THE CONTROL OF AIR-BORNE BACTERIA AND FUNGUS SPORES BY MEANS OF AEROSOLS

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In a previous communication we (Pulvertaft, Lemon & Walker, 1939), gave an account of a method of sterilizing air by means of finely atomized antiseptic solutions, founded on the principles outlined by Trillat (1938). His claims were fully confirmed.

The mists used in this method may be produced by a number of different appliances, and in the experiments here described were produced by the "Phantomyst" (André Components, Ltd.). The essential appliances are: (1) a mechanical compressor, electrically driven, and (2) an atomizer, usually of scent-spray type, provided with (3) a baffling system which returns to the reservoir droplets above a certain size, thus avoiding much wastage.

A mist produced in this way has been called an aerosol by Trillat. There are indications that in an effective bactericidal aerosol the radius of the individual droplet is of the order of 1–2 μ. Larger droplets rapidly fall to the ground. The effect of atomizing the antiseptic solution is to fill the room so treated with a very large number of minute droplets, each initially containing the same concentration of antiseptic as the parent solution. Convection currents maintain these particles in suspension for a period of many hours, and during that time there will be opportunities for contact between bacteria and antiseptic droplets, which vastly outnumber the bacteria, and are of the same order of magnitude.

It is necessary to emphasize the fact that a vaporized antiseptic produces a different state of dispersion in the air because its final state is one of solution in air and its bactericidal concentration comparable to a solution in water. In the case of aerosols, however, the total amount of antiseptic in the air is exceedingly small, and often scarcely appreciable to the senses. Consequently, aerosols may be safely used where the use of vaporized antiseptics would be undesirable or impossible, especially if continuous air sterilization was desired.

When a parent solution is atomized, its original characteristics may, however, be quickly changed. Though experimental proofs have not been obtained the following explanations are suggested to account for the changes. The minute droplets of the aerosol probably lose fluid by evaporation, and the antiseptic itself, if of high vapour pressure, may in consequence pass into the atmosphere. Apparently for this reason a great many antiseptics, effective under

ordinary conditions, are useless as aerosols; indeed, those available are very few, especially if a product imperceptible to the senses is aimed at. To be satisfactory the antiseptic must possess a low vapour pressure, and be soluble in water or in an innocuous solvent, preferably of low vapour pressure. The addition of a hygroscopic substance such as glycerol to the water is desirable in order to prevent the evaporation of all the water from the aerosol droplets. In the absence of such a hygroscopic substance the aerosol may consist in a very short time of semi-solid particles of no bactericidal value. As aerosols such standard antiseptics as phenol, lysol, and monsol, have little bactericidal value even at concentrations sufficiently high to be most unpleasant. In contrast, resorcinol, although possessing a relatively low lethal coefficient, produces a highly efficient bactericidal aerosol at a very low concentration, especially when glycerol is incorporated in the aqueous solution.

METHODS OF ESTIMATING BACTERIAL CONTENT OF AIR

In these experiments, unless otherwise stated, bacteria have been introduced into the air by a throat spray in the form of saline or broth suspensions. A known volume of culture, of known bacterial concentration, was atomized in a room and as a control the same suspension was always used in another room of the same size and shape.

The number of organisms in the air was estimated by counting the colonies growing on agar plates exposed for fifteen minutes. The results were checked on several occasions with a Wells air-centrifuge, which gave comparable results. Usually the test organism was *B. prodigiosus*.

In calculating the concentration of the antiseptic in it, the air has been considered as a diluent of the fluid antiseptic. Thus a concentration of 1:100,000,000, implies that one part of fluid antiseptic is suspended in the form of a mist in 100,000,000 parts of air. The concentration of the antiseptic in the parent fluid was varied as shown in the experiments quoted.

THE VOLUME OF THE OUTPUT FROM THE ATOMIZER

It has already been stated that the total volume of fluid atomized is no measure of the efficiency of the atomizer, since in some cases the bulk of the output may be wasted in the form of large droplets. The mechanical effect of the air stream and the viscosity of the parent fluid can be altered at will, within certain limits, and affect the results. Heating the parent fluid produced a large increase in output but experiments showed that, with an aqueous solution, the additional output was mainly water, and no increased efficiency of the aerosol formed was obtained. These facts were determined by measuring the specific gravity and by chemical titration of the solutions remaining after the experiments.

The value of air-mixing devices

In the absence of mechanical disturbance of the air, convection currents must be relied upon to bring the bacteria and the aerosol droplets into contact.

An electric fan placed behind the outlet of the apparatus is very useful in hastening this contact. A fan, with the object merely of distributing the mist more evenly, was first used in these experiments when very minute quantities of an aerosol were being tested in a large room. The result showed that a very small quantity of an aerosol, when distributed by a fan, had an efficiency equal to a far larger quantity when no fan was used.

Table I shows the results of a test on *B. prodigiosus*, with the resorcinol-glycerine mixture, sold under the trade name of "Aeryl". The organisms were introduced into the air from a throat spray. Fifteen minutes elapsed between the time the "Phantomyst" was stopped and the beginning of the exposure of the agar plates for a period of fifteen minutes.

Control tests were performed in a room of identical shape and dimensions, and with equal numbers of bacteria. Results are expressed as percentages of the control.

Table I

	Concentration of aerosol	Result %
Fan in use	1:100,000,000	0·2
Fan not in use	1: 20,000,000	2·0

The efficiency of the droplets of antiseptic is apparently at its maximum when they are first formed and any method which accelerates their contact with the bacteria is advantageous. Certain forms of forced ventilation, such as the Plenum system, can be usefully incorporated in an air-sterilizing technique without the use of a fan, but such ventilating systems cause a rapid loss of aerosol from the room. Under these conditions a cheap aerosol is highly desirable.

The effect of atmospheric temperature

The effect of raising the temperature of the parent fluid has been mentioned. The influence of a rise in the atmospheric temperature on an existing aerosol is of great importance, because it is accompanied by a corresponding rise in the rate of vaporization of both solvent and solute, and a corresponding fall in the efficiency of the aerosol. This was illustrated by experiments carried out in rooms of similar shapes and dimensions maintained at temperatures of 4 and 38° C. respectively. The result with "Aeryl" was that the concentration of aerosol in the room at 38° C. was twice that in the room at 4° C. but the bactericidal efficiency was less than half. On the other hand in experiments conducted at 19 and 4° C. there was little difference in the efficiency.

In the latter experiment two factors were operating in contrary directions at the higher temperature, (*a*) the fall in efficiency of an aerosol, owing to

vaporization, and (*b*) the increase in the lethal coefficient of the antiseptic. At the lower temperature, though the lethal coefficient of the antiseptic was lower, the efficiency of the aerosol was higher because vaporization was diminished.

We have had no opportunity of determining the effect of decreasing the atmospheric temperature below zero. While within the temperature ranges of the temperate climates the effects of vaporization and lethal coefficient of temperature cancel each other out, it might be anticipated that both at very high and very low temperatures aerosol efficiency would be much diminished.

HYPOCHLORITE SOLUTIONS AS AEROSOLS

Masterman (1938) described the use of a proprietary solution of sodium hypochlorite for air sterilization, using a mechanically operated vulcanite atomizer. Trillat (1938) also stressed the value of hypochlorite solutions. Aerosols of this type are in many ways superior to resorcinol, because they are very cheap, and produce a pleasantly fresh atmosphere, as well as being completely innocuous to mucous membranes. Theoretically, however, the life of such aerosols should be short, owing to the high vapour pressure of the solution. Claims are made that certain proprietary hypochlorites are more stable than the B.P. product.

Masterman found that his arrangement gave very good results for the sterilization of the atmosphere of workshops, offices and other types of rooms. Under experimental conditions in which the atmosphere is artificially infected with known bacteria and in heavily contaminated air we have found that the aerosol prepared from this proprietary liquid had a relatively low bactericidal value but that the addition of 20% glycerine to the preparation so greatly increased the efficiency of the resulting aerosol that the number of colonies appearing on a test plate after the exposure of a heavily contaminated atmosphere to a concentration of 1:6,000,000 for 30 min. amounted to about only 2% of the control.

Experiments were continued with a solution of sodium hypochlorite at a concentration of 20% (10% available chlorine). This solution was diluted with water and glycerine so that the final product contained 2% sodium hypochlorite and 20% glycerine. This strength was decided on because it was found to be the greatest concentration that provided an aerosol which was not irritant to the mucous membrane. In experiments carried out with this concentration under the conditions previously mentioned, it was found that the solution without the addition of glycerine was approximately 98% effective while that with glycerine added was 99·8% effective. The test organisms for these experiments were *B. prodigiosus* and *Streptococcus haemolyticus*.

Although the effective concentrations for hypochlorite aerosols are high as compared with the very low effective concentrations for resorcinol preparations they have certain advantages which are worth recording. Hypochlorite is

extremely cheap when compared with resorcinol preparations which are costly. While at effective concentrations resorcinol aerosols are scarcely noticeable, the effective concentration of a hypochlorite aerosol is, on the other hand, quite pleasant and imparts what has been described as a pleasant "Sea Breeze" odour to the atmosphere and, what is more important, it has been observed to have a marked deodorizing effect upon the atmosphere of rooms into which it is introduced.

The efficiency of hypochlorite preparations for the purpose of sterilizing surfaces was investigated in a series of experiments. We have shown previously (1939) that for the sterilization of surfaces with resorcinol the addition of a surface-tension reducer is essential and we have always incorporated 0·5% "Lissapol" (sulphonated oleic acid). The standard test, described previously (Pulvertaft, Lemon & Walker, 1939, p. 10), was used in which the concentration of aerosol within the test chamber was raised to 1:30,000 and the smooth rubber test surfaces were exposed to the aerosol for periods of 15, 20 and 25 min. At the conclusion of the test period each test surface was applied to an agar plate. Table II gives averages of the results obtained, which are expressed as percentages of control growth. The test organisms used were *B. prodigiosus* and *Streptococcus haemolyticus* but separate figures for each organism are not given as no significant difference was observed between them.

Table II

Period of exposure min.	With 0·5% Lissapol %	Without Lissapol %
15	75	15
20	50	10
25	25	3

The results are somewhat remarkable in that the addition of a surface-tension reducer greatly reduced the efficiency of a hypochlorite preparation. Hypochlorite-glycerine solutions must be made up immediately before use.

The Rideal-Walker coefficient of a solution of hypochlorite as used in these experiments was 0·4.

A RESORCINOL MIXTURE FOR BOTH ATMOSPHERIC AND SURFACE STERILIZATION

The original preparation devised by Pulvertaft *et al.* (1939) for the sterilization of surfaces was known as "G.M.2" and consisted of a 5% solution of chloramine T in a mixed liquid composed of water, alcohol, acetone and glycerine, containing 0·5% of a surface-tension reducer. Although the preparation could also be used for sterilizing the atmosphere comparatively large concentrations had to be used and the time necessary to produce a sufficient quantity of aerosol for a large room was inconveniently long.

In view of this disadvantage and because it was desirable to have one liquid that would efficiently sterilize both the atmosphere and surfaces, an

amalgamation of "Aeryl" and "G.M.2" was decided upon. Owing to its comparatively low solubility chloramine T has the disadvantage that it tends to deposit upon the jet of the injector and therefore it was discarded and resorcinol was substituted, while the glycerine in the solvent was replaced by ethylene glycol. In other respects the solution was similar to "G.M.2".

The final product was known as "Aeryl II" and was subjected to a series of tests for atmospheric sterilization. It was ascertained that an exposure of 15 min. to a concentration of 1:50,000,000 resulted in a test growth amounting to only 0·1% of that in the control. A series of tests of its ability to sterilize surfaces showed that after an exposure of 10 min. it was 99% efficient and that after an exposure of 20 min. 100% efficient. The exposure in each case was to a concentration of 1:30,000. Before glycols are recommended for air sterilization proof of their harmlessness in low concentrations over long periods is essential.

SPORE-BEARING ORGANISMS

The most favourable result obtained with a 48 hr. broth culture of *B. subtilis* was the destruction of 70% of the inoculum. It is probable that the survivors represented the spores and that the vegetative forms were destroyed.

AIR-BORNE FUNGUS SPORES

The growth of fungi in undesirable places is the cause of almost incalculable loss in industry and of less serious, but still extensive, damage in many households. If any source of mould infection exists the spores will be carried through the atmosphere by means of air currents. Particularly in dusty areas, it has been considered advisable to purify the air entering such buildings as factories producing or using materials which may act as substrates for the growth of fungi and be damaged thereby. Up to the present time the efficient and permanent purification of the air in such places has presented so many practical difficulties that its application has been limited. The only example of its practical application on a large scale is in the food-canning industry where the air in contact with the material is heated to a temperature sufficient to destroy any bacteria or fungus spores. The only other practical method of preventing contamination by such organisms is the incorporation of a toxic substance or antiseptic in the final product. Since in a large number of products this method is undesirable the introduction of any other method which would prevent their growth would be advantageous.

One example of the destruction which may be brought about by the growth of such fungi as *Cladosporium herbarum* is that which occurs in meat refrigerators. In refrigerators the infecting spores are almost certainly air borne as the manner of their incidence cannot be reconciled with any other explanation. An analysis of the atmosphere of a cold-storage installation showed the presence of an average of three fungus spores per litre. These were mostly *Cladosporium herbarum* and various species of *Penicillium*.

Apart from these industrial considerations, Fraenkel (1938, 1939) has

shown that frequently air-borne fungus spores may act as allergens causing asthma and other allergic diseases, and that their exclusion often relieves the patient.

The control of air-borne fungus spores

In order to remove mould spores from the atmosphere, Smith (1938) advocated the use of a fine spray of thymol solution in a culture room that is to be used for the subculture of fungus species. A considerable proportion of the mould spores in the entering air are doubtless removed by the filter bags of the air-conditioning plants now installed in many buildings, but the plants have no influence on air-borne mould infection otherwise introduced. While ultra-violet radiation has been shown to be entirely successful in the destruction of mould spores and bacteria it is effective only on organisms which are directly exposed so that the sterilization by this means of the atmosphere of places of irregular shape presents considerable difficulty. There are, in addition, the well-known disadvantages of working in the presence of ultra-violet radiation and the necessity for wearing goggles to prevent conjunctivitis, etc. James (1936) claimed that the circulation of ozonized air was sufficient.

The control of air-borne fungus spores by aerosols

The control of air-borne fungus spores by means of aerosols presents special problems.

Firstly the fungus spore is a much larger body than the average bacterium, which has an average volume of 1–2 cu.μ while that of a fungus spore may be anything from 100–200 cu.μ or more. According to the hypothesis put forward a droplet of aerosol should contain sufficient bactericide to destroy a single bacterium but a single droplet would hardly be capable of destroying a fungus spore. Since it is extremely undesirable that the size of the droplets should be increased owing to the rapidity with which large ones fall out of suspension, it seems desirable that the size of the droplets should remain constant but that their number should be greatly increased so that the chances of contact between a spore and a number of droplets either simultaneously or within a short space of time should be multiplied.

Secondly, substances which are good bactericides are frequently of little value in the destruction of fungus spores (Smith, 1938). Phenol, for example, although an excellent bactericide has a relatively low toxicity, particularly in alkaline solution, towards fungus spores. Certain substances have been developed for the specific control of mould growth and the most important of these, known as "Shirlan", the anilide of salicylic acid, was produced by the Cotton Industry Research Association (1928). This substance is almost insoluble in water but its sodium salt, which is reported to be equally effective, is soluble.

Preliminary aerosol tests were carried out with the various parent fluids which have been mentioned and the results obtained indicated that "Aeryl"

was the most satisfactory. A new liquid was made up containing the sodium salt of the anilide of salicylic acid known as "Shirlan" sodium and glycerine, but the aerosol of this preparation proved to be completely ineffective, having no fungicidal action whatsoever. Further experiments have since been carried out with this "Shirlan" sodium, but an active aerosol preparation has not been obtained.

Experiments were then continued in order to obtain information regarding the action of "Aeryl" upon the spores of varous fungi.

TECHNIQUE

The fungus was cultured upon 2% maltose agar until a sufficient growth had been obtained when the plates were left open at 37° C. for 16 hr. so that they became thoroughly dry. The cultures were then powdered as finely as possible and divided into two equal parts. One part was distributed about the test chamber by means of a strong current of air so that the spores were left floating in the atmosphere. After an interval of 30 min., during which the chamber was left undisturbed, plates were exposed for a period of 15 min. These plates constituted the controls. After the chamber had been cleaned carefully the other part was distributed in the same way and an aerosol to a concentration of 1: 500,000 was introduced. The chamber was again left undisturbed for a period of 30 min., after which further plates were exposed for 15 min. These constituted the test plates. The plates were incubated at 20° C. for 100 hr. before the colonies were counted.

Table III

Strain of fungus	No. of colonies on test plates (average)	No. of colonies on control plates (average)
*Cladosporium herbarum**	38	Confluent growth
*Mucor racemosus**	4	Confluent growth
*Thamnidium elegans**	2	Confluent growth
*Cladosporium herbarum** (another strain)	24	Confluent growth
*Wardomyces anomala**	2	35
Monillia sitophilia	About 10% of control accurate count impossible owing to type of growth	Confluent growth
Penicillium commune	65	Confluent growth
Mucor adventitius	6	Confluent growth
*Torula botyroides**	Nil	184
*Sporotrichon carnis**	Nil	275
Aspergillus niger	8	450

An asterisk is placed against strains which have been isolated from refrigerators used for the storage of meat.

It will be observed that there was complete lack of growth on two only of the test plates, but it must be remembered that the original inoculum was very heavy and grossly in excess of any infection likely to occur under practical conditions. In many cases it was not possible to count the colonies on the

control plates owing to these becoming a continuous mass of mycelium; in all cases, however, the term "confluent growth" may be taken to indicate a number of colonies in excess of 500. It will be noticed that a number of the strains of fungi employed were originally isolated from cold storage installations where meat was stored and we have ourselves isolated several of these from the atmosphere of such refrigerators.

SUMMARY

1. The optimum conditions, chemical, physical and mechanical, for the production of a bactericidal aerosol are discussed.

2. Experiments with sodium hypochlorite indicate that it may be used as the active principal of a bactericidal aerosol and that, although it is less efficient than resorcinol preparations, it has the advantage of cheapness and the power of deodorization.

3. A new parent liquid with a base of resorcinol is described which is suitable for both atmospheric and surface sterilization.

4. Experiments are described which indicate that it is possible to destroy air-borne fungus spores by means of aerosols.

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