# THE USE OF ANTISEPTIC SPRAYS FOR AIR STERILIZATION

## A SUMMARY OF A REPORT TO THE MEDICAL RESEARCH COUNCIL

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### MATERIAL AND METHODS

Experiments on the bactericidal properties of mists were performed in a closed room of 850 cu. ft. capacity. The test organism was sprayed into the room through an *Atmozon nebulizer operated from a mechanically driven* Austin diaphragm compressor; the pressure was maintained at 7 lb./sq. in. Air to the compressor was taken through rubber tubing from an adjoining room to avoid accidental admixture of antiseptics into the air used to atomize the bacteria. The output of the nebulizer was 0·22 c.c./min. In the majority of the experiments the test organism was sprayed for 2 min. To ensure that an adequate mist was being emitted, the culture was nebulized in the dark against a dark background, and examined frequently with a light beam. Even distribution of the mist in the room was ensured by convection currents from a large hot-water pipe which ran along the length of two walls about 2 ft. from the floor, and by a small electrically driven fan placed immediately behind the nebulizer.

For the antiseptics a number of different nebulizers has been used. The author, in collaboration with Drs E. Schuster, R. B. Bourdillon and O. M. Lidwell, tested a number of nebulizers of different design. Those that were found satisfactory have been used in this work. It is obvious that mists of greatly different physical qualities will be produced by slight variations in construction of the nebulizer and especially in the degree of baffling produced. From a unit volume of fluid antiseptic the number of mist particles can be varied to any desired degree. Comparisons of the efficacy of different antiseptics have, therefore, always been made with the same nebulizer. A nebulizer so arranged as to give an output of 1·0 c.c. of antiseptic solution per minute has generally been used.

A satisfactory routine method for testing bactericidal mists has been decided upon to be as follows: (*a*) Control: nebulize bacteria for 2 min., expose culture plate for 5 min., cover first plate and expose second plate for 5 min., cover second plate and expose third plate for 5 min.; then clear the air of the room by ventilation. (*b*) Test of antiseptic: nebulize antiseptic for 5 min. (5·0 c.c.); into this mist nebulize bacteria for 2 min.; expose plates as in control.

The collection of organisms from the air by filtration has been found impracticable, owing to collection of antiseptic from the air in sufficient concentration to be bacteriostatic. Wells's air centrifuge was tried, and, although it collects the bacteria from the air more effectively, there are disadvantages connected with its use. These disadvantages are mainly in the special preparation of the media cylinders, in the uneven distribution of colonies in the cylinders which makes counting difficult, and coalescence of colonies due to 'streak' formation by condensation water. Exposed plates were incubated overnight and the colonies counted with the naked eye. A photographic plate (the size of a Petri dish) marked off into segments of a circle, was of value in counting crowded plates.

*Test organism.* In the majority of experiments a β-haemolytic *Streptococcus* of Lancefield's serological group C has been used. This organism was originally isolated by Mr R. E. Glover from the respiratory tract of a ferret. It is grown overnight in 10% horse serum Hartley broth at 37° C. It is diluted 1/20 to 1/50 in the same medium immediately before use. This organism does not grow in this medium at room temperature in the time taken for these experiments. Care must be taken to use smooth cultures only. The organism occasionally produces 'rough' growth. It is advisable, therefore, to have a store of dried cultures to revert to in case of strains in everyday use showing a tendency to produce granular growth. On very moist blood agar plates the organism sometimes forms large mucoid colonies which makes counting difficult. This can be avoided by using plates dried for 1–2 hr. at 37° C. When sprayed from saline suspension the organism dies very quickly and shows greater susceptibility to antiseptic mists than when sprayed in serum broth.

This β-haemolytic *Streptococcus* has been chosen not only because of its distinctive growth, but also on account of its probable similarity to human pathogenic group A strains. It is apparently non-pathogenic to man. Results obtained with a strain of *Staphylococcus albus*, have been much more irregular, and they were found to be unduly resistant to most antiseptic mists.

### RESULTS

(1) *Antiseptic† mists which have been found effective*

*Eugenol carbinol.* This substance is mentioned by Bechhold as an aerial antiseptic in his patent specifica-

* On taking up a military appointment, Dr Pulvertaft submitted a report of his work at the National Institute for Medical Research at Hampstead, during the year 1940–1, to the Medical Research Council. It has proved desirable that the results of this work should be published. As it has been impossible for Dr Pulvertaft to prepare the summary for publication, the task has been undertaken in his absence by one of his colleagues. It is hoped that a detailed description of various aspects of the work will be published at a later date.

† Many of the antiseptics tested were prepared by Dr R. K. Callow and tested by the author in collaboration with Dr Callow.

https://doi.org/10.1017/S0022172400013073 Published online by Cambridge University Press

tion (1935). At first it seemed very promising. It is easily procurable, rapid in its action, and effective at concentrations as low as 0·25% when dissolved in propylene glycol. Result of a typical experiment with eugenol carbinol in propylene glycol is recorded in Table 1. Eugenol carbinol is, however, less effective in solution with 7·5% glycerine in water. Disadvantages associated with eugenol carbinol are: it rapidly deteriorates in solution, results obtained with it have not been uniform; and it is slightly irritating to the eyes and nose. It is of particular interest that eugenol carbinol was found to be an effective aerial antiseptic, while eugenol itself was not. Allyl para-cresol carbinol was also found to have some bactericidal effect, while allyl meta-cresol did not. A more detailed study of these and other carbinols, in particular catechol, would be of great interest.

*Sodium hypochlorite.* This antiseptic has the advantage of being inexpensive, non-toxic and highly effective as an aerial antiseptic even at low concentrations: 5·0 c.c. of a 1% solution in the air of a 1000 cu. ft. room. It deserves special consideration for its deodorizing properties which will always be a desirable attribute under conditions of overcrowding and bad ventilation. Mists of 1% sodium hypochlorite were effective against suspensions of haemolytic streptococci atomized into the air of the test room, and usually also effective against streptococci emitted by ferrets suffering from mixed infection with influenza virus and a group C β-haemolytic *Streptococcus*. In some experiments with ferrets less satisfactory results have been obtained, most probably because the state of activity of a ferret under test and the number of times it sneezes are uncontrollable. Many experiments, and especially those performed in cubicles with *Streptococcus*-infected ferrets, have indicated that concentrations as low as 0·2%, as recommended by Baker, Finn & Twort (1940), were inadequate. When hypochlorite is used, attention must be paid to a number of details. Thus, nebulizers emitting large drops must be avoided owing to harmful effects on coloured fabrics. Also materials used to construct the nebulizers must resist the corrosive action of hypochlorite.

*Hexyl resorcinol in propylene glycol.* A 0·25% solution of hexyl resorcinol in propylene glycol was found to be effective, although slower in action than a similar solution of eugenol carbinol or 1% aqueous hypochlorite.

*Cathechol.* Catechol is completely non-irritant in the form of a mist, and the author has experienced no ill-effects on breathing heavy mists of a 20% solution for repeated short periods. It has very striking bactericidal properties for sprayed organisms when used as weak as 2·5% in solution in propylene glycol. It has been remarkable that almost complete kill of sprayed organisms occurs within 15 min. with catechol, even when it is used in aqueous solution. Results with hexyl resorcinol are much less striking. The killing effect is probably due largely to the speed of action of catechol as compared with hexyl resorcinol. Catechol is, however, relatively toxic, and, therefore, cannot be used in human habitations. It may, however, find application in horticulture and industry.

Efficient aerial antiseptics sometimes have a marked effect on colonial appearances of organisms. Differences in the degree of haemolysis produced by β-haemolytic organisms, irregularities in size of colonies, variations in the degree of pigmentation when pigment-producing organisms were used, have all been observed. These observations strongly suggest that, when organisms are not killed by the antiseptic, they may be altered in characteristics of growth or even virulence. In the case of the *Streptococcus* (group C) used in these experiments, striking differences in colony appearance, depending on the state of dryness of the medium, have been observed (van den Ende, unpublished observations). At the time that this work was done the variations had not been adequately investigated. The variations observed with some of the antiseptics may not, therefore, be directly related to the action of the antiseptics.

Table 1. *Showing the effect of eugenol carbinol and hexyl resorcinol in propylene glycol on haemolytic* Streptococcus *(group C) sprayed in* 10% *horse-serum broth*

| Antiseptic | 0–1 min. | 1–2 min. | 2–3 min. | 3–4 min. | 4–5 min. | Total 0–5 min. | 5–10 min. |
|---|---|---|---|---|---|---|---|
| | | | | No. of bacteria | | | |
| Control (no antiseptic) | Conf. (1920+) | 1920 | 1960 | 1990 | 1740 | 9530 | 7160 |
| Eugenol carbinol 0·25% in propylene glycol 0·5 c.c. in 800 cu. ft. | 1230 | 590 | 196 | 152 | 95 | 2263 | 133 |
| Hexyl resorcinol 0·25% in propylene glycol | Conf. (2240+) | 2240 | 2200 | 1610 | 1140 | 10430 | 1740 |

The antiseptics tested differ markedly in their speed of action. Eugenol carbinol and sodium hypochlorite, for instance, show significant action within 2 min. Others such as hexyl resorcinol have no appreciable bactericidal effect in less than 8 min. No antiseptic efficient after 10 min., while ineffective earlier, has been found and, in general, effects after 15 min. have not been studied; obviously such effects would have little practical value. The results recorded in Table 1 clearly show the difference in the speed of action between 0·25% eugenol carbinol and 0·25% hexyl resorcinol in propylene glycol. In general there appeared to be a negative correlation between speed and duration of antiseptic activity.

Aerial antiseptics differ also in the length of time after spraying that they remain effective. Hexyl resorcinol in propylene glycol is almost as effective 30 min. after as immediately after spraying. The lack of activity of a euginol carbinol mist after the same delay is in marked contrast (see Table 2). A large number of substances was tested and found to be ineffective as aerial antiseptics whether used in aqueous solution or in propylene glycol.

https://doi.org/10.1017/S0022172400013073 Published online by Cambridge University Press

### (2) *The effect of antiseptic mists on dry and dust-borne organisms*

Early experiments were performed with the late Sir Patrick Laidlaw on bacteria sprayed into aspirator flasks which contained a small volume of saturated calcium chloride solution. The partial drying of the mist, which results from the presence of calcium chloride, resulted in a greater resistance to antiseptic aerosols. Twort has also reported that drying of bacterial mists results in greater resistance to antiseptics.

In order to simulate more closely the dry dust-borne organisms, such as are encountered in hospital-ward air after bedmaking, use was made of (1) streptococci dried in high vacuum over $P_2O_5$, ground to a fine powder in a mortar and distributed into the air of the test room with the aid of a fan, and (2) spraying cultures of a group C *Streptococcus* on to a blanket, drying overnight in the incubator and distributing the organisms in the air of the test room by beating the blanket. In both tests no significant bactericidal effect could be demonstrated even when concentrations of antiseptics were used that were highly effective against organisms sprayed in serum broth. This failure to destroy dry dust-borne organisms may, of course, at least in part have been due to the fact that the organisms were probably present in clumps rather than as single organisms, and that the presence of a relatively large volume of foreign matter may render the antiseptic ineffective.

*Tests in occupied spaces.* Experiments performed in hospital wards have shown that the organisms liberated from bed clothes during bedmaking were resistant to the effect of antiseptic mists in concentrations known to be rapidly lethal to sprayed moist organisms. Tests were performed in hospital wards (general medical wards) and in overcrowded large rooms. The number of organisms which could be recovered from the air was small, except when beds were made. Only on one occasion was a pathogenic micro-organism—a β-haemolytic *Streptococcus*, group G—recovered from the air, viz. from the air of a ward in which a patient dying with a malignant recto-vesical fistula was nursed. A similar *Streptococcus* was present in the faeces of the patient.

Tests performed in rooms occupied by normal people seemed to indicate that, even under conditions of overcrowding, the air contained few organisms referable to the respiratory tract. The majority of organisms encountered appeared to be dust-borne.

### (3) *The effect of solvents*

Glycerine was a constituent of the antiseptic solution containing resorcinol, which was used by Trillat, and, theoretically, its presence should prevent the drying of the mist and thus enhance its action. With the nebulizers commonly employed the addition of glycerine in concentrations higher than 25 % greatly reduced the output of mist. For concentrations of 0–25 % of glycerine the output was reasonably uniform. In none of the experiments, in which glycerine was used, was there an improvement of the bactericidal effect of the mist—in fact with many concentrations the bactericidal effect diminished owing, no doubt, in part to the poorer output. The addition of glycerine to hypochlorite solution results in the formation of chloroform, and this mixture can, therefore, not be used.

Propylene glycol, which was introduced by Twort, is probably the only organic solvent worth considering. It cannot be used with hypochlorites, but the majority of other efficient aerial antiseptics are probably more efficient in this solvent. Propylene glycol alone has been shown to be an effective aerial antiseptic (Robertson, Bigg, Miller & Baker, 1941; Robertson, Bigg, Puck & Miller, 1942). It has repeatedly been shown, however, that solutions of antiseptics, such as hexyl resorcinol or eugenol carbinol, are more effective in solution in propylene glycol than in aqueous solution. No direct comparisons of propylene glycol alone and the antiseptics dissolved in propylene glycol were made. However, many experiments were performed in which effective and ineffective aerial antiseptics, both dissolved in propylene glycol, were compared. In no case did an antiseptic, which was entirely ineffective in aqueous solution, prove effective when used in propylene glycol. Thus, an antiseptic like phenol, which has no appreciable bactericidal effect when sprayed in aqueous solution, is also not effective when used in solution in propylene glycol. It may be that the value of propylene glycol as solvent depends at least in part on its own antiseptic power—which, under the conditions of these experiments, was very slight. Unfortunately, propylene glycol is costly in this country at present.

Table 2. *Showing the effect of ageing on hexyl resorcinol and eugenol carbinol mists*

| Antiseptic | 0–5 min. | 5–10 min. | 10–15 min. |
|---|---|---|---|
| | | No. of bacteria | |
| None | 400 | 280 | 180 |
| None | 721 | 650 | 590 |
| Hexyl resorcinol 0·25 % in propylene glycol. Fresh | 5 | 0 | 0 |
| Ditto, 30 min. old | 67 | 0 | 0 |
| Eugenol carbinol 0·25 % in propylene glycol. Fresh | 46 | 0 | 0 |
| Ditto, 30 min. old | Semi-conf. | 250 | 100 |

Ethyl phthalate has given satisfactory results, but is undesirably irritating to the respiratory tract.

### (4) *The cumulative action of antiseptic mists*

Twort has reported that, when several experiments were performed in the same small chamber, concentrations of antiseptic initially ineffective are found to be highly efficient in later experiments. This effect could be explained by the presence of antiseptic adhering to the walls. A similar effect was not seen in the larger (1000 cu. ft.) experimental room used for most of this work, in spite of the fact that the walls were washed down very infrequently. A cumulative action was, however, suggested by experiments performed in a

https://doi.org/10.1017/S0022172400013073 Published online by Cambridge University Press

cubicle containing ferrets infected with influenza virus and group C haemolytic streptococci. Thus, eugenol carbinol was, in most experiments in which this was tested, found to lose its bactericidal effect within 30 min. after spraying. The presence of even 'old' inactive mist, however, apparently increased the efficacy of newly introduced mist. It was, as previously mentioned, however, difficult to control the experimental conditions with doubly infected ferrets, so that this point will require further investigation. It is suggested that the cumulative effect of an antiseptic mist may be explained on an assumption that ageing mist still contains a certain percentage of active antiseptic molecules per particle, or that the antiseptic may 'age' either through chemical change, e.g. oxidation, or through volatilization of antiseptic. In the case of an antiseptic with low vapour pressure, the amount escaping to the vapour phase may be greatly modified by pre-existing antiseptic vapour.

Although the phenomenon has not been adequately investigated, it is obviously of great practical importance. As long as the antiseptic mist persists in the air, it may be necessary to add new mists only in small amounts. Replacement once in 30 min. has been adequate in many experiments. It may be possible to obtain the required effect even with less frequent spraying. On the other hand, experiments, in which both test organisms and antiseptic were nebulized continuously, suggest that the continuous output of small amounts of antiseptic may be as effective, or more so. Thus, in an experiment, in which both the bacteria (group C *Streptococcus*) and the antiseptic (0·25 % hexyl resorcinol in propylene glycol) were sprayed continuously for half an hour, there was marked reduction in the numbers of bacteria in the air as compared with a control period when only the bacteria were sprayed (see Table 3). The amount of antiseptic sprayed per minute was very much less than in the usual type of experiment.

Table 3. *Showing the effect of continued spray of small amount of antiseptic solution on haemolytic streptococci sprayed in* 10 % *horse-serum broth. The antiseptic spray in B was commenced 5 min. before spray of bacteria*

| Antiseptic | 0–5 min. | 5–10 min. | 10–15 min. | 15–20 min. | 20–25 min. | 25–30 min. |
|---|---|---|---|---|---|---|
| | | | No. of bacteria | | | |
| A. None | 35 | 112 | 153 | 255 | 290 | 330 |
| B. 0·25 % hexyl resorcinol in propylene glycol | 16 | 9 | 14 | 8 | 16 | 10 |

### (5) *The effect of surface-tension reducing agents on the efficiency of bactericidal mists*

Surface-tension reducers were originally added to antiseptics used in the form of mists, for the purpose of enhancing their value in surface sterilization (Pulvertaft, Lemon & Walker, 1939). Twort published a formula of an effective aerial antiseptic which includes a surface-tension reducer. In the present series of investigations, a wide variety of surface-tension reducing agents were tested, either alone or in mixtures with antiseptics. No improvement in the bactericidal effect of the atomized antiseptics was observed in the presence of a surface-tension reducing agent.

### (6) *The effect of breathing antiseptic mists on subsequent inhalation infection*

(This work was done in collaboration with M. van den Ende and D. G. ff. Edward.)

It was originally intended to determine whether the inhalation of antiseptic aerosols used for air sterilization has any appreciable effect in preventing respiratory infection. Hypochlorites and hexyl resorcinol were tested in this way. Later, the investigation was expanded to include acriflavine and proflavine, and, after the demonstration of the effectiveness of sulphonamides in wound infections when applied locally, sulphathiazole. Mice were exposed to high concentrations of these substances in the form of mists of aqueous solutions, or, in the case of sulphathiazole, dense clouds of insufflated fine powder. Quantitative examinations showed that less than 10 % of the amount aspirated reached the periphery of the lungs. Nevertheless, the concentrations reached in the peripheral lung were in many experiments at least equivalent to concentrations which *in vitro* proved to be bacteriostatic for the test organism—*Pasteurella muriseptica*. In the case of proflavine, tests were also conducted with a strain of influenza virus A. In no case did treatment with an antiseptic significantly reduce the incidence or severity of infection which resulted from exposure to the test virus or bacterium immediately after the treatment.

## CONCLUSIONS

As criteria of a good antiseptic for use as a bactericidal mist are suggested: 90 % reduction of bacteria, compared with the control when not more than 5 c.c. of the antiseptic solution, at a strength not greater than 1 %, is nebulized into a room of 1000 cu. ft. capacity, concentration in air = 1 g. in 20,000 cu. ft. It is probably true that, as for many antiseptics, the same amount of antiseptic has a greater bactericidal effect when a large volume of a weak solution is nebulized, than when a small volume of a correspondingly stronger solution is used. But in practice, a compromise is necessary, as spraying of large amounts of fluid produces a visible cloud and undesirable humidity. Stronger concentrations, on the other hand, may be irritating to the eyes or nose.

The tests performed in occupied spaces have shown that, in the absence of epidemic respiratory disease, relatively few organisms referable to the respiratory tract are encountered in the air. Under these circumstances, there would be no need of antiseptic mists; in fact the antiseptic mists, in the concentrations found effective against moist sprayed bacteria, would have no effect on the organisms encountered. At times of epidemic respiratory disease, on the other hand, circumstances may quite well be different. Large numbers of

infective droplets disseminated from the respiratory tract may then be present in the air, and on these antiseptic mists may well be expected to have a lethal effect.

When antiseptic sprays are used in occupied spaces, the distribution of the mist is obviously of importance. Mist emitters must be placed near air inlets, but it may not prove suitable to incorporate them in duct systems, owing to adherence of mist particles to the ducts which operate as a baffling system. In large rooms the best layout may prove to be the use of several small units, rather than a single large one. A large unit almost inevitably produces an undesirably high concentration of mist in the vicinity of the nebulizer, and too low concentrations elsewhere. It is urgently desirable that simple methods of air analysis for mist concentrations should be available, so that the uniformity of distribution, especially in large rooms, can be controlled.

In view of the apparent cumulative action of mists, and in view of the continuous emission of infectious droplets from the respiratory tract, a continuous or semi-continuous mist emission is desirable. This forms an argument for the use of mechanically operated nebulizers. Small foot compressors will probably prove impracticable except in small spaces, where the need for air disinfection will not be so urgent.

The output of the nebulizers must, of course, be sufficient to maintain an adequate concentration of antiseptic. In calculating the required output of nebulizers for any particular room, the ventilation and the number of occupants in the room must be taken into account.

The author is in agreement with Baker *et al.* (1940) on the efficacy of electrolytic sodium hypochlorite. For general and extensive use in the prevention of human and animal infection, sodium hypochlorite seems the best suited to present conditions. For special cases, the more expensive and more efficient glycol solutions of phenolic compounds may be employed. The advantages of sodium hypochlorite are its extreme cheapness, non-toxicity, its efficiency in low concentrations, its deodorizing property which is a desirable attribute, especially where overcrowding and bad ventilation exist. When hypochlorite is used, special care is necessary to avoid nebulizers which emit large droplets (damage to fabrics).

For other purposes, e.g. in horticulture and industry, other antiseptics, such as eugenol carbinol, hexyl resorcinol, and catechol, may be useful. Although the possible toxicity of some of these must be kept in mind, this danger should not be overrated. An individual breathing a maximum of 1000 cu. ft. in 24 hr. will inhale at the most 5 c.c. of a 1% solution of the antiseptic, i.e. about 0·02 g. The total amount of antiseptic inhaled will be retained, but at least 90% will be held up in the upper respiratory passages whence a good deal will be removed by blowing the nose.

The work has been greatly assisted by the generous loan of apparatus by André Components, Milton, Austin Compressors, to whom the author wishes to express his gratitude.

The author wishes to express his appreciation to Dr Twort and others at the Portslade Laboratories for their help and the great interest they have shown in his work.

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(*MS. received for publication* 2. vi. 43.—Ed.)