beneficial so that we are meanwhile keeping an open mind on the question.

(2) If heat be the method of choice for the generation of our phenolic mists then the usefulness of the solvents apart from technical reasons appears to be very doubtful. Hexyl resorcinol, resorcinol and benzyl cresol when heated all gave very good results. From our previous experience with mixtures of hexyl resorcinol in propylene glycol we had thought that probably a 10% solution approximated roughly a constant boiling mixture; nevertheless, the ultimate 'kill' obtained seemed to depend almost entirely upon the amount of hexyl resorcinol volatilized and not upon the presence or absence of the propylene glycol. It has to be remembered that although polyhydric alcohols

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such as those with which we are concerned here are in the pure state themselves germicidal to a moderate degree, they would most probably tend to damp down the lethal effectiveness of more powerful germicides with which they were mixed, despite possible benefits derived from their hygroscopicity. Therefore, it is not surprising to find that the results given in Table 9 are no great improvement on those in Table 8. For the changes in relation to time which the mist particles of S^2 and R.G.G. undergo, see Twort *et al.* (1940, pp. 265–78).

(3) The results given by mechanical nebulization and heat volatilization of the two phenolic solutions containing the polyhydric alcohols were on the whole slightly in favour of the former method when utilizing the hexyl resorcinol solution and in favour of the latter when utilizing the resorcinol solution. This reversal may be due to the 'Atmozon' atomizer we used not generating large enough particles of the resorcinol solution. Weight for weight of phenolic base, however, heat volatilization of the pure phenols in spirit, as we have seen, gave quite good results, and although it is not within our province to discuss from the technical point of view the relative merits of mechanical and heat generation of phenolic mists, it must, nevertheless, be an advantage to be able to dispense with the lowly volatile solvents for several reasons, and it would appear that these advantages might outweigh the disadvantages.

The superiority of mechanical nebulization, properly carried out, over heat volatilization of hypochlorite solutions is, of course, to be expected if hypochlorous acid in solution is more effective than as a free gas. When heating there will also be some loss of hypochlorous acid due to formation of chlorates.

(4) Considered solely as regards lethal effectiveness on air-borne bacteria, the smokes generated from the phenolic substances under review compare favourably, weight for weight, with the smokes generated from balsams, etc. It could not be very well otherwise unless the latter smokes contained substances of extraordinarily high bactericidal activity, because almost certainly but a tithe of the original crude substance heated eventually is present in the air as a germicide particle. As a matter of fact, if the first distillate of Peru balsam be considered as a crude material, for such it really is, and the detailed results obtained with it be compared with those obtained with resorcinol, there is apparently not a very great difference in favour of the latter substance. Even from the meagre data given in the tables the reader will glean that this distillate is a very powerful aerial disinfectant of the flora of the saliva. In Table 4 it has been seen that, under the favourable experimental conditions of a relative humidity of 64% and at a concentration of smoke equivalent to that generated by heat volatilizing 1 g. of the substance per 300 cu.m. of air, more than 80% of the test bacteria were destroyed within 5 min. and more than 99% (not shown in the table) within half an hour. About half this quantity of resorcinol gives almost identical resultant 'kills'.

(5) The gap between the 'kills' of broth emulsions of the 'F' coccus and the flora of the saliva by smokes does not appear to be so wide as was thought originally, probably due to our lack of appreciation of the importance of the presence of moisture in the test atmosphere. What it comes to is that our smokes generated from such substances as balsams act somewhat like resorcinol in this respect, in contradistinction to the action of hexyl resorcinol and benzyl cresol which have an exceptionally high lethal effectiveness on broth emulsions of laboratory cultures.

It is not possible for the moment to give with any degree of confidence an explanation for the improved 'kill' of bacteria by germicidal aerosols in the presence of excess moisture. As a matter of fact, a great excess of moisture does not appear to be beneficial, and there is possibly an optimum degree of relative humidity above which the 'kill' begins to fall off. Attempts are being made to ascertain this hypothetical optimum.

The water may act primarily on the germicide and/or bacterial particles, before or after coalescence. As it had been established that the rapidity of evaporation of the mist particles of germicidal aerosols plays such an important part in the ultimate elimination of viable bacteria from the air, it was anticipated that an aerosol of S^2 (containing hygroscopic propylene glycol) or of 'Aéryl' (containing glycerol and water) would fail to give regular results unless the humidity of the air were standardized, although it was thought unlikely that there would be any precipitation of hexyl resorcinol out of solution. Similarly the amount of water vapour in the air must influence the rate of drying of the bacterial particle and thus influence the viability of the bacterium itself as well as the size of the particle of which it forms a part. We assume that increase of moisture favours survival in the vegetative state of air-borne bacteria.

We are inclined to the view, although without experimental proof, that the increased capacity of germicidal aerosols to kill bacteria suspended in the air when suitably humidified may be related to the more favourable conditions prevailing after fusion of the germicide and bacterial particles, i.e. conditions favouring more intimate contact and absorption, to the disadvantage of the bacterium. That the bacterium has not had to adjust itself to an unfavourable environment and has remained in the vegetative state renders the task of the germicide all the more easy. However, we must not lose sight of the fact that in all tests the substitution as solvent for hexyl resorcinol of watery glycols, etc. in place of pure glycols decreased the resultant 'kill' by the mechanically atomized mixture (Twort *et al.* 1940).

Finally, it must be emphasized, and this is very important, that we have unfortunately, meanwhile, no knowledge of the physical characteristics of the particles making up the mists generated by heat comparable to that which we have regarding the particles resulting from mechanical atomization, and we know little enough about the behaviour of the latter. Data concerning composition, size distribution, rate of evaporation and coagulation might easily suffice to explain why in our experiments mechanical atomization of solutions of hexyl resorcinol in propylene glycol seemed to be the method of choice while in the case of resorcinol in watery glycerol heat volatilization gave the better result. Matters might be reversed were there modifications in our technique.

Conclusions

1. The humidity of an atmosphere being disinfected by means of germicidal aerosols influences markedly the success or otherwise of the operation, the number of air-borne bacteria able to survive for a given time being often decreased many fold by raising the relative humidity from 40 to 60% or higher.

2. The effect of increasing the humidity was noted on the survival rate of both atomized broth emulsions of stock laboratory cultures and the flora of atomized normal saliva, but the magnitude of the effect was not similar with all the test organisms.

3. The effect was noted when using as germicidal aerosols heat volatilized or mechanically nebulized solutions of pure phenolic substances, hypochlorites and balsams, but again the magnitude of the effect was not similar with all types of germicidal aerosols, either as regards germicide base or method of producing the mist.

4. No precise recommendations can be given as to percentage relative humidity, germicide and method of generating aerosol in order to obtain maximum disinfection of air, until more data are available concerning the chemical composition and physical behaviour of the operative germicide particles.

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