THE SIGNIFICANCE OF VOLATILITY AND WATER SOLUBILITY IN DISINFECTION PROCESSES

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From the commencement of the researches in these laboratories the aim has been disinfection as opposed to sterilization, because the breathing of air devoid of its normal, saprophytic flora over a long period might eventually lead to a lowering of the powers of resistance of the respiratory epithelium to the invasion of bacteria in general, and, therefore, was to be avoided as far as possible. The ideal conditions for controlling cross-infection in respiratory diseases would appear to be those in which attenuation of the causal organism results, death then being more easily accomplished within the animal body. By this means immunity of the animal to a subsequent infection would possibly be higher than if there had been inhalation of organisms which were already dead.

Although our chief consideration of the effect of germicides on airborne organisms has been from the bactericidal aspect, bacteriostatic effects have not been overlooked. Absence of colonies on the culture media is taken as evidence of the former and size of colonies of the latter. Absence of colonies does not necessarily imply death of the organism, as can be demonstrated by using richer culture medium than usual. The fact that in air experiments, designed to test the sensitivity of several organisms to a particular germicide, different types of culture media may be employed does not matter so much when the results are calculated on a percentage survival basis. Another factor is the possibility of effect of the germicide on the organism after it has settled on the medium. This effect may be a continuation of that initiated in the air or it may be an indirect one, resulting from an alteration of the constitution of the medium by the collection of mist particles or the absorption of vapours. These considerations hardly apply when using ordinary gravity plates, but it may be otherwise where suction apparatus is in use, involving concentration of germicide and bacteria on the culture medium.

Already it has been shown that vapour pressure of germicides is an important factor in air disinfection, and the present series of investigations was mainly designed to correlate four factors, viz. vapour pressure, water solubility, air phenol coefficient and test-tube phenol coefficient. Hence, a study was made of twenty substances, comprising eighteen phenols and two organic acids. Throughout this paper volatility will sometimes be found expressed as air solubility. Some relevant physical characteristics of these compounds are given in Tables 1 and 1A.

Table 1. *Some physical characteristics* (° C.)

Germicide	Solubility: Water at 20° g./100 g.	Solubility: Air at 20° mg./m.^{3}	b.p.	m.p.
Hexyl resorcinol	0·08	0·855	325·0	65·5
Benzyl phenol	0·01	2·122	321·0	84·0
Pentachlorphenol	0·0015	2·5	300·1	190·2
Cinnamic acid	0·055	2·783	300·0	133·0
Hydroquinone	7·1	5·83	286·0	170·0
Orcinol	> 10·0	6·676	288·5	107·5
Resorcinol	140·0	8·43	276·5	110·0
Vanillin	1·0	8·67	285·0	81·0
Amyl-*m*-cresol	0·009	20·34	265·0	< 0·0
Benzoic acid	0·29	37·2	249·0	121·5
p-Chlor-*m*-xylenol	0·02	54·41	251·0	115·0
Chlorthymol	0·01	62·38	261·0	59·0
Isothymol	0·1	91·45	232·8	45·0
Catechol	41·5	110·5	245·0	105·5
p-Chlor-*m*-cresol	0·35	144·8	232·5	55·5
Thymol	0·083	268·6	232·0	50·0
p-Cresol	1·94	800·7	202·5	34·0
m-Cresol	2·18	844·3	202·0	11·5
Phenol	5·0	1668·0	182·0	42·5
o-Cresol	2·45	1772·0	191·5	30·0
Additional substances				
Diethylene glycol	Misc.	28·1	244·5	< 0·0
Propylene glycol	Misc.	443·5	187·3	< 0·0
Maleic anhydride	—	1344·0	200·0	55·0
Mercuric chloride	6·1	2·86	305·0	287·0
Iodine	0·0285	4534·0	185·3	114·0

A determination of the amount of each substance required to give an equal bactericidal effect (near sterility) was made on air seeded with *C. xerosis* emulsified in sterile saliva. In conformity with previous observations the quantities used tended to be proportional to vapour pressure (White, Baker & Twort, 1944; Twort, Baker & White, 1944).

https://doi.org/10.1017/S0022172400019264 Published online by Cambridge University Press

Further tests were made with the germicides at an equal degree of saturation. From these two series there were varying concentrations and varying bactericidal effects available for comparison. These biological results were correlated by the simple ranking method with boiling-points, vapour pressures and amounts in mg./m.^{3} of air required to saturate. There was no significant difference in the

Table 1 A. *Vapour pressures deduced from the formula of Clausius and Clapeyron,* $\log_{10} p = A - \frac{B}{T}$

Germicide	Vapour pressure at 20° C.	Constants A	Constants B
Hexyl resorcinol	0·0000805	9·5738	4004
Benzyl phenol	0·0002108	9·2638	3791
Pentachlorphenol	0·00017	—	—
Cinnamic acid	0·0003434	9·5258	3808
Hydroquinone	0·0009679	9·3758	3631
Orcinol	0·0009835	9·3028	3607
Resorcinol	0·0014	9·4318	3600
Vanillin	0·00104	9·3668	3619
Amyl-*m*-cresol	0·002088	9·5298	3578
Benzoic acid	0·005569	9·4558	3432
p-Chlor-*m*-xylenol	0·006351	9·3328	3381
Chlorthymol	0·006177	9·0678	3306
Isothymol	0·01114	9·4668	3346
Catechol	0·01835	8·8938	3115
p-Chlor-*m*-cresol	0·01857	9·2588	3221
Thymol	0·03273	8·9148	3047
p-Cresol	0·1355	8·8998	2863
m-Cresol	0·1429	8·8818	2850
Phenol	0·3242	8·9768	2774
o-Cresol	0·2997	8·6978	2702
Diethylene glycol	0·00484	9·6648	3511
Propylene glycol	0·1066	9·6278	3106
Maleic anhydride	0·2505	8·5478	2681
Mercuric chloride	0·000193	9·7244	3938
Iodine	0·3264	—	—

The figures for iodine and pentachlorphenol were taken from the literature.

three results of each series, but the number of samples was insufficient to demonstrate whether weight of the molecule *per se* was of any importance. The values obtained indicated, (1) that the greater the air solubility the greater the amount of substance required to obtain a given kill, and (2) with the particular contact time allowed and with 25% saturation the kill tends to become less as volatility decreases. These two points were further examined by testing for extreme effects, the experiments in this connexion being now briefly described.

MAXIMAL AND MINIMAL EFFECTS

The last column of Table 2 shows the approximate amounts used in the air to obtain our standard

Table 2. *The comparative activity of germicides in the test-tube, droplet and air*

Germicide	Test-tube (mg./ml.)	Droplet mg./ml.	Droplet mg./m.^{3}	Air (mg./m.^{3})
Hexyl resorcinol	0·067	0·062	0·025	0·43
Benzyl phenol	0·167	0·62	0·25	0·53
Pentachlorphenol	0·1	0·62	0·25	0·65
Cinnamic acid	1·1	0·83	0·333	0·7
Orcinol	20·0	3·33	1·33	2·0
Resorcinol	20·0	1·67	0·667	2·0
Hydroquinone	2·0	1·67	0·667	3·0
Vanillin	10·0	8·33	3·33	3·0
Benzoic acid	1·1	1·25	0·5	9·3
Amyl-*m*-cresol	0·025	3·33	1·33	10·0
Mean	5·46	2·17	0·87	3·16
Catechol	12·5	0·52	0·208	10·0
p-Chlor-*m*-xylenol	0·222	8·33	3·33	16·0
Chlorthymol	0·05	0·83	0·333	20·0
Isothymol	1·1	8·33	3·33	22·0
p-Chlor-*m*-cresol	0·667	1·67	0·667	30·0
Thymol	0·4	2·78	1·11	65·0
m-Cresol	2·2	5·2	2·08	200·0
p-Cresol	2·2	5·2	2·08	250·0
Phenol	10·0	32·5	13·0	300·0
o-Cresol	5·5	5·2	2·08	400·0
Mean	3·48	7·06	2·82	131·3
Mean of whole	4·5	4·6	1·8	67·0
Diethylene glycol	> 500·0	—	—	7·0
Propylene glycol	> 500·0	—	—	55·0
Maleic anhydride	0·278	3·33	1·33	4·0
Mercuric chloride	0·002	0·208	0·083	0·4
Iodine	0·022	0·052	0·021	1·5

(95% kill on the 5th to 8th min. plate). Actually, in most cases, the amounts shown are somewhat greater than the standard result demanded.

Grading the germicides according to maximal and minimal effects necessitates the adoption of other standards, which are: 100% kill in a minimum of time and, at least, a 50% kill within 15–30 min. A 50% kill is, however, not considered significant unless substantiated by several tests. Thus, for comparison there are available three grades:

Bactericidal effect	Contact time (min.)	Survivors allowed
Minimum (Table 3)	15–18 or 30–33	50%
Standard (Table 2)	5–8	5%
Maximum (Table 3 A)	0–1	0

The germicides in Table 3 are arranged according to the lowest percentage saturation required to demonstrate a definite bactericidal effect on airborne *C. xerosis* (broth emulsion). The first three members stand alone at 1% saturation; the next ten require 5% saturation; of these the first four

https://doi.org/10.1017/S0022172400019264 Published online by Cambridge University Press

require 15 min. and the remainder 30 min. contact. Of the last seven germicides in this table, only vanillin failed at 12% saturation in 15 min.

Sterility in a minimum of time (maximal effect) is illustrated by the results in Table 3A, the germicides being again listed in order of merit. The conditions under which there was slight growth were also taken into account when grading. The best results with the lowest degree of saturation were those in which sterility occurred in 0–1 min. It will be seen that the more volatile substances gave immediate sterility with 12·5% saturation (more

Table 2A. *The variation in phenol coefficients* (C. xerosis) *according to the experimental conditions*

Germicide	Test-tube	Droplet	Air
Hexyl resorcinol	150	524	700
Benzyl phenol	60	53	566
Pentachlorphenol	100	53	460
Cinnamic acid	9	40	430
Hydroquinone	5	20	100
Orcinol	0·5	10	150
Resorcinol	0·5	20	150
Vanillin	1	4	100
Amyl-*m*-cresol	400	10	30
Benzoic acid	9	26	32
Mean	73	76	272
p-Chlor-*m*-xylenol	45	4	19
Chlorthymol	200	40	15
Isothymol	9	4	14
Catechol	0·8	62	30
p-Chlor-*m*-cresol	15	20	10
Thymol	25	12	4·5
p-Cresol	4·5	6	1·2
m-Cresol	4·5	6	1·5
Phenol	1	1	1
o-Cresol	1·8	6	0·8
Mean	31	16	10
Diethylene glycol	<0·02	—	43
Propylene glycol	<0·02	—	5·5
Maleic anhydride	36	10	75
Mercuric chloride	4500	157	750
Iodine	450	625	200

molecules = more collisions), whereas the less, such as hexyl resorcinol and vanillin, needed more than 8 min. contact with full saturation. In this series of tests smaller amounts of some of the substances than were required in the standard test were utilized. This is because the experimental conditions were unavoidably different. As a rule it is found that more reliance can be placed on an analysis of the results given by members of a group tested at one time than on an analysis of the results of different groups tested at different times.

For correlation purposes the arrangement of the germicides as in Tables 1, 2, 3 and 3A were utilized. To repeat:

In Table 1 the order is in increasing amounts required to saturate the air.

In Table 2, in increasing amounts to give the arbitrary standard effect.

In Table 3, in increasing percentage saturations to give a minimal effect, and

In Table 3A, in increasing percentage saturations to give a maximal effect.

It is important to note that the figures in the last column of Table 2 refer to results already available obtained with salivary emulsions of the test organism. Figures in the other two tables refer to broth emulsions. The positions of hexyl resorcinol and resorcinol in the lists indicate, at least, two samples which are likely to vary considerably according to the emulsifying agent in use. When 1 or 2% of gelatine in saline solution was substituted for Reddish broth the bactericidal activity of hexyl resorcinol in the air was considerably damped, but resorcinol and *p*-chlor-*m*-cresol remained unaffected. This may be due to a difference in the absorbability of hexyl resorcinol and resorcinol by gelatine. In this connexion we endeavoured, in the first instance, to ascertain whether there was a difference in the absorption of hexyl resorcinol by gelatine and broth, but there was difficulty in the subsequent estimations of the phenol, and the investigation was temporarily abandoned.

VAPOUR-PRESSURE DETERMINATIONS

The vapour pressures at 20° C. (Table 1A) were deduced from the Clausius and Clapeyron formula

$$\log_{10} p = A - \frac{B}{T},$$

where p = vapour pressure, A and B are constants (calculated from boiling-points at different pressures), and T = temperature ° Abs. Although the vapour pressures so obtained are those of the liquid phase, the figures served as a working basis for the biological experiments. The boiling-points were determined by the bubbling method of Smith & Menzies (1910), and the volatility, expressed as mg./m.^{3}, was calculated from the formula

$$\frac{10^6 p \times M \times 273}{760 \times 22{\cdot}4 \times (273+t)},$$

where p = vapour pressure at t° C., M = molecular weight.

The values obtained by correlating the results depicted in Table 1 with those in Tables 2 and 3A were respectively 0·983 and −0·878, while the results in Table 3 gave no significant correlation with those in any of the other three tables. These values were better than anticipated, for although most of the germicides were solid at room temperature the melting-points were very divergent; and

where the melting-points are much higher than 20° the error introduced is likely to have some significance, since the vapour pressure of a solid is necessarily less than that of the supercooled liquid at the same temperature, and the more so the further that temperature is removed from the melting-point. Nevertheless, no marked difference was observed on dividing into two groups, one, containing the ten highest and the other the ten lowest melting-points, and comparing the kills obtained in several series of tests. Much better results in the former group might have been expected if the vapour-pressure figures used were higher than the true ones, and the percentage saturation thus higher than intended. Our former colleagues Finn and Powell showed ultramicroscopically that a number of phenols which they tested formed supercooled liquid, and, hence, the error in using vapour pressure over liquid instead of solid may not be great.

It is not to be inferred from these remarks that the results with every phenol within the vapour-pressure range at 20° C. of 0·0001 to 0·3 mg. Hg would comply with those in Table 2. Outside these limits it might be necessary in making comparative tests to select a more sensitive or more resistant organism than *C. xerosis*: more sensitive where vapour pressure or molecular potency is very low and more resistant where the opposite conditions prevail.

Taken as a whole, the findings recorded confirm previous isolated observations pointing to a relatively abrupt termination of bactericidal activity of many substances of high vapour pressure.

Since it was desirable to determine the absolute minimal time required for maximal bactericidal effect, it appeared that the 'slit-sampler' of Bourdillon, Lidwell & Thomas (1941) might be suitable, as it records the death-rate from zero time. Unfortunately, there is introduced an error in the possible collection of a large quantity of germicide together with the bacteria. Therefore, absence of colonies after incubation may not be due so much to death in the air as on the medium. Although previous indications of this had been obtained (Baker & Twort, 1944) the twenty chemicals were, nevertheless, tested for maximal effects by this method. In most instances sufficient germicide was collected on the plates to show bacteriostatic action.

To ascertain the concentration of germicides in the agar, capable of producing bacteriostasis, plates

Table 3. *Minimal effect. Minimal percentage saturation of the air in which the germicides are demonstrably operative on* C. xerosis *emulsified in broth*

	Percentage survivors					
	15 min. contact			30 min. contact		
Germicide % sat....	1	5	12	1	5	12
p-Chlor-*m*-cresol	3·2	0·7	0	3·5	0	0
Catechol	7·4	1·3	2·5	12·0	0	0
Hexyl resorcinol	*10·6*	0·8	0	18·0	0	0
Pentachlorphenol	—	37·0	3·3	—	36·0	1·7
Phenol	—	40·0	0	—	4·5	0
o-Cresol	—	44·0	0	—	6·2	0
Chlorthymol	—	*48·0*	0	—	15·0	0
Benzyl phenol	—	57·0	0	—	14·0	0
Orcinol	—	100·0	33·0	—	28·0	6·7
p-Chlor-*m*-xylenol	—	69·0	50·0	—	40·0	13·3
p-Cresol	—	74·0	0	—	43·0	0
Resorcinol	—	81·0	1·7	—	46·0	0
m-Cresol	—	77·0	0	—	*49·0*	0
Thymol	—	80·0	0	—	100·0	0
Amyl-*m*-cresol	—	100·0	1·9	—	95·0	0
Hydroquinone	—	100·0	4·5	—	50·0	0
Benzoic acid	—	100·0	5·8	—	100·0	4·6
Isothymol	—	100·0	25·0	—	100·0	12·5
Cinnamic acid	—	70·0	*26·0*	—	100·0	0
Vanillin	—	100·0	62·0	—	86·0	33·0

	% sat.	15 min. contact	30 min. contact
Diethylene glycol	2·5	83·0	40·0
Propylene glycol	2·5	59·0	20·0
Maleic anhydride	0·005	100·0	40·0
Mercuric chloride	1·0	41·0	22·0
Iodine	0·001	30·0	1·1

https://doi.org/10.1017/S0022172400019264 Published online by Cambridge University Press

were prepared containing known quantities of the substances. Seeding with *C. xerosis* was carried out aerially. As in most tests of this nature the standard was phenol, which gave complete sterility in agar at half the strength required in the Reddish test. Fifteen other germicides acted more or less similarly, so that their phenol coefficients approximated the test-tube figures. Benzoic and cinnamic acids and benzyl phenol each had a considerably lower phenol coefficient in agar, but hydroquinone and catechol were highly bacteriostatic.

Table 3A. *Maximal effect. Minimal percentage saturation of air in which the germicides give sterility of* C. xerosis *emulsified in broth*

	Sterile		Not sterile		
Germicide	% sat.	min.	% sat.	% survivors	min.
p-Cresol	12·5	1	6·25	25	1
Phenol	12·5	1	6·25	31	1
m-Cresol	12·5	1	6·25	34	1
o-Cresol	12·5	1	6·25	75	1
Thymol	12·5	1	6·25	75	1
Chlorthymol	12·5	1	—	—	—
p-Chlor-*m*-cresol	25·0	1	12·5	0·9	1
p-Chlor-*m*-xylenol	25·0	1	12·5	2·1	1
Benzoic acid	50·0	1	25·0	1·7	1
Catechol	100·0	1	50·0	1·2	1
Isothymol	100·0	2	50·0	0·9	1
Amyl-*m*-cresol	100·0	3	50·0	1·7	1
Pentachlorphenol	100·0	3	100·0	20·0	1
Benzyl phenol	100·0	8	100·0	1·2	3
Cinnamic acid	100·0	8	100·0	4·2	3
Resorcinol	100·0	8	100·0	15·0	3
Hydroquinone	100·0	8	100·0	42·0	3
Orcinol	100·0	8	100·0	48·0	3
Vanillin	100·0	>8	100·0	0·8	8
Hexyl resorcinol	100·0	>8	100·0	1·3	8
Diethylene glycol	100·0	>8	100·0	50·0	8
Propylene glycol	100·0	>5	100·0	4·3	5
Maleic anhydride	2·0	1	1·0	13·0	1
Mercuric chloride	50·0	1	25·0	0·8	1
Iodine	0·3	1	0·15	1·2	1

DROPLET TESTS

The droplet tests were devised in order to obtain some idea of the actual amount of germicide responsible for the kill of organisms in the air, although in these tests the bactericidal effect was visualized as taking place partly under test-tube and partly under air conditions. Briefly, the technique consisted of adding the usual volume (0·2 ml.) of *C. xerosis* in broth to 1 ml. of a suitable dilution of the germicide. This mixture was immediately sprayed into the air, and plates exposed as usual. The tests were controlled by atomizing 0·2 ml. of emulsion plus 1 ml. of the diluent. The most suitable solvent was 40 % alcohol in water, concentrations higher than this tending to be lethal to the test organism. As an alternative to alcohol dilute propylene glycol was tried, but was only satisfactory as a solvent in strengths which were bactericidal.

Dilutions of the germicides to be atomized with the test organism were so arranged as to give concentrations in the air varying from 0·1 to 100 % saturation as calculated from the vapour pressures. The aim was to obtain a 95 % kill within 5–8 min. after spraying the mixture into the air; a plate was also exposed 15 min. later. It was observed during these experiments: (*a*) that the kill on the 1 and 5 min. plates was often as good as, and sometimes better than, that on the 15 min. one; (*b*) that the amount of germicide needed to give the required kill was not sharply defined. The end-points shown in Table 2 must be considered as very approximate, as at times little better kills resulted from increasing the strength even sixteenfold.

An analysis of the figures in Tables 1A and 2A shows that the low vapour-pressure members (upper group) incline to high phenol coefficients. This is not so noticeable under test-tube conditions, 73 : 31, as it is when the mixture (germicide and bacteria) is dispersed in the air, 76 : 16, but is most marked when germicide and organism are dispersed separately, 272 : 10. The respective correlation values when using the arrangement in Table 1A, and test-tube, droplet and air results shown in Table 2, are 0·309, 0·586 and, as has already been seen, 0·983.

The value 0·309 relates to test-tube phenol coefficients based on the results obtained with the standard Reddish test. In order, however, to get a better picture of the relation of the activity of a germicide in the air to that in the test-tube the basis upon which activity is assessed should vary on each side of the arbitrary standards employed as a routine in these laboratories. Accordingly, as in the air, minimal and maximal effects in the test-tube were examined.

To determine the maximal effect in the test-tube saturated watery solutions were first prepared, and tested by the Reddish technique, with the object of obtaining sterility in 1 min. Where necessary these solutions were diluted $\frac{1}{2}$, $\frac{1}{4}$, etc., until they failed to sterilize in 1 min., but did so in 2 min., the end-point being taken as that of the weakest solution capable of sterilizing in 1 min. Similar tests were carried out with dilutions prepared from 10 % alcoholic solutions. Comparison of the two sets of results showed that where water solubilities were known the end-points coincided fairly closely within the limits of titration errors, so that it was possible to calculate the approximate water solubility of those substances which were previously in doubt or unknown.

There were now available six series of results, three under test-tube (water) and three under air

conditions. Each series was listed in order of merit as to degree of saturation and actual amount used (phenol coefficient), the fifteen intercorrelation values of each series being compared. Some of these values have already been discussed, and it will suffice if a few general remarks are passed on the results as a whole.

Both in the air and the test-tube the phenol coefficient intercorrelation values between minimal, standard and maximal results were high, but there was no significant intercorrelation between air and test-tube results. This means that for grading disinfectants there is no advantage in making the experimental conditions more or less severe than the standards adopted in the air and the test-tube, and that activity in the former cannot be predicted from that in the latter. When graded according to degree of saturation required in the three test-tube series the intercorrelation values were again high, contrary to what had already been found in the air. These findings have to be considered when applying the partition law to explain the mechanism of action of germicides.

THE EFFECTS OF SOLUBILITY IN THE MEDIA

The correlation of water solubility and test-tube activity of the twenty samples was 0·88. Scrutiny of the data in Tables 1 and 2 shows clearly that the rise in air activity follows more closely decrease of vapour pressure (air solubility) than does rise of test-tube activity with decrease of water solubility. Provided the phenol has a reasonable solubility in water, say 1 %, activity in the test-tube was low. The outstandingly feeble action of phenol in the droplet is not surprising as both water and air solubility are high, but so are the solubilities of catechol, and yet this substance is exceedingly potent in the droplet. Actually, catechol, viewed from the solubility angle, gives a better all-over performance than any other phenol tested.

The bases of comparison were for droplet and air milligrams of germicide per cubic metre of air, and for droplet and test-tube milligrams per ml. of fluid; that three times more organisms were used in the Reddish test than in the droplet was ignored. From Table 2 it will be seen that the test-tube phenol coefficient is little indication of efficacy in the air, but is of more importance in the combined droplet. In the droplet rapid evaporation of the solvent might especially affect bactericidal activity of compounds of low solubility, and highly volatile germicides may themselves evaporate before having much effect on the test organism. When making comparisons between air and test-tube results it should be borne in mind that the initial concentrations of germicide are very different. This is clearly shown by the following w/w concentration figures derived from Table 2:

Germicide	Air	Test-tube	Ratio
Hexyl resorcinol	1 in 3×10^6	1 in 15×10^3	1 : 200
Amyl-*m*-cresol	1 in 13×10^4	1 in 4×10^4	1 : 3
Resorcinol	1 in 65×10^4	1 in 5×10	1 : 13,000

Lack of parallelism between the air and test-tube efficiency is not very easy to explain, especially as there is no obvious reason for assuming that the mechanism of action is different in the two cases. For instance, weight for weight chlorthymol and hexyl resorcinol (both very slightly water-soluble) in an aqueous medium give approximately similar results, while in the air the ratio of amounts required is about 50 : 1. When the activity of catechol and orcinol (both water-soluble) is compared with that of amyl-*m*-cresol (almost water-insoluble), the concentration ratios in the test-tube are 500 : 800 : 1, in the air 5 : 1 : 5. Thus, in air disinfection water solubility does not seem of much importance.

Table 4. *Solubility groupings. Mean amounts abstracted from Table 2*

		Droplet			
		Test-tube (mg./ml.)	mg./ml.	mg./m.^{3}	Air (mg./m.^{3})
Mean	(20)	4·5	4·6	1·8	67·0
Air:					
Insoluble	(10)	5·5	2·2	0·9	3·2
Soluble	(10)	3·5	7·0	2·8	131·0
Water:					
Insoluble	(10)	0·4	2·7	1·1	16·5
Soluble	(10)	8·6	6·5	2·6	118·0

The ratios simplified

	Test-tube : Droplet	Droplet : Air	Test-tube : Air
Mean	1 : 1·0	1 : 37·0	1 : 37·0
Air:			
Insoluble	1 : 0·4	1 : 3·6	1 : 1·5
Soluble	1 : 2·0	1 : 47·0	1 : 94·0
Water:			
Insoluble	1 : 6·8	1 : 15·0	1 : 102·0
Soluble	1 : 0·8	1 : 45·0	1 : 36·0

	Air	Water
	Insoluble : Soluble	Insoluble : Soluble
Test-tube	1 : 0·65	1 : 21·5
Droplet	1 : 3·1	1 : 2·4
Air	1 : 41·0	1 : 7·2

The results under the three heads test-tube, droplet and air are summarized in Table 4. The twenty germicides were divided into groups of ten each under air solubility and water solubility. This grouping method clearly bears out the conclusions noted in the preceding paragraph.

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Bactericidal power of phenols *in vitro* is generally recognized as being related to water solubility (partition law). Substitution of a good solvent for a particular germicide usually does not improve activity, and when such solvent is miscible with water may even lead to decreased action. This principle of high activity being coincident with low solubility would appear to be true also in air disinfection. There are, however, exceptions, both in the air and test-tube, which will be referred to later, where high solubility and high bactericidal activity occur together.

If it be assumed that the mechanism of germicidal action is the same under all our test conditions, then the amount of germicide required to kill each organism in the combined droplet, after evaporation of the solvent, should approximate to that picked up by an organism from the air where germicide and organism are separately atomized. On the whole, smaller amounts (but very much greater on a w/w basis) of germicide were required in the droplet than in the air, the relevant figures being 1 : 37 when the twenty germicides are considered together. By division into groups of low and high vapour pressure the difference becomes 1 : 47 and 1 : 3·6 respectively; so that in high vapour-pressure (air-soluble) compounds wastage is the greater, not only absolutely but also relatively. Again, while the total amount of germicide used in the test-tube was approximately the same as in the droplet, the water-soluble compounds gave the greater wastage in the test-tube, 1 : 1·25, and the water-insoluble in the droplet 1 : 6·8.

OTHER COMPOUNDS

At the end of Tables 1–3A are added five non-phenolic substances which were examined to ascertain if they behaved similarly to the phenols. In addition, a number of further compounds (Table 5) were tried. Some of them are not mentioned in the text, but most failed to show any promise and were temporarily discarded. The three resorcinol compounds were manifestly active, but since their purity was doubtful a more thorough examination of them was deferred until a later date.

Glycols. In the early days of the investigations carried out in these laboratories on air disinfection the glycols provided the chief type of solvent for the phenols under test. Being miscible with water, it was not surprising to find that they had extremely low test-tube phenol coefficients, but it was demonstrated that in the air propylene glycol slightly surpassed phenol in activity (Twort, Baker, Finn & Powell, 1940). Recent tests have shown that propylene and diethylene glycol have air phenol coefficients of the order of the phenols of similar vapour pressure, but tri- and tetra-ethylene glycols seem to be very ineffective aerial bactericides, even near full saturation. Their high hygroscopicity would undoubtedly affect bactericidal activity (Robertson, 1943). The consequent absorption of water would dilute them below their test-tube activity.

Maleic anhydride. This proved to be the most exceptional organic compound tested, only 0·25 % saturation was needed to give the standard kill as against about 25 % saturation of phenols. The difference was more marked when testing for minimal, but less when testing for maximal effects. This is one of the exceptions previously mentioned as having high air solubility and high activity.

Table 5. *Some additional substances tested*

Benzophenone	Resazurine
Benzoquinone	Adipic acid
Ethyl resorcinol	Malic acid
Amyl resorcinol	Maleic acid
Resorcinol benzoate	Fumaric acid
Pentachlor resorcinol	Succinic acid
Benzyl benzoate	Citric acid
Benzyl cinnamate	Mucic acid
p-Aminophenol	Lactic acid
Triethanolamine	Ethyl lactate
Tetraethylenepentamine	Phthalic acid
Heptadecanol	Naphthalene
Triethylene glycol	Diethyl phthalate
Erythritol	Salicylic acid
Thymotic acid	2-4-Dihydroxy benzoic acid
1-2-Dihydroxy-4 : *n*-amyl-phenyl-methyl-ketone	'Izal'
Formaldehyde	'Carbowax 1500'

Phthalic anhydride. In view of the high boiling-point, 295° C. compared with 200° C., of maleic anhydride, more phthalic anhydride was required to give the standard kill than anticipated: 1 mg./m.^{3} gave more survivors than did 1 mg./m.^{3} of maleic anhydride. This, in addition to its strong odour and high melting-point (130·6), excludes phthalic anhydride for practical purposes.

To ascertain the relative merits of resorcinol and maleic anhydride as aerial disinfectants an analysis was made of the results when the experimental conditions were similar as to initial concentration and age of mist, test room, test organism, etc. In all, seventy-one pairs were available for comparison, and of these the figures for each germicide were equal on eleven occasions. Of the remainder, resorcinol gave the better result in the proportion of 3 : 2. Thus, there appears to be little to choose between the two germicides from the point of view of bactericidal efficiency on the airborne test organism used.

Lactic acid. This acid was tested by Lovelock, Lidwell & Raymond (1944) as an aerial bactericide, and their results were, in the main, confirmed in

these laboratories. It was further tested in parallel with maleic anhydride and resorcinol, in hot-plate and mechanical atomization experiments. Fresh and aged mists, in concentrations initially of 1, 4 and 10 mg./m.^{3}, were used, but only in the last concentration, when mechanically atomized and fresh, was the acid really efficient. Less effective mists were produced from the hot plate, undoubtedly due to decomposition.

Salicylic acid. This acid, on mechanical atomization, was more active than lactic acid, but few of the test organisms survived for 5 min. in an atmosphere containing 1 mg./m.^{3}.

Maleic acid. The germicidal aspects of maleic acid were examined, since it was thought that the rapid decay of resorcinol might be due to oxidation to maleic acid. Kar (1942) showed that resorcinol is oxidized by hydrogen peroxide in the presence of tungstic acid sol to carbon dioxide and maleic acid: $C_6H_4(OH)_2 + H_2O_2 = C_4H_4O_4 + 2CO_2 + H_2O$. No CO_2 is formed in the absence of the sol. Oxidation of the acid to CO_2 would account for the lack of activity, even in large concentrations, when the acid is mechanically atomized, although another possible explanation, in view of its high melting-point, is that there are not sufficient vapours produced to give a killing concentration in a short time. Heat-produced mists were very effective, no doubt due to conversion of the acid to the anhydride. This led to the investigation of the bactericidal properties of the anhydride. Presumably, the vapours of the anhydride contact the bacterium without being appreciably affected by the presence of water vapour in the air, but after contact change to the acid occurs in the contained water of the bacterium, in simulation of test-tube conditions, and a lethal effect becomes manifest.

Other acids. When mechanically atomized in amounts of from 10 to 100 mg./m.^{3} of air the other acids listed in Table 5 also proved to be devoid of bactericidal action. On heating they had some activity, evidently due to decomposition. The test-tube phenol coefficients bore no relation to water solubility, but, as is known, depend upon the degree of dissociation (pH) of the acid. The droplet results resembled those found in the test-tube, the difference in the phenol coefficients being within the experimental error.

INORGANIC SUBSTANCES

Iodine has a high test-tube phenol coefficient, in accordance with its low water solubility, but it also has a high air phenol coefficient, although its vapour pressure approximates that of phenol.

Mercuric chloride, on the other hand, is exceptional in that while its water solubility is of the order of that of phenol its test-tube phenol coefficient is high, although behaviour in the air is in fairly close conformity with that of the eighteen phenols.

To sum up, low solubility in either medium is a fair pointer to good bactericidal potency in the case of phenols and, perhaps, glycols, but may not be so as regards some other types of substances.

GENERATION AND DECAY OF THE GERMICIDE IN THE AIR

The likely factors responsible for loss of germicide are: (1) falling out of the large particles produced on generation and by coagulation, (2) adsorption and/or absorption by the surround, and (3) in some cases chemical change. Air changes will decrease concentration, and will be operative on all compounds irrespective of their vapour pressures and physical state.

Thus, when it is desired to maintain a given concentration of germicide in the air allowance must be made for losses due to the above factors. Nevertheless, bactericidal activity does not always correspond with concentration of germicide. While the importance of relative humidity of the air is unquestionable (Baker & Twort, 1941), the degree of saturation of the air with the germicide and the rate at which the mist particles evaporate are governed by the operating temperature.

Decay of particles. The decay of germicide particles (diminution of size) can be measured ultramicroscopically within the usual limits of resolution (Twort *et al.* 1940).

Loss of vapours. We know of no method of estimating the loss of vapours alone when the original generation was from a mist, since it is impossible to determine when all the substance has become molecularly dispersed, and submicroscopic particles cease to exist. Chemical methods give a good criterion of the rate of loss of the germicide as a whole. They do not, however, necessarily reflect results obtained by biological methods.

Biological methods. Biological methods may be efficient indicators of the total amount of airborne germicide, and under certain conditions may show whether the bulk of the substance is present as a mist or vapour. Early work with the ultra-microscope indicated that the life span of mist particles varied among the germicides tested. Substances which showed long mist persistence had correspondingly long biological activity in the air, so it was assumed that cessation of activity was indicative of the change to the vapour state. Recent chemical tests carried out with resorcinol, however, show that there is a rapid loss of total germicide from the air, and with this a parallel decline in biological action. So that in tests where mists were aged, in an attempt to produce an atmosphere containing vapours only, there was, in fact, present

only a small percentage of the original concentration. However, in spite of the rapid disappearance of resorcinol vapours from the air, failure to demonstrate a difference in the kill of organisms when sprayed into the test chamber 1 min. before and after that of the germicide has been consistently recorded. Painting tests (Baker & Twort, 1944), were initiated in an effort to produce vapours only, and more recently evaporation from glass plates at 66° C. was tried with the same end in view.

Evaporation from glass plates. An area equivalent to 0·075 m.²/m.³ of air was painted with an alcoholic solution of each of the twenty germicides, in quantities sufficient to saturate a closed space, if all the substance evaporated. Three separate tests were carried out with each compound, 5, 15 and 30 min. being allowed before inoculation of the emulsion of *C. xerosis* in sterile saliva; after which plates of media were exposed at 5, 10 and 15 min. intervals. The germicides were grouped according to the observed time required for complete evaporation and, as the table below shows, the best results were obtained by inoculating the test organism immediately on completion of evaporation of the substance:

Evaporation (min.)	No. of samples	Best result at 5 min.	10 min.	15 min.
Complete in 5	6	6	.	.
Complete in 10	10	7	3	.
Complete in 15	2	1	1	.
Incomplete 15	2	.	.	2

Substances which evaporated completely within 5 min. showed rapid loss of activity, which was demonstrated by delaying the inoculation of the test organism for 5 or 10 min. beyond the 5 min. allowed for evaporation. The view that loss of activity is directly related to rate of evaporation is further supported by these tests.

TESTS IN ROOMS

Resorcinol. In attempts to conform more closely to practical conditions two rooms of 15 and 44 m.³ capacity were chosen, and variations introduced by having in one series doors shut and in the other partly open.

Preliminary tests were carried out with *C. xerosis* emulsified in broth. A specially designed, thermostatically controlled, electric heater was used to generate the resorcinol continuously at the rate of 0·75, 5·5 and 17 mg./m.³/hr. Judging by the number of plate colonies obtained, an equilibrium was apparently reached within 2 hr., and chemical tests verified this. The middle concentration gave a 95 % kill after 5 min. and sterility after 15 min. contact. The results were better than anticipated because organisms emulsified in broth tend to be relatively resistant to the action of resorcinol, and in ordinary rooms results are, for several reasons, usually inferior to those obtained when using the twin, lead-lined chambers.

Later, sterile saliva was substituted for the broth as emulsifying agent, and in some cases the natural flora of the saliva served as test organism. The emulsions were sprayed into the air 0·5, 1, 2, 3 and 20 hr. after commencement of the experiment, and plates exposed in different positions 1 and 5 min. subsequent to the introduction of the organisms. In most cases where there was no fluctuation in output of germicide from the heater, equilibrium was reached in about 1 hr. The number of survivors on the 5–8 min. plate when equilibrium had been reached, excluding some inconsistences where sterility was obtained, is herewith shown. It was occasionally observed that excessive draught created by stormy weather was apparently responsible for unusually poor kills. Included for comparison are the mean results of forty-five experiments in the closed, twin, lead-lined chambers, where 1 mg./m.³ of resorcinol was generated within a minute or so from a metallic strip rapidly heated electrically. In some cases the saliva had been diluted with equal parts of water.

Doors	mg./m.³/hr.	% survivors *C. xerosis*	mg./m.³/hr.	% survivors saliva A
Shut	1·25	25	2·6	18
	5·0	3	4·0	10
	15·0	1	12·0	5
Open	1·5	20	2·6	24
	2·0	10	7·0	4

Chamber tests

C. xerosis: In 50 % saliva in water gave 3 % survivors
In neat saliva gave 7·5 % survivors

Saliva A: 50 % in water gave 7·5 % survivors
Neat gave 15·0 % survivors

Hexyl resorcinol. The method of generation was as for resorcinol, but owing to the small amount of hexyl resorcinol required the container was so narrow that output was irregular, decreasing as the level of the fluid fell. In spite of this there was a gradual build-up of concentration when doors were kept closed, as evidenced by the increased bactericidal effect. At the commencement, when output was at a maximum (0·65 mg./m.³/hr.) the kill of *C. xerosis* emulsified in broth was 80 % after 5 min. contact, and increased to 99 % when the output, 24 hr. later, was at a minimum (0·3 mg./m.³/hr.). Within half an hour, when the initial output was approximately doubled, the 5 min. plate was sterile. After 24 hr. generation there was about a 90 % kill in the first minute.

https://doi.org/10.1017/S0022172400019264 Published online by Cambridge University Press

Some results with three further substances when using a similar test emulsion of bacteria were:

Germicide	mg./m.³/hr.	% survivors
Maleic anhydride	4·0	33·0
	10·0	2·5
	20·0	0
Benzyl phenol	0·4	33·0
	2·5	3·0
Hydroquinone	0·5	100·0
	0·75	100·0
	2·5	25·0

MISCELLANEOUS EXPERIMENTS

Because it was considered an advantage in practice to have a fluid rather than a solid, resorcinol and diethylene glycol were mixed in the proportion of 1 : 3 (roughly the relationship between their vapour pressures) in the hope that the outputs would remain constant at the same ratio. Tentative laboratory tests indicated that difficulties would ensue on the practical scale, so the investigation was discontinued.

As the application of heat to phenols leads to a gradual darkening, the residues from their prolonged heating were examined biologically. The original bactericidal activity was retained in the case of (1) resorcinol heated at 160°/110 hr., (2) resorcinol and diethylene glycol mixture at 135°/140 hr., (3) hexyl resorcinol at 110°/950 hr. Maleic anhydride also retained its activity after heating at 65°/270 hr.

The effect of alkalinity of the walls of the test room was also examined. A weak acid or alkali sprayed into the air was without apparent influence on the bactericidal activity of resorcinol when operating in the air.

With a view to accelerating the oxidation of resorcinol and to have some evidence that this phenol is easily oxidized in the air, mixtures of solutions of resorcinol and 20 vol. hydrogen peroxide were sprayed into a room. The results, however, were vitiated, as hydrogen peroxide interfered with the chemical estimation of the resorcinol, and greatly enhanced the effects on the test organism. Control experiments proved that the peroxide itself was highly germicidal in the air. So marked was its action that a systematic study of this substance is in progress. At this stage of the investigation it can be stated that as little as 1 mg. (0·05 ml.) per m.³ of air may show a considerable effect in 15 min. and 3 mg./m.³ a 90–95 % kill in 5–10 min. Abramson (1942) reported that hydrogen peroxide persisted for at least 1½ hr. when mixed with 50 % glycerol. In our experience, after 2 hr., with no addition of glycerol, 15 mg./m.³, atomized in a room, are capable of exerting a bactericidal effect to the extent of showing nearly an 80 % kill of *C. xerosis* on the 5 min. plate, and in bleaching the diazo dye used in the chemical estimation of resorcinol.

DISCUSSION

Both in air disinfection and the test-tube solubility is of some significance. With no knowledge to the contrary, it is assumed that death of the micro-organism is accomplished by the same mechanism under either condition, and that the same amount of germicide is absorbed in each case. In order to kill an organism the degree of saturation of the germicide in the operating medium must reach a certain figure. It is very much open to discussion how the lethal dose is transmitted to the organism, and what constitutes a lethal dose. Does the organism receive its quota of germicide by condensation and then subsequent absorption, the latter being solely responsible for the biological effect? The amount condensed *ceteris paribus* will obviously depend upon the degree of saturation, and the amount absorbed upon the solubility of the germicide in the tissues of the living organism, if there be no selective action. To obtain condensation it is necessary for the medium (air) to be supersaturated. When slight supersaturation occurs, permanent droplets cannot be formed because the vapour pressure is higher over a sphere than over a plane surface. If condensation takes place below the saturation point, the surface of the organism must either be porous or of such a nature to cause condensation. One can visualize a 'scrubbing' action by the organism of the germicide from the air, and the higher the degree of saturation the more germicide would be scrubbed out. When similar amounts of substances of different vapour pressures are present, the low vapour-pressure substances would be scrubbed out the easier; but if the substances were present in proportion to their vapour pressures, more, weight for weight, of the high vapour-pressure substances would be removed. Thus, in comparing different germicides at a similar degree of saturation, the amount condensed on the organism will depend upon the vapour pressure of the substance, and it is to be expected that at a high degree of saturation substances of high vapour pressure would operate relatively more efficiently than those of low vapour pressure. These expectations were realized experimentally. The superiority of the high vapour-pressure substances cannot be accounted for by the formation of a monomolecular film on surrounds, which would require approximately similar amounts where molecular weight does not differ much, so that a greater proportion of the low vapour-pressure compounds would be removed from the air to this end. In some cases the effective weight of a substance is far less than that theoretically required for the formation of a monomolecular film.

In the test-tube the partition law probably plays an important part. The amount of germicide re-

ceived by the organism may depend upon how the substance distributes itself between the water of the medium and the lipoid of the organism. If a substance, such as resorcinol, is very soluble in water, then a large amount would be required before sufficient would be absorbed in the lipoid to give a kill, unless the substance was also extremely soluble in the lipoid or was chemically very toxic. Thus, if *Cw/Cl* (concentration in water and lipoid) is of a high order, then a large amount of substance will be required to give a kill.

Injection of animals has led one to conclude that the divergence in molecular potency among many members of the phenol group is not so great as would seem to be implied from the results of ordinary disinfection processes. If the molecular potency of the germicides be equal, then the same number of molecules of each substance would be necessary to give a kill. This means that, although the amount condensed upon the organism may vary, the molecular amount responsible for the kill is the same, and more of one substance than of another must be used when a similar effect on the organism is the aim. If it be assumed that the partition law is the fundamental factor involved, search has to be made for factors other than solubility to explain some of the observed differences in bactericidal activity.

To consider the reaction in the test-tube we have (1) water solubility, (2) molecular potency, and (3) lipoid (bacterium) solubility of the germicide. If among phenols (2) and (3) are, for all members, of the same order, then *ceteris paribus* the bactericidal activity should vary inversely as (1). This, however, is not strictly so and, therefore, (2) or (3) or both must vary. They should vary to the same extent when air is substituted for water on one side of the equation, but again experimental results fail to satisfy theory completely. Put briefly, there seems to be more evidence of a variation in (2) or (3) judged by the results of test-tube experiments than there is from the results of air experiments, but only when the latter are carried out under certain experimental conditions. This point may be illustrated by comparing some of the characteristics of the other phenols with those of phenol itself, each characteristic of the latter being taken as unity. For example, in the case of resorcinol water solubility becomes 28, but the test-tube activity (Reddish test) is the reciprocal of 2, not of 28, so that it requires a lipoid solubility of 14 or a molecular potency of 14 to conform to the partition law. Such a divergence seems to be very unlikely, and is not borne out by air tests where the figure was 0·76 instead of 14. In the test-tube the divergence figures for catechol, hydroquinone and vanillin were 6·6, 7 and 0·2 respectively, with relatively little divergence in the air when the standard test conditions were employed. We incline to the view that among the phenols tested lipoid solubility is likely to vary to a greater extent than molecular potency, although the results of air tests indicate no great difference. It is true that the same may be said of most of the test-tube results, but where there are great discrepancies, viewed from the partition law, the operation of further factors has to be envisaged.

With regard to exceptional cases, such as maleic anhydride and iodine, the mechanism of action is probably different from that of the phenolic substances and glycols. In these two cases the chemical probably 'tacks' on to the organism by virtue of its unsaturated character. Both maleic anhydride and iodine couple on to substances with conjugated bonds. For example, according to the well-known Diels and Alder reaction, maleic anhydride condenses spontaneously with conjugated diolefines. It has been explained already in the text that if maleic anhydride be dissolved in the water medium of the organism, then it is the maleic acid produced that is responsible for the kill. If the lethal effect be a physical one, then the two substances in question must have a higher lipoid solubility than the phenols; the value $\frac{Ca\ (\text{vapour})}{Cl}$ must be low.

While the above remarks may help to explain the relation of vapour pressure of germicide to bactericidal activity, experience shows that in most instances the air is rapidly depleted of vapours and, in consequence, no bactericidal activity is demonstrable. The vapours are, thus, not retained by the air for long, whatever the conditions of generation, and in the absence of leakage from the experimental room other factors must be responsible for the loss. Detoxication of the germicide either before or after condensation and absorption are possible contingencies, but no proof was obtained that one sample was affected more than another in this way.

When examining the question of the rate of disappearance of germicide from the air it is the durability of bactericidal or bacteriostatic effectiveness which is important from the practical point of view. In our experience with resorcinol, rate of disappearance and decrease of action seemingly run parallel. The same cannot necessarily be said to apply to all germicides. A chemical change in the germicide might not show itself on the organism, while a physical change might only be shown biologically. For lining up chemical with biological tests the germicides should be compared both at a similar degree of saturation of the air and at similar amounts per unit of space. Measured chemically, 1 mg./m.^{3} of phenol disappears more slowly than 1 mg. of resorcinol, but phenol has about 200 times the vapour pressure of resorcinol.

https://doi.org/10.1017/S0022172400019264 Published online by Cambridge University Press

It is problematical whether disappearance would be so different were the germicides used in the proportion of 200 : 1, for the results of a limited number of biological tests have not given indications of this.

The inoculation of the air with the germicide by using a hot-plate or spray may be a satisfactory procedure for carrying out mist persistence tests, but can hardly be so when testing germicides of low vapour pressure where the decay of the mist particles to vapour may introduce a serious time lag. For comparing persistence of vapours it is probably better to evaporate the germicide slowly from a large, relatively cool surface, in a thermostatically controlled room. Provided the total amount evaporated is insufficient to saturate the air, local saturation would be unlikely to occur except in the neighbourhood of the evaporation surface.

Let it be assumed, for the moment, that condensation of a germicide vapour be equal on all types of surface, including bacterial. A quantity of germicide dispersed equably in the air as a vapour, and incapable of showing a lethal effect owing to the percentage saturation being too low, might show an effect when dispersed as a mist. In the latter case, owing to local points of higher saturation in the neighbourhood of the particles, the airborne bacteria are in a more favourable position than the surround in the competition for the vapours. On the other hand, when the amount dispersed as a vapour just suffices to provide a lethal degree of saturation in the whole of the experimental space, one should have, if there be no loss, a superior result to that of a similar amount dispersed as a mist, when there will be points where the degree of saturation is sublethal until the particles have completely decayed. When the germicide is evaporated from a large, flat surface (floor), the degree of saturation throughout the air will vary until equilibrium is reached, so that the position factor becomes more complicated.

SUMMARY

1. Solubility of a germicide in the medium (water or air) in which it is operating appears often to be the most important physical characteristic governing degree of activity on bacteria (partition law).

2. Evidence of the importance of solubility has only been derived from a study of the action of phenols and glycols, a high bactericidal activity never being registered unless solubility was low.

3. Other types of germicide were exceptional in that high solubility coincided with high bactericidal activity: iodine and maleic anhydride in the air; mercuric chloride and many organic acids in the test-tube.

4. Mixtures of germicide and bacteria as used in the test-tube were sprayed into the air, the bactericidal effect being usually increased or decreased according as to whether it was originally low or high in the test-tube.

5. The vapour pressures of most of the germicides used were deduced from the Clausius and Clapeyron formula, the boiling-points being determined by the bubbling method.

6. When the amount of a phenol used in the air is approximately proportional to the vapour pressure, the bactericidal effects of the different samples are more nearly parallel when the degree of saturation of the air is low than when it is high.

7. No phenol with a vapour pressure lower than 0·0021 mg. Hg, and tested at full saturation, sterilized the air during the first minute of the experiment, while all samples with, at least, 10 times this vapour pressure did so when at 12·5 % saturation.

8. Among the phenols tested, especially those having low vapour pressures, the concentration (w/w) in air is very much less than the concentration in water in the respective experimental conditions, where but few survivors is the criterion of efficacy. Owing to a number of different factors, the rapid decline in the initial concentration is more likely to be met with under air than under test-tube conditions.

REFERENCES

Abramson, H. A. (1942). A stable hydrogen peroxide aerosol. *Science*, **96**, 238.

Baker, A. H. & Twort, C. C. (1941). The effect of humidity of air on the disinfection capacity of mechanically atomized and heat volatilized germicidal aerosols. *J. Hyg., Camb.*, **41**, 117–30.

Baker, A. H. & Twort, C. C. (1944). Germicidal mists and vapours in air disinfection. *J. Hyg., Camb.*, **43**, 382.

Bourdillon, R. B., Lidwell, O. M. & Thomas, J. C. (1941). A slit sampler for collecting and counting airborne bacteria. *J. Hyg., Camb.*, **41**, 197–224.

Kar, B. C. (1942). Oxidation of resorcinol by hydrogen peroxide in the presence of tungstic acid sol as catalyst. *J. Indian Chem. Soc.* **19**, 499.

Lovelock, J. E., Lidwell, O. M. & Raymond, W. F. (1944). Vapourization of lactic acid as an aerial disinfectant. *Nature, Lond.*, **153**, 743.

Robertson, O. H. (1943). Sterilization of air with glycol vapours. *Harvey Lect.* Series 38, pp. 227–54.

Smith, A. & Menzies, W. C. (1910). Studies in vapour pressure. *J. Amer. Chem. Soc.* **32**, 907.

Twort, C. C., Baker, A. H., Finn, S. R. & Powell, E. O. (1940). The disinfection of closed atmospheres with germicidal aerosols. *J. Hyg., Camb.*, **40**, 253–344.

Twort, C. C., Baker, A. H. & White, L. J. (1944). Air disinfection. *Brit. Med. J.* no. 4361, p. 190.

White, L. J., Baker, A. H. & Twort, C. C. (1944). Aerial disinfection. *Nature, Lond.*, **153**, 141.

(*MS. received for publication* 23. viii. 45.—Ed.)