AN INVESTIGATION OF THE MERITS OF OZONE AS AN AERIAL DISINFECTANT

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(With Plates 8 and 9 and 4 Figures in the Text)

The investigation described in this report has been directed primarily towards acquiring experimental evidence as to the value of ozone as an aerial disinfectant. An answer has been sought to the question: 'Does ozone, when present in the atmosphere in concentrations safely tolerated by man, exert bactericidal action on micro-organisms dispersed as aerosols, i.e. as minute particles suspended in the air, and on such micro-organisms when they have settled on various types of surfaces as dust, and are liable from time to time to be redispersed in the air?' The problem is one of considerable importance and urgency in view of the claims made for ozone as a means of mitigating conditions arising through overcrowding in ill-ventilated enclosures.

The powerful disinfecting action of ozone has long been recognized, but a search of the literature on the subject reveals considerable differences of opinion as to its value as an aerial disinfectant, when the gas is present in concentrations that could be breathed during prolonged spells with safety. Some authors have been led to dismiss ozone as being quite ineffective, while others have heralded it as having great potential value in ventilation problems. The general problem of aerial disinfection as a practical adjunct to ventilation has many inherent difficulties, and makes demands upon the combined resources of physiological, bacteriological and physico-chemical knowledge. The particular problem of ozone is no exception, and it is evident that failure to appreciate fully its several aspects has led to the conflict of opinion.

Ozone is essentially a new association product of oxygen atoms. It is an extremely reactive gas possessing great oxidizing potential, and is toxic for the living organism. Consequently proper control of output of ozone becomes a matter of prime importance when considering its use as an aerial disinfectant. It may be well, therefore, to review briefly the development in the design of ozonizers. The earliest form consisted simply of an arrangement for generating a spark discharge between two electrodes in oxygen or air. The energy of this discharge sufficed, however, to ionize not only oxygen but nitrogen also. The oxides of nitrogen, so formed in addition to ozone, are very dangerous gases, owing to the insidious nature of their toxic action upon the respiratory organs. Like ozone they are powerful oxidizing agents, and can liberate iodine from potassium iodide; and they exhibit bactericidal and fungicidal properties (Williams & Hartgraves, 1939). Their presence will confuse the issue in any

study of the properties of ozone, and it is imperative to eliminate them. The trend in ozonizer design has been towards better control of the electric discharge in order to secure maximum output of radiation in the ultra-violet, which is most effective for the production of ozone; to avoid the formation of oxides of nitrogen; and to minimize the heat energy, which, in the case of the spark discharge, is sufficient to decompose the ozone. A big step forward was made by inserting a suitable dielectric (glass or mica) between the electrodes, leading to the design incorporated in the ozonizer investigated in the present study. This consists in having one electrode completely enclosed in a cylindrical glass tube, containing an inert gas, viz. neon, at low pressure, while the second electrode, of metal gauze, forms a jacket in contact with the exterior surface of the dielectric. When an alternating electric field of some few thousand volts potential is applied between the internal and external electrodes, a silent glow discharge is excited, generating electrons of suitable energy to ozonize the air passing over the surface of the tube. An ozonizer of this general design is reputed to yield ozone of high quality with little, if any, oxides of nitrogen, and heating effects are negligible. This assumes that the characteristics of the dielectric do not become impaired under prolonged subjection to high electric stress, or by the superficial accumulation of moisture or dust. It is clearly desirable that dry, dust-free air should pass the ozonizer tube to ensure a uniform output of ozone. If the normal circulation of the air is relied upon, the efficiency of an ozonizer may be expected to vary according to the prevailing atmospheric conditions (cf. later notes on ozonizers used in present study). Recently, a new dielectric—a modified, phenol-formaldehyde plastic—has been claimed to be superior to glass for use in ozonizer tubes (Thorp, 1941).

WHAT CONSTITUTES A SAFE CONCENTRATION OF OZONE?

The physiological properties of ozone, or, more correctly, of ozonized air produced by various forms of ozonizer, have been fairly widely studied. It appears that most people find ozone irritating to the throat when breathed for long spells with the concentration at about 0·05 part per million (p.p.m.) by volume. At higher levels, viz. 0·3–0·5 p.p.m., aggravated symptoms of respiratory irritation and headache develop. In small animals 15–20 p.p.m. brings death within a matter of hours from respiratory congestion (Hill & Flack, 1912). From the recorded evidence, and their own observations, Witheridge & Yaglou (1939) concluded that the safe upper limit of ozone concentration for man is 0·04 p.p.m. This appears to be the only conclusion warranted by experimental work so far adequately recorded.

Claims that higher concentrations of ozone may be safely tolerated have been made, but, unfortunately, they are not supported by detailed scientific evidence. Olsen (1914) considers 0·3 p.p.m. as the maximum concentration of ozone permissible for breathing. Riesbeck (1939) states that experience with ozone in dwelling rooms and schools has shown that concentrations up to several parts per million can be tolerated without ill-effect. Very recently,

Thorp (1941), in a paper contrasting the toxic properties of air containing pure ozone with those of an atmosphere containing a mixture of 53 % ozone and 47 % oxides of nitrogen (as found to be produced by a particular ozonizer employing glass as dielectric), has stated that pure ozone is non-toxic in concentrations below 20 p.p.m., whereas the toxic limit for the impure product is about 1 p.p.m. This raises anew the question of the quality of ozone generated by ozonizers, and further scientific details of Thorp's work should be made available. We are not primarily concerned, however, with the toxic limit for short exposures, but with the concentration of ozone tolerable over prolonged periods without any irritation or other harmful effect. This level must be taken, on the evidence, to be a concentration not exceeding 0·04 p.p.m.

Critical concentrations of oxides of nitrogen

Henderson & Haggard (1927), citing data established by Lehmann & Hasegawa (1913), give the following table in their monograph on noxious gases:

Physiological response to various concentrations of nitrous fumes

	Parts NO_2 per million parts of air
Maximum concentration allowable for prolonged exposure ...	39
Least amount causing immediate irritation to the throat ...	62
Least amount causing coughing	101
Dangerous for even short exposure	117–154
Rapidly fatal for short exposure	240–775

A recent study of the toxicity of oxides of nitrogen has been made by La Towsky, MacQuiddy & Tolman (1941). See also a Bulletin on the toxicity and potential dangers of nitrous fumes by Oettingen (1941), just to hand.

Disinfecting action of ozone

Of the many recorded studies on the disinfecting action of ozone, few have dealt with aerial disinfection. The value of ozone in destroying bacteria contained in water is well established, and the inhibiting action of the gas on the growth of moulds has been applied with success in cold-storage compartments. The influence of ozone on bacteria dispersed in the air is more difficult to investigate, and, although this is all-important in relation to air-borne infection and ventilation problems, the amount of direct experimental evidence is meagre. The type of experiment that has been most frequently reported has consisted in dipping blotting paper strips, glass rods, cotton threads or cheese cloth into cultures of various organisms, and then exposing in moist and dry condition to the influence of ozonized atmospheres (Konrich, 1913; Sawyer, Beckwith & Skolfield, 1913; Jordan & Carlson, 1913; Franklin, 1914). Agar and gelatine surfaces have been streaked with bacterial cultures and likewise exposed (Jordan & Carlson, 1913; Franklin, 1914; Wulliémoz, 1938). Tests of this nature have yielded the uniformly definite finding that the concentration of ozone necessary to have any significant killing effect is well beyond the limit

that could be safely tolerated by man. Organisms are found to vary in their resistance to the action of ozone, and bacteria in moist films are more readily killed than when in a thoroughly dry state. Hartman (1925) has stressed the need for differentiating between an inhibitory and a germicidal concentration. He states that ozone in a concentration 0·5–1 p.p.m. is decidedly inhibitory towards nearly all forms of micro-organisms, but reports having found by experiment that 13–14 mg. ozone per l. (6500 p.p.m. by volume) is non-germicidal for certain mould spores.

The tests having the closest relation to our present problem are those conducted by Jordan & Carlson (1913), Olsen (1914) and Franklin (1914) on the effect of ozone on air-borne bacteria. Jordan & Carlson studied the effect of ozone on the bacteria in the air of their experimental room, after the dust had been well stirred up. Their conclusions from these tests were as follows:

'(1) No surely germicidal action on certain species of bacteria could be demonstrated by the usual disinfection tests with amounts of gaseous ozone ranging from 3 to 4·6 parts per million.' (These figures for the ozone concentration may be slightly high, since the absorption was conducted in acidified potassium iodide solution, as Olsen & Ulrich (1914) point out.)

'(2) The alleged effect of ozone on the ordinary air bacteria, if it occurs at all, is slight and irregular, even when amounts of ozone far beyond the limit of human physiologic tolerance are employed.'

Olsen, at the Fourth International Congress on School Hygiene, New York, 1913, reported on tests made on the influence of ozone on the count of viable bacteria in the air of schoolrooms. The bacteria present were estimated by drawing a known volume of air through a tube containing sterilized sand, then washing the sand with sterile water and plating the fluid (*a*) on agar which was incubated at 37·5° C., and (*b*) on gelatine, incubated at 20° C. Counts were made of total bacteria and moulds. Ozone was found to exert a definite sterilizing action, but no figures for the ozone concentration were specifically quoted for these tests. However, as Olsen states in his paper that the maximum concentration permissible for breathing is 0·3 p.p.m., and since the above tests were conducted during class-time, it is to be presumed that this limit for the amount of ozone was not exceeded. Franklin, at the same Congress, also reported having successfully demonstrated the bactericidal power of ozone, in concentrations varying from 1 to 10 mg./cu. m., i.e. 0·5–5 p.p.m. against the small number of organisms in the air of a closed room of 200 cu. ft. capacity.

Wells & Wells (1936), in a paper on general aspects of the problem of air-borne infection, refer to work by Wagner at Harvard, in which ozone in tolerable concentrations, at four times the threshold concentration for olfactory detection, i.e. at 0·04–0·06 p.p.m., caused a threefold increase of the rate at which bacteria disappeared normally from the air. Unfortunately, other details of these ozone experiments have not been available.

Thus the recorded evidence regarding the effects of ozone on air-borne bacteria is also conflicting.

In the present study the action of low concentrations of ozone on artificially disseminated aerosols of bacteria has been directly estimated. Aerial disinfection would in practice be directed against pathogenic organisms known to be invaders of the respiratory tract. The following bacterial strains have been chosen for study: (1) *Streptococcus salivarius*, (2) *Streptococcus* 'C'—a haemolytic *Streptococcus* isolated from a ferret suffering from a mixed influenzal and streptococcal infection (Glover, 1941), and (3) *Staphylococcus epidermidis albus*. *B. prodigiosus* has been employed for preliminary tests when developing new techniques.

Experiments

The experimental tests have been conducted in a room of 750 cu. ft. capacity; floor area 80 sq. ft. The two adjacent outside walls contained windows 3 ft. 6 in. × 3 ft., hinged to open outwards, and beneath these ran lagged hot-water pipes. The air of the room was kept in movement by means of a large fan revolving at 60 rev./min. and distant 13 in. from the ceiling. The spindle carrying the fan penetrated the centre of the ceiling, through an airtight oil seal, being driven by an electric motor in the loft above. One inside wall of the room contained (1) the door, (2) a small glass window for observational purposes, and (3) a number of tubes through which aerosols might be introduced into the room and samples of the atmosphere withdrawn (see Pl. 8). Electric light was available; no daylight was admitted during an experiment. Inside the room and situated conveniently near the observation window was a circular table (height 3 ft. 4 in.), which could be rotated from without by means of the rod used to lift the covers from the Petri dishes arranged around the table (see Pl. 9, fig. 1). A series of agar-plate exposures could thus be made without entering the room. Also in the room, and situated about two-thirds of the way across from the observation window, was a wooden stand (height 3 ft. 8 in.) which carried the ozonizer. A thermometer and a hair hygrometer hung from the side of this stand and were readable through the window. The walls and floor of the room were coated with an enamel paint. The room, with the windows and door closed, was without ventilation.

Temperature and humidity

No facilities for automatic regulation and control of temperature and humidity existed. Experiments at low humidities had to await naturally dry conditions. High humidities were secured artificially either by introducing steam and allowing at least half an hour for a steady state to develop, or, preferably, by spraying into the room a copious fine mist of distilled water which enabled a 20% increase in humidity to be achieved within 10 min. A wet and dry bulb hygrometer of the whirling type was used for measurement immediately before an experiment, and any subsequent variation in humidity was followed by means of the hair hygrometer. Such artificially humidified

atmospheres adjusted prior to each experiment are not wholly satisfactory, because conditions of true equilibrium cannot be attained so quickly. Temperature differences result in condensation, and there is a gradual falling of humidity to normal level. The ideal conditions for this work would require two identical independently air-conditioned rooms.

Estimation of ozone

The limiting concentration of ozone with which these studies would be concerned being 0·02–0·05 p.p.m., it appeared that provided the ozone present in 20 l. of the atmosphere could be absorbed in 5 or 10 c.c. of liquid, then the classical method of estimating ozone (Ladenburg & Quasig, 1901) might be applied. This method consists in absorbing the ozone directly in a neutral solution of potassium iodide followed by titration of the liberated iodine with standard thiosulphate, after first acidifying the iodine solution.

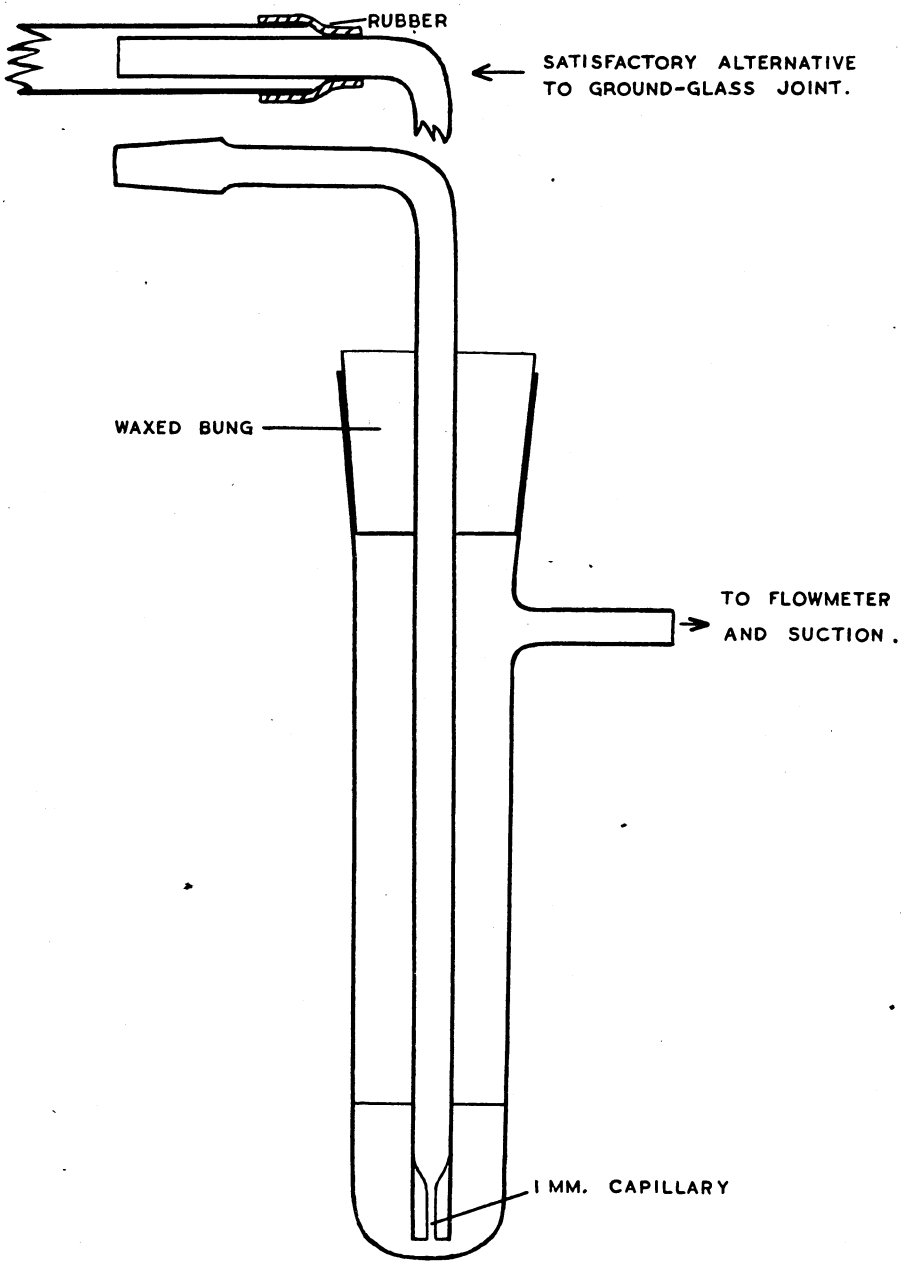
The several factors influencing the interaction of ozone with potassium iodide solution have been carefully investigated by Guéron, Prettre & Guéron (1936). An important practical point emerged from their studies, namely, that exposure of the reacting system to a large glass surface, as, for example, by bubbling through a sintered glass disk, favours the formation of iodate and also accelerates the spontaneous decomposition of ozone.

A number of control tests were made on various aspects of the method before adopting a procedure for use in the present study.

(*a*) Concentration of potassium iodide: Early experiments were made with a 20 % solution of potassium iodide (A.R. grade) for absorbing ozone, as usually advocated. Unfortunately, the colour given when starch was added lacked stability, becoming more intense with time, suggestive of some auto-oxidative process. Control tests showed that the passage of ordinary air through 20 % potassium iodide solution sufficed to liberate iodine. Solutions less concentrated than 5 % remained colourless under the conditions of test, and 1 % solutions have been adopted for routine analyses with satisfactory results.

(*b*) Formation of iodates: Tests for the presence of iodates following the absorption of dilute (less than 0·1 p.p.m.) ozone have given consistently negative results. When more concentrated ozone is absorbed in potassium iodide, however, the resulting alkalinity is appreciable, and the system should be buffered at *p*H 7 with phosphate to minimize the formation of iodate.

(*c*) Absorption of ozone: Some of the requirements of the present study have demanded that ozone samples should be taken speedily, as, for instance, in following the variation in ozone concentration with time of operation of the ozonizer, or, when data for the decay curves of an ozonized atmosphere were needed. The sampling errors for rates of flow ranging from 0·1 to 8 l./min. were studied. The form of sampling tube shown in Text-fig. 1 and Pl. 9, fig. 2 has been used, two and often three being connected in series for the tests now being considered. The gas makes contact with the absorbing fluid at high velocity through a capillary, 0·5 cm. long and 0·1 cm. in diameter, set 3 mm.



Text-fig. 1. Sampling tube for ozone estimation.

from the bottom of the tube. Errors may arise owing to (1) incompleteness of the primary absorption of ozone, and (2) the entrainment of iodine in the current of air as it passes through the solution. All the evidence has indicated that, under the prevailing conditions, the absorption of ozone has always been reasonably complete, better than 95%. Paneth & Glückauf (1941) have reported good absorption of atmospheric ozone under presumably similar conditions. The serious error involved is that due to the carrying over of iodine.

The percentage loss due to entrainment when air was drawn at known rates of flow through dilute iodine solutions, ranging from 10^{-3} to $10^{-4}M$, in 1% potassium iodide was found to be largely independent of the rate of flow over the range 0·1–8 l./min., and of the iodine concentration, but was a function of the volume of air passed and of the volume and temperature of the solution. Thus, at 18° C. for 20 l. of air passing through 5 c.c. of solution a loss of 38% was involved, and when 10 c.c. were used the loss was 25%, while at 0° C. the corresponding losses were 14 and 6% respectively.

Ozone estimations by absorption in 1% potassium iodide solution at 0° C. have been found to be 20, 30, and 50% higher than those conducted at 15, 18 and 25° C. respectively. Our adopted procedure, therefore, has been to absorb the ozone from 20 l. of air directly in neutral 1% potassium iodide, preferably at 0° C., and to titrate the iodine liberated with 10^{-3} M sodium thiosulphate in the presence of starch. The titrations were made with the aid of a micrometer-syringe ('Agla' Burroughs Wellcome—titration readings to 0·0001 c.c.), or, alternatively, with specially calibrated micro-pipette. All estimated ozone concentrations have been corrected for absorption at 0° C.

We had hoped to check our estimations of ozone, and of nitrogen dioxide, by the method recently described by Edgar & Paneth (1941), but so far this has not been possible.

Estimation of oxides of nitrogen

The standard phenol-disulphonic acid method being found too insensitive for our purpose, the diphenyl-benzidene reagent (Letts & Rea, 1914) was used to estimate the oxides of nitrogen present. This reagent gives a blue colour in the presence of as little as 0·0002 mg. nitric nitrogen per c.c. Absorptions from the ozonized atmospheres were made in $N/10$ sodium hydroxide, and also in $N/10$ sulphuric acid containing 0·03% hydrogen peroxide. The purest chemicals are essential—sodium hydroxide from sodium drippings, sulphuric acid of A.R. quality, Merck's 'Perhydrol', and high-grade distilled water were used. After absorption the sulphuric acid was neutralized and boiled before testing.

A typical analysis of the air in the experimental room, in which a two-bulb ozonizer had operated for some hours, showed it to contain 0·22 p.p.m. of ozone and less than 0·0075 p.p.m. oxides of nitrogen (as NO_2).

Other experiments were made with a single ozonizer tube mounted in a glass chamber, as shown in Pl. 9, fig. 2. Tests were conducted at different rates of flow and at different humidities. When air at 80% R.H. and 22° C. passed the

tube at 0·25 l./min., the issuing ozonized air was found to contain 0·05 p.p.m. oxides of nitrogen and 40 p.p.m. ozone. Tests with drier air, at 50 % R.H., showed an even smaller proportion of oxides of nitrogen.

Control tests were made with ozonized oxygen (pure for inhalation), and conditions were established for the complete retention of ozone by manganese dioxide and asbestos, as used by Usher & Rao (1917). The efficiency of this trap depends on the ozone concentration and the rate of flow. Ozonized air passing the trap under conditions previously proven for the complete retention of ozone, was found to contain negligible amounts of oxides of nitrogen, confirming earlier tests. Potassium iodide solution, in a tube backing the first absorption tube, gave no trace of colour with starch and acid.

It appears, therefore, that the ozonizer under investigation yields ozone with very little oxides of nitrogen even in a relatively humid atmosphere.

Ozone and nitrogen dioxide in the normal atmosphere

The amount of ozone present in the untreated air is found to vary according to the locality and the prevailing atmospheric conditions. The following figures have been obtained recently by Edgar & Paneth (1941):

Ozone	0·005–0·045 p.p.m.
Nitrogen dioxide ...	0·001–0·02 p.p.m.

Ozonizers used

The ozonizers used in the present studies were kindly placed at our disposal by the makers, C. and R. Research, Ltd.,[1] whose ready co-operation at all times is here acknowledged.

The tubes were of the enclosed electrode type with glass dielectric, as already described, and the ozonizer operated on the 210 V. 50 cycle a.c. mains with the transformer incorporated in each model. Most of our experiments have been conducted with an ozonizer having one or two tubes, but in the early tests, in which the effects of relatively high concentrations of ozone were studied, four and eight tubes were employed. The possibility of varying the number of tubes provided the only means of controlling the output. The air subjected to the ozonizing action was not dried or filtered. The level of ozone concentration attainable in any instance depended, accordingly, upon the prevailing temperature, humidity, and purity of the atmosphere. Low humidity favoured the relatively rapid and liberal generation of ozone, while with a high humidity prevailing the ozone concentration was much lower, and a steady equilibrium level was reached. The persistence of ozone in atmospheres of low humidity was found to be much better than at high humidities, the rate of decay at 50 % R.H. being about one-half that at 80 % R.H.

[1] C. and R. Research, Ltd., 1 Kensington Place, London, W. 8.

Estimation of bacteria

(*a*) *In the air.* The variation of the bacterial concentration in the air has been followed in terms of estimations of viable organisms by two methods:

(1) The exposure of nutrient agar plates for a given period—usually 2 min. Some initial difficulty was experienced when the Petri dishes were simply fitted with metal lids removable by means of a lever arm. Considerable contamination occurred, due to organisms passing under the lid in spite of the deep flange. The trouble was eliminated by placing the uncovered Petri dish within a large copper dish (depth $\frac{3}{4}$ in.; diam. $5\frac{1}{2}$ in.), the cover of which fitted into an annular peripheral trap containing soap solution (see Pl. 9, fig. 1). A slot, 1 mm. in width, was cut in the rim of the cover at a point opposite the lever arm attachment to facilitate the steady removal of the lid and its replacement at the end of the prescribed period of exposure. The procedure has been highly satisfactory, and produced very consistent series of analyses.

(2) Sampling the atmosphere by means of the slit machine described recently by Bourdillon, Lidwell & Thomas (1941). A known volume of the atmosphere is drawn through a slit and caused to impinge at high velocity on the surface of the agar in a Petri dish which rotates slowly. In all our tests the organisms caught in half a minute, during one revolution only of the plate, have been counted. Agar previously exposed to ozonized air showed no inhibitory effect on the growth of bacteria. Also bacteria allowed to settle on agar plates and then exposed, during one revolution in the slit machine, to the action of the impinging jet of ozonized air, developed normal colonies.

It may be well to point out just what is measured by these two methods. The plate exposure test collects those organisms which by settling under gravity, or through the normal air movement, make contact with the agar surface. It will, therefore, tend to be selective, particularly in the case of aerosols heterogeneous in particle size, in that, of the particles settling out, the numerical distribution will favour those of greatest mass. In the slit sampling method all the organisms contained within the volume of air sampled impinge at high velocity against the agar, and, under the conditions prevailing in these tests, 90 % will be trapped. The mass/numerical distribution, therefore, should more closely approach that in the atmosphere. An aerosol, homogeneous in particle size, should yield identical decay curves by these methods, but in the case of a hetero-disperse aerosol differences may be anticipated (cf. curves for uniform and coarse aerosols later).

(*b*) *On surfaces.* The influence of ozone on bacteria which had already settled on various types of surface—agar, glass, filter-paper, woollen serge—has been studied. The following methods of test were used:

(1) Agar surface—plates directly incubated.

(2) Glass surface—the small Petri dishes used were washed out with a known volume of broth and this was then plated on agar.

(3) Filter-paper and woollen serge—an impression technique was used: The exposed surface on which the organisms had settled was laid in contact with appropriate nutrient agar. The uppermost surface of the material was then stroked backwards and forwards with a glass spreading rod in two directions at right angles. The material was then carefully stripped from the surface, the Petri dish closed and incubated. This method has provided surprisingly consistent counts.

Atomizers used

The majority of tests have been made on aerosols produced by atomization of suitable bacterial suspensions in a modified form of the atomizer incorporated in the Collison Inhaler.[1] This atomizer, which for convenience will be referred to as the 'P' spray, produces, by reason of the very effective trapping of all coarse droplets, an exceedingly fine uniformly disperse aerosol, in which upwards of 90 % of the bacteria have been shown to exist as singletons (microscopical analysis). Coarse aerosols, more representative of those occurring naturally in a sneeze, have been produced by means of an all-glass atomizer of the scent-spray type made by Mr L. Ward.

Experimental procedure

Two general procedures have been adopted in the experiments to be described:

A. A bacterial suspension of appropriate concentration is sprayed into the experimental room (humidity and temperature recorded) under definite conditions, i.e. at a known pressure and for a fixed period. Then the bacterial concentration in the air of the room is tested at selected intervals by the methods already indicated. In this way data are obtained, enabling the normal control decay curve of the aerosol to be drawn. The windows of the room are then opened from the outside and the room thoroughly aerated for an hour with the help of the Martindale blower which delivers 60 cu. ft. air/min. The windows are then closed and the room left for at least half an hour for conditions to become steady, the humidity being adjusted, if necessary, to the value previously obtaining during the control run. Then a fresh bacterial aerosol is sprayed under conditions akin to those of the control. Two, or maybe three, samples are taken to establish the commencement of the decay curve, and then the ozonizer is switched on. Bacterial tests are continued at the same intervals as in the control, and so the 'experimental decay curve' is determined.

B. This procedure consists in first building up a known concentration of ozone in the experimental room before spraying the organisms. Experimental and control decay curves are established as in A. The order of control and experimental runs is reversed from time to time.

[1] To be obtained from the Inhalation Institute, 87 Eccleston Square, London, S.W. 1.

OBSERVED EFFECTS OF OZONE

(1) *On bacterial aerosols (fine; uniform)*

This section deals with aerosols produced by spraying bacterial suspensions from spray 'P' at 20 lb./sq. in. pressure. The concentrations of bacteria in the aerosols have generally been within the range 50–500 viable organisms per litre of air. Results of analyses made at chosen intervals after spraying are expressed as percentages of the initial concentration (5 min. allowed for initial mixing in the earlier experiments, but later 2 min.). Any killing effect due to the action of ozone is seen by comparing the curves for decay with and

Table 1 *a*. *Ozone versus* Streptococcus salivarius *aerosols (fine; uniform)*

Ozone p.p.m.	Medium of sprayed suspension	Experimental procedure	Method of analysis	R.H. %	*T* °C.	Results: Percentage kill	Results: Contact time min.
1·5–2·4	Serum broth	B	P	60	15	70 95	5 10
1·6	,,	B	P; S	40	15	? slight	15
1·4	,,	B	S	42	15	50	20
0·48	,,	B	P; S	45	16	Nil	20
0·6–0·4	,,	B	P; S	77	17	90 98 >99	5 10 15
0·15	,,	B	P	66	21	90 >99	5 15
0·12	,,	B	P; S	54	22	80	15
0·08	,,	B	P	40	16	Nil	30
0·08	,,	B	P; S	80	22	90	15
0·05	,,	B	P; S	42	18	Nil 30	30 90
0·05	,,	B	P; S	64	18	70	15
0·04	,,	B	S	61	22	56	15
0·025	,,	B	P	46	20	Nil	30
0·025	,,	B	S	80	20	37 >90	15 30

P = plate exposure; S = slit-machine sample.

without ozone, and its magnitude may be conveniently given in terms of the percentage reduction in viable organisms relative to the control. Exploratory tests were made with ozone concentrations ranging from 2 p.p.m. down to 0·025 p.p.m. Tables 1 *a–c* contain summaries of the findings for the three organisms, *Streptococcus salivarius*, *Streptococcus* 'C' and *Staphylococcus albus* respectively.

The outstanding fact established in these early tests was the dominant role played by humidity in determining the action of ozone, particularly when the gas is present in low concentration. A partial answer to our opening question was also provided, for it was clearly evident that in relatively dry air, at humidities less than 45%, ozone, even in concentrations considerably in

excess of the tolerance limit, exerts no appreciable disinfecting action on the bacterial aerosols studied. Quite other relationships hold, however, for humidities above 50 %, when, in increasing measure as the humidity is raised, the ozone was found to reduce the count of viable bacteria in aerosols sprayed from aqueous, or serum broth suspensions of the organisms. Ozone in concentration less than 0·025 p.p.m. showed definite bactericidal action at 60–80 %

Table 1 *b*. *Ozone versus* Streptococcus 'C' *aerosols* (*fine; uniform*)

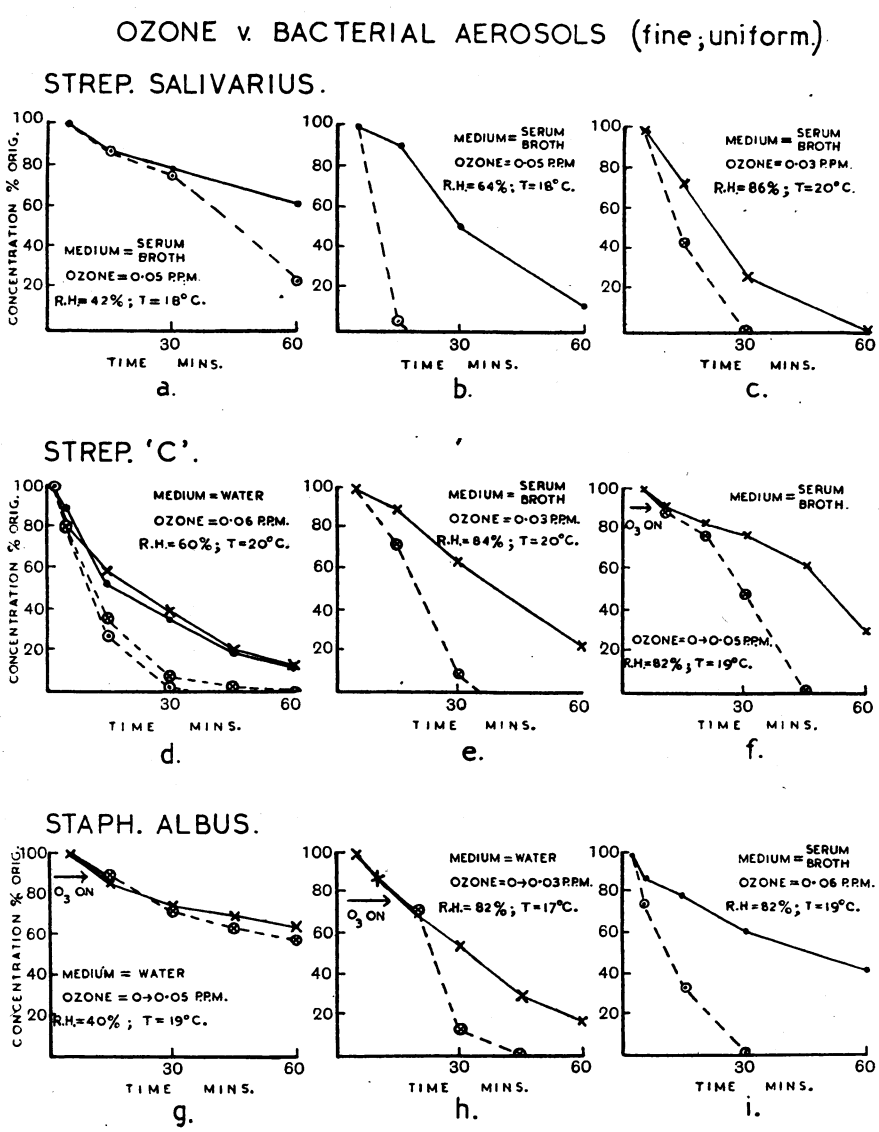
Ozone p.p.m.	Medium of sprayed suspension	Experimental procedure	Method of analysis	R.H. %	*T* °C.	Results: Percentage kill	Results: Contact time min.
1·40	Serum broth	B	S	40	16	25	5
						60	10
						78	15
0 → 0·032	,,	A	S	54	23	80	30
0·032 → 0·08	,,	A	S	54	23	95	90
0 → 0·048	,,	A	S	82	18	95	45
0·025	,,	B	S	60	20	94	30
0·025	,,	B	P; S	83	20	88	30
0·022	,,	B	P; S	84	20	90	30
0·05	Water	B	P; S	56	20	55	15
						80	20
						90	60

Table 1 *c*. *Ozone versus* Staphylococcus albus *aerosols* (*fine; uniform*)

Ozone p.p.m.	Medium of sprayed suspension	Experimental procedure	Method of analysis	R.H. %	*T* °C.	Results: Percentage kill	Results: Contact time min.
0·9	Water	A	P	38	15	Nil	15
0·66	,,	B	P	47	15	Nil	15
0·27	,,	B	P	76	18	90	15
0·19	,,	B	P	37	18	Nil	60
0 → 0·08	,,	A	S	83	17	40	20
						>90	35
0 → 0·08	,,	A	S	40	17	Nil	45
0·08 → 0·14	,,	A	S	40	17	Nil	60
0 → 0·025	,,	A	S	40	18	Nil	105
0 → 0·025	,,	A	S	80	18	75	30
0·03	Serum broth	B	P	79	18·5	95	30
0·06	,,	B	P	82	19	59	15
						>95	30

P = plate exposure; S = slit-machine sample.

humidity. More detailed studies were subsequently made and the curves in Text-fig. 2 *a–i* serve to indicate the speed and course of the action of ozone against bacterial aerosols at different humidities, and when water or serum broth constituted the medium of the sprayed suspension. The limiting size and composition of the aerosol particles depend largely upon the medium of the bacterial suspension sprayed. Thus, with water as suspension medium, the



Text-fig. 2.

Key to lettering, Text-figs. 2–4

- • Concentration by plate exposures in absence of ozone.
- ⊙ Concentration by plate exposures in presence of ozone.
- × Concentration by slit-machine samples in absence of ozone.
- ⊗ Concentration by slit-machine samples in presence of ozone.

The continuous lines, in all instances, are those given in absence of ozone, while the broken lines are those given in presence of ozone.

- ▲ log (100 % original concentration) by plate exposures in absence of ozone.
- ◭ log (100 % original concentration) by plate exposures in presence of ozone.

This way of representing the results emphasizes the divergence of the two curves with time.

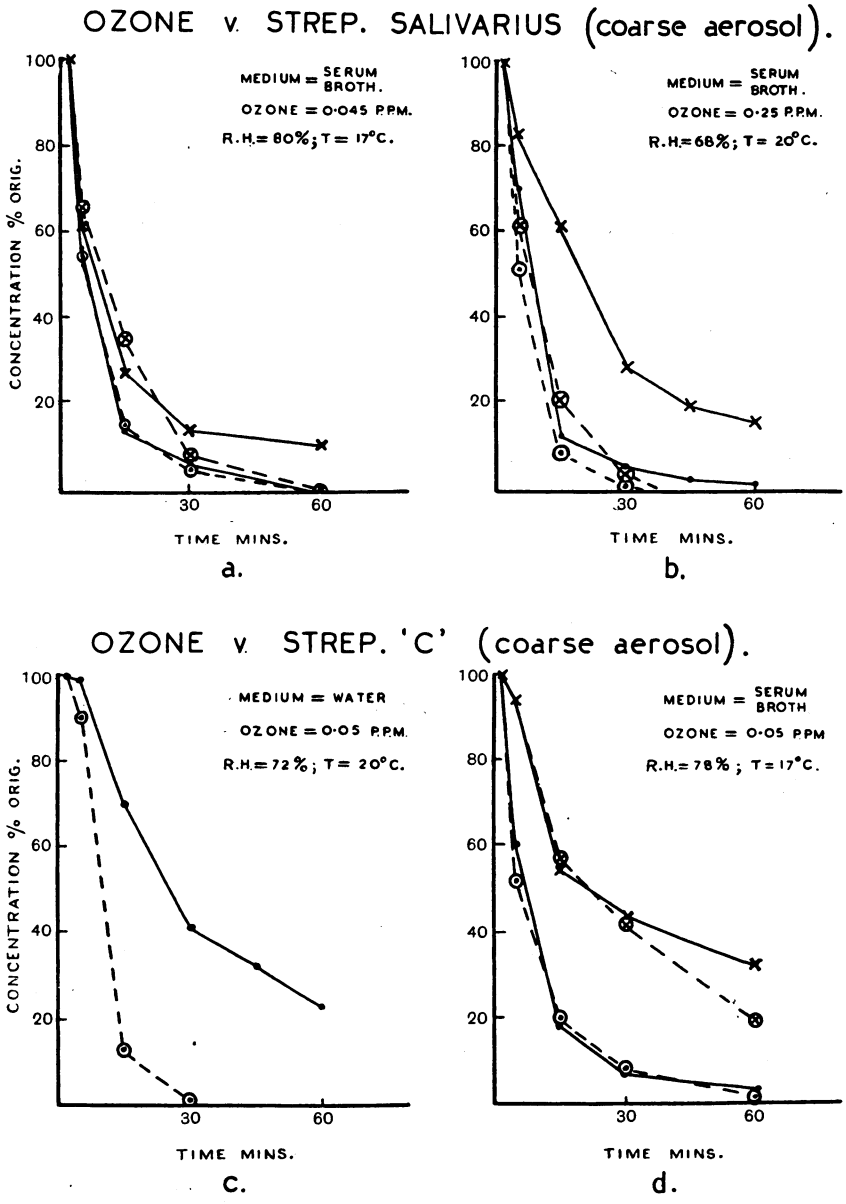
bacteria in the air will be practically naked, whereas with serum broth they will be protected by a coating of serum and broth proteins. It is interesting that with these fine, uniform aerosols, little difference could be discerned between the action of ozone on aerosols from aqueous and those from serum broth bacterial suspensions. This is presumably attributable to the minimal thickness of the protective coating in the case of the very finely dispersed aerosols (cf. later data with coarse aerosols). It is to be noted that the decay curves by plate exposures and slit-machine samples are practically identical with these relatively homodisperse aerosols, although in experiments prolonged up to 2 hr., a slight divergence is frequently manifested when the organism has been sprayed from serum broth suspension.

(2) *On coarse heterodisperse bacterial aerosols*

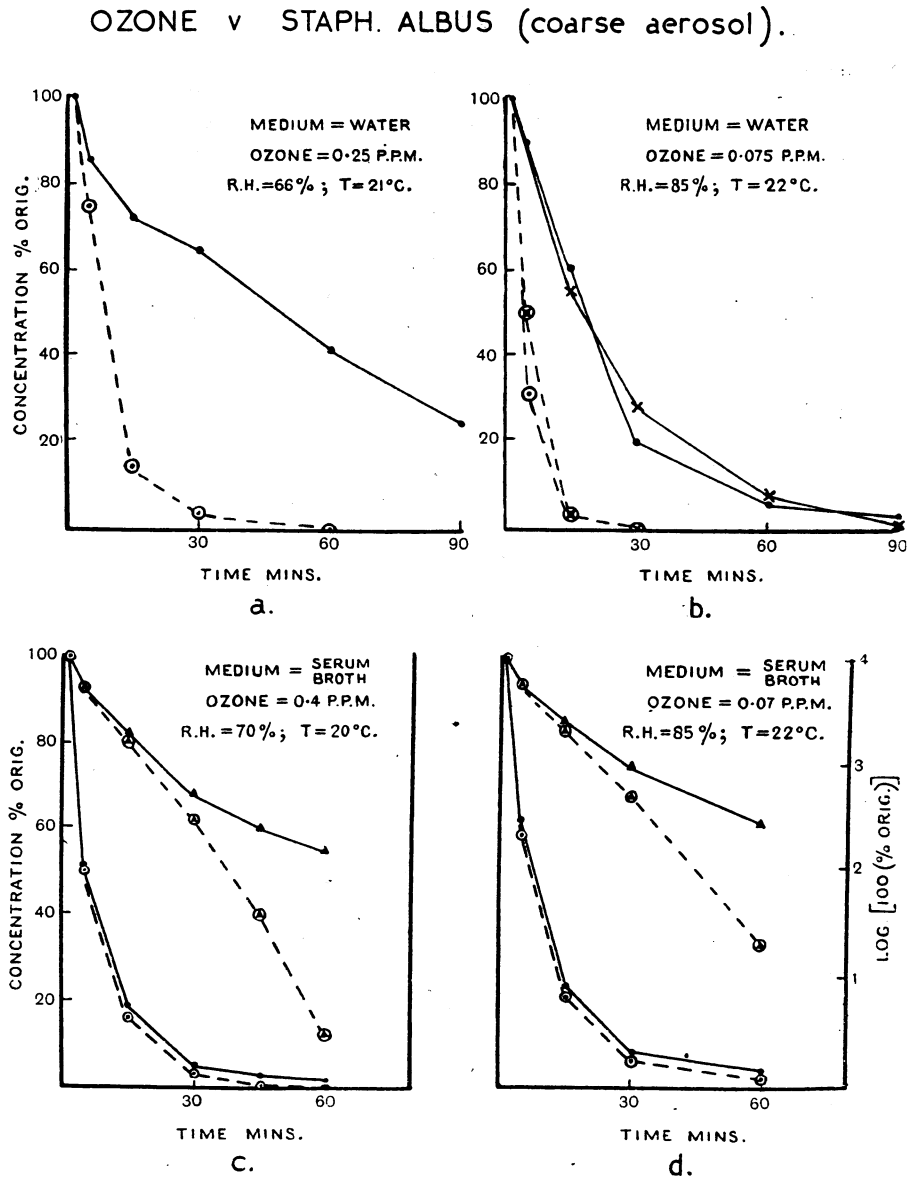
The practical significance of the results of the preceding section may be doubted, since aerosols naturally emitted, as in a sneeze, are relatively coarse and very heterodisperse. The question will be raised: 'Has ozone any influence on such systems?' Evidence on this point has been sought in experiments with coarse, heterogeneous bacterial aerosols produced by the all-glass spray already described. Representative analyses of a large number of tests on the action of ozone upon such non-uniform aerosols are given in the experimental curves of Text-figs. 3 and 4.

It will be seen from curves, Text-fig. 3 *c* and Text-fig. 4 *a*, *b* for *Streptococcus* 'C' and *Staphylococcus albus* respectively, that when these organisms are sprayed from aqueous suspensions, ozone is still able to exert a good killing effect in concentration 0·05 and 0·075 p.p.m. When serum broth is the suspension medium, however, as illustrated in Text-figs. 3 *a*, *d*, 4 *c*, *d*, ozone in tolerable concentrations has a negligible effect within half an hour, but a small effect is in some instances evident after 1 hr. Much higher concentrations are required to achieve any rapid inactivation of the organisms.

The ratio P/S, of the counts of viable organisms by plate exposure to those by slit-machine sampling, remained steady throughout the experiment when the organisms were sprayed from aqueous suspension, the value being $\frac{1}{15}$ to $\frac{1}{20}$, the same as with the uniform aerosols. When serum broth, on the other hand, was the suspension medium, the coarse aerosols exhibited quite a different ratio. Soon after spraying, say 2 min., the ratio P/S would be 4 or 5, decreasing to unity in about half an hour, and thereafter becoming fractional, viz. $\frac{1}{2}$ to $\frac{1}{3}$ in a further half-hour. This effect is reflected in the divergence shown in the die-away curves given by the two methods of analysis, e.g. Text-fig. 3 *d*, and is evidence of the heterodispersity of the equilibrated nuclei in the coarse aerosols, when these are derived from a serum-broth suspension of bacteria. On the other hand, aqueous suspensions under similar conditions yield relatively uniform aerosols of bacteria, the limiting particle size in this case being largely independent of the initial droplet diameter, so long as this is less than about 0·1 mm., the water evaporating rapidly to leave an unprotected


OZONE v. STREP. SALIVARIUS (coarse aerosol).
MEDIUM = SERUM BROTH.
OZONE = 0·045 P.P.M.
R.H.= 80%; T= 17°C.
MEDIUM = SERUM BROTH.
OZONE = 0·25 P.P.M.
R.H.=68%; T= 20°C.
CONCENTRATION % ORIG.
TIME MINS.
a.
b.
OZONE v. STREP. 'C' (coarse aerosol).
MEDIUM = WATER
OZONE = 0·05 P.P.M.
R.H.= 72%; T= 20°C.
MEDIUM = SERUM BROTH
OZONE = 0·05 P.P.M.
R.H.= 78%; T= 17°C.
c.
d.

Text-fig. 3.


OZONE v STAPH. ALBUS (coarse aerosol).
MEDIUM = WATER
OZONE = 0·25 P.P.M.
R.H. = 66%; T = 21°C.
MEDIUM = WATER
OZONE = 0·075 P.P.M.
R.H. = 85%; T = 22°C.
CONCENTRATION % ORIG.
TIME MINS.
a.
b.
MEDIUM = SERUM BROTH
OZONE = 0·4 P.P.M.
R.H. = 70%; T = 20°C.
MEDIUM = SERUM BROTH
OZONE = 0·07 P.P.M.
R.H. = 85%; T = 22°C.
LOG [100 (% ORIG.)]
c.
d.

Text-fig. 4.

organism. The further deduction may be made that the organisms are largely single, as in the fine aerosols, the atomizing process being such that the probability of more than one particle being trapped per droplet is very small for the relatively weak bacterial concentration in the suspensions sprayed (10^5–10^6/c.c.). (N.B. When using spray 'P' concentrations 10^{10}/c.c. were used.)

(3) *On naturally emitted bacterial aerosols*

The *Streptococcus* 'C' used in the earlier experiments was originally isolated from a ferret suffering from a mixed influenzal and streptococcal infection (Glover, 1941). Two experiments have been conducted in collaboration with Mr R. E. Glover, to see whether any killing effect due to ozone could be detected in the case of naturally emitted *Streptococcus* 'C'. Two ferrets having mixed influenzal and streptococcal infections were placed in a cage and introduced into a cubicle of 250 cu. ft. capacity. A fan kept the air in movement and an ozonizer was in the centre of the cubicle. Blood-agar plates were placed in pairs at different parts of the room and exposed for 1 hr. periods. After two such exposures, the ozonizer was switched on and a further series of three plate exposures made. Ozone samples were taken at the end of each hour. The protocol of one of these experiments is given in Table 2. The ferrets showed

Table 2. *Ozone versus naturally emitted* Streptococcus *'C'*

Two ferrets suffering with influenza and *Streptococcus* 'C' infection—in cubicle. R.H. = 66%. $T = 17°$ C.

	Position of plates								
	In front of cage		4 ft. from cage		4 ft. from cage, 3 ft. above cage		Window ledge 5 ft. from cage		
	Plate counts *Streptococcus* 'C' colonies								Ozone p.p.m.
Control (1) 1 hr. exposure	150,	175	50,	39	28,	29	12,	18	—
(2) „ „	20,	29	7,	5	11,	15	6,	12	—
Ozonizer on (3) 1 hr. exposure	34,	15	11,	18	13,	12	7,	2	0·22
(4) „ „	22,	72	14,	8	5,	11	8,	8	0·30
(5) „ „	21,	8	9,	2	3,	4	5,	3	0·34

increased activity and had spasms of sneezing soon after the ozonizer was switched on. However, they quickly became acclimatized and slept, appearing little affected in the 3 hr. by the concentration of ozone developed.

Clearly the ozone at 0·2–0·3 p.p.m. has very little, if any, effect on the *Streptococcus* 'C' organisms under these conditions. This type of experiment, however, in which the bacterial content of the atmosphere is not steady, but fluctuates according to the frequency of sneezing, can only give a conclusive result when the disinfecting agent acts rapidly and with lethal effect on the majority of organisms. When one considers the nature of the natural sneeze and the gross heterogeneity of the resulting aerosol, it is possible, in view of the earlier experimental evidence in this study, that the bacteria contained in any fine poorly protected particles may become inactivated by the ozone.

This would at best represent only a very small percentage effect and could not be read from the plate-exposure counts.

(4) *On bacteria that have settled on surfaces*

Viable bacteria contained in aerosol particles may settle on to surfaces and constitute a potential danger as a source of infection through redispersal in the air, or even through contact when carried on clothes. Accordingly, a number of tests have been made to discover whether ozonized air has any disinfecting action on such deposited organisms. Aerosols were sprayed into a large metal box and allowed to settle on to various surfaces—agar in 4 cm. Petri dishes; no. 50 or no. 1 Whatman filter paper as 4·2 cm. disks; sterile glass 4 cm. Petri dishes; woollen serge cut as 1 or 2 in. squares—which were arranged on a tray which could be slid on the floor of the box as a shallow drawer. The several types of surface with the deposited organisms were then placed in the experimental room under known conditions of humidity (range 60–85 %), temperature and ozone concentration. After being exposed for a given period, the viable bacteria on the surfaces were estimated by the method already outlined and compared with the numbers on controls not exposed to ozone. The results are summarized in Table 3 *a*, *b*. The percentages represent the mean of at least three, and sometimes six, separate tests under the given conditions. The data point to the following conclusions:

(1) Ozone in a concentration 0·2 p.p.m. in a moderately humid atmosphere exercises a very definite killing effect against certain bacteria deposited on surfaces from aerosols, but below this level of concentration it rapidly ceases to have any significant action.

(2) The actual degree of killing depends on (*a*) the type of surface, inasmuch as some materials may provide more protection than others (thus moist agar, no. 1 Whatman and woollen serge are more favourable to survival than glass or no. 50 Whatman paper); (*b*) the medium in which the organisms are contained before being sprayed, a higher protein content favouring protection of the bacterium in the ultimate particle; (*c*) the different resistance to ozone of different types of organism; in our experience, for organisms deposited on surfaces also, the resistances to ozone are in the following order:

Staphylococcus albus > *Streptococcus salivarius* > *B. prodigiosus*.

Discussion

The foregoing experiments have clearly shown that ozone, in concentrations that can be safely tolerated, i.e. not exceeding 0·04 p.p.m., does, in appropriately humid atmospheres, exert a disinfecting action on certain bacteria—*Streptococcus salivarius*, *Streptococcus* 'C', *Staphylococcus albus* and *B. prodigiosus*—dispersed in aerosol form as single naked organisms. The disinfecting power of such dilute ozone is still effective against the same organisms, similarly dispersed, but individually coated with a minimal protective covering

Table 3 *a*. *Ozone versus bacteria deposited on surfaces from aerosols*

Organism	Character of aerosol	Ozone p.p.m.	R.H. %	*T* °C.	Exposure min.	Observed percentage kills on various surfaces: Agar	Glass	Filter paper	Woollen serge	Remarks
B. prodigiosus (aqueous suspension)	Fine; uniform	0·21	68	20·5	30	Nil	>99	98	—	2% nutrient broth agar Filter paper = no. 50 Whatman
					60	12	>99	99	—	
					90	15	>99	>99	—	
	Coarse	0·06	89	21	45	—	—	98	95	
Streptococcus salivarius (serum broth suspension)	Fine; uniform	0·21	65	20	30	19	73	31	—	
					60	50	80	45	—	
					90	84	97	48	—	
		0·22	78	20	30	28	80	45	—	
		0·21	82	18	75	98	>99	>99*	—	
		0·12	84	20	30	42	78	60	—	
					60	84	>99	85	—	
					90	99	>99	84	—	
		0·18	80	20	30	71	>99	25	—	2% blood agar Filter paper = no. 1 Whatman except * = no. 50 Whatman
					60	96	>99	37	—	
					90	98	>99	54	—	
		0·025	72	20	30	Nil	Nil	Nil	Nil	
	Coarse	0·20	70	20	30	22†	64	Nil	Nil	
					60	33†	57	Nil	Nil	
					90	60†	85	Nil	Nil	
		0·09	78	19	30	Nil	25	Nil	—	
					60	Nil	85	Nil	—	
					90	Nil	95	Nil	—	

† These blood-agar plates were dried in error for 6 hr., instead of normal 1 hr., before exposed to aerosol.

Table 3 *b*. *Ozone versus bacteria deposited on surfaces from aerosols*

Organism	Character of aerosol	Ozone p.p.m.	R.H. %	*T* °C.	Exposure min.	Observed percentage kills on various surfaces: Agar	Glass	Filter paper	Woollen serge	Remarks
Staphylococcus albus (aqueous suspension)	Fine; uniform	0·075	66	22	30	—	—	Nil	Nil	2 % blood agar
		0·075	85	20	30	Nil	—	Nil	Nil	No. 1 Whatman filter paper
		0·06	85	20	30	Nil	—	Nil	Nil	
		0·09	87	20	30	Nil	—	Nil	Nil	
					60	Nil	—	Nil	Nil	
					90	Nil	—	Nil	Nil	
		0·44	70	20	30	15	86	22	23	
					60	35	95	70	30	
					90	78	>99	80	54	
Staphylococcus albus (serum broth suspension)	Fine; uniform	0·075	86	22	50	Nil	—	Nil	Nil	
		0·18	79	19	30	Nil	—	Nil	Nil	
					60	Nil	—	Nil	Nil	
					90	Nil	—	Nil	Nil	
	Coarse	0·5	70	21	30	Nil	Nil	Nil	Nil	
					60	Nil	Nil	Nil	Nil	
					90	Nil	Nil	Nil	Nil	

of protein. As this protective coating increases in thickness however, for instance, with coarse aerosols from suspensions containing appreciable amounts of serum protein, the influence of ozone rapidly diminishes and becomes negligible within the permitted range of concentration. Much greater concentrations of the gas are then necessary to achieve any appreciable killing effect on the organisms. This explains why ozone has been found ineffective against naturally emitted aerosols on the basis of plate-exposure tests. Such aerosols are grossly heterodisperse and the organisms are initially contained in a medium of saliva or of mucus and other constituents of nasal secretion, which will vary according to the nature and stage of the particular infection. In the final, equilibrated aerosol each organism, or group of organisms, will find itself protected, by a layer of semi-solid organic matter, from attack by ozone.

Should the aerosol naturally sneezed contain among its heterogeneous assortment of particles a significant proportion (as regards responsibility for infection) of extremely fine nuclei, then, although the percentage may be so small that any killing effect due to ozone may be imperceptible by the plate exposure test, the elimination of these most minute nuclei from the sum total of normally infectious particles might conceivably reduce the incidence of infection, were such particles potentially more dangerous than the coarse ones. Clear-cut evidence upon the latter point is as yet lacking, although we have evidence that bacteria and viruses dispersed in this finely particulate state can be the means of conveying infection. Thus, while the experimental data obtained with relatively coarse aerosols, and the two experiments with *Streptococcus* 'C' naturally emitted by an infected ferret, make it very improbable that ozone in tolerable concentration can be of practical value in countering the danger of air-borne infection, this conclusion should be finally checked by direct animal infection tests. This is another direction in which the present study might be extended.

The supplementary tests, made to discover whether ozone has any effect on bacteria that have settled on various types of surface, have furnished data that show parallelism with those for the aerosols themselves. In general, an appreciably higher concentration of ozone is necessary to kill an organism that has settled on a surface than that found to be sufficient against the original aerosol. This is to be attributed to two factors: (1) those particles that settle out represent the coarser fractions in a non-uniform aerosol, and (2) some surfaces offer additional protection to settled particles, by reason of mechanical sheltering due to the nature of the surface structure. This latter point is well illustrated by the relative ease with which organisms settling on a glass surface are inactivated by ozone, compared with those settling on to a moist agar surface or, again, by the more rapid inactivation on the relatively smooth surface of a hardened filter paper (no. 50 Whatman) compared with the ordinary more absorbent pads (no. 1 and no. 5 Whatman). The limited number of tests conducted have sufficed to indicate that ozone in tolerable

concentrations cannot be expected to diminish the danger of infection lurking in settled bacterial aerosol particles, whether carried on clothes or present as dust that may be redispersed. There is evidence, however, suggesting that ozone in considerably higher concentration can accomplish disinfection of surfaces on which certain bacteria have settled. Factors determining the amount of ozone necessary include (1) the kinds of organisms, (2) the nature of the protective sheath, (3) the humidity, and (4) the nature of the surface on which the particle has settled. The data available point to a concentration of ozone in excess of 1 p.p.m. in an atmosphere of 60–80 % humidity, as requisite to produce good surface sterilization. Unprotected singleton organisms have been shown to succumb most readily, as in the original aerosols, while organisms embedded in a relatively thick film of organic matter have correspondingly high resistance. This agrees with the recorded evidence, cited earlier, based on tests upon partially dried infected films. Again, it must be emphasized that in fully dried films, organisms generally show an exceptionally high resistance to any disinfecting action of ozone. Haines (1935) has reported that, for organisms in the vegetative state on nutrient agar, ozone in concentration greater than 200 p.p.m. was needed to accomplish sterilization. In some tests we designed to test the penetrative power of ozone, *Staphylococcus albus* was dispersed in serum agar and subjected to action of a slow current of ozonized oxygen past the surface, and compared, after incubation at 37° C., with a similar culture treated with oxygen only. An ozone concentration of 330 p.p.m. achieved complete surface sterilization, but there was no appreciable penetration of disinfecting action below the surface. This would be expected in view of the rapid interaction of ozone with organic matter.

A few experiments were made to compare the action of ozone against bacterial aerosols in darkness and when an electric light illuminated the experimental room. No significant differences could be demonstrated.

Some further discussion is desirable of the observation that humidity favours the disinfecting action of ozone. Wells and his co-workers (see Wells & Wells, 1936; Wells, 1940), in their studies on air-borne infection, have come to regard humidity as a basic factor governing (*a*) the viability of organisms dispersed as droplet nuclei in the air, and (*b*) the bactericidal action of ultra-violet radiation on such bacterial aerosols. It is interesting to note, however, that with ultra-violet light the bactericidal action is greatest at low humidities; for instance, Wells finds that against *B. coli* in an atmosphere of 45 % R.H., it is about ten times as lethal as at 90 % R.H. Very recently, Baker & Twort (1941) have reported upon tests which have shown the prevailing humidity to have an important bearing on the disinfecting power exhibited by certain smokes. They have also found that high humidity promotes the bactericidal efficiencies of some phenolic vapours, and likewise of some antiseptic aerosols but here much depends on the nature of the solvent involved.

The effects exerted by humidity on such aerosol systems as those here being considered can be summarized under three headings:

(1) The purely physical effect on limiting particle size and settling rate. This has been discussed by Wells (1934).

(2) The effect on the normal viability of air-borne organisms, survival being favoured by low humidity (Kirstein, 1900, 1902; Wells & Riley, 1937; and findings in the present studies). Not only bacteria, but certain viruses also, have, in recent years, been shown to lose their activity rapidly in the partially dried state, but will remain active for long periods when thoroughly dry. Reinberger & Bailley (1940) have attributed this, in the case of loss of virulence of rabies virus from brain tissue, to proteolytic action for which moisture is essential.

(3) The interaction between ozone and the organisms, as reflected in the increased death-rate of the latter, and favoured by high humidity. This is probably due to the fact that certain determinant chemical groups (enzymic?) responsible for the metabolic activity of the organism, are more vulnerable to attack by ozone in the presence of water than in its absence. Ozone is not unique in this respect. The effectiveness of hypochlorous acid gas in disinfecting bacterial aerosols has in our experience also been found to be conditioned by the factor of humidity. For instance, at 0·3–1·0 p.p.m. hypochlorous acid gas, as generated by bubbling a mixture of air and carbon dioxide through a dilute aqueous suspension of bleaching powder, has manifested but slight killing effect against certain streptococci and staphylococci in atmospheres of 30–40 % R.H., but, other factors remaining constant, the raising of the humidity to 60–80 % has resulted in more than 99 % of the organisms being killed in less than 5 min. Humidity clearly plays an essential part in determining the underlying chemical reactions of the disinfecting processes due to these gases. The speed with which ozone exerts its disinfecting action is slower than that of hypochlorous acid gas with which the killing effect can be very rapid and complete [cf. Masterman (1941)].

Data, for which we thank our colleagues Drs Bourdillon and Lidwell, made available during the past two years indicate that in practice the average relative humidities prevailing in unheated and non-ventilated shelters of various types range from 50 to 80 %, according to climatic conditions; while, if the shelters are at all crowded, the figure may reach 90 %. In the London underground tubes, on the other hand, which are ventilated and heated, an average figure is 40–50 % R.H.

The power of ozone to disinfect (it is also recognized as a good deodorizer) an appropriately humid atmosphere, when present in such extreme dilutions as a few parts per hundred million parts of air, is to be attributed to its great oxidizing power. It is possible that in these extreme dilutions, it may catalyse the oxidizing processes of the cell to such an extent as to destroy the normal oxidation-reduction balance. It is well known, however, that ozone has a particular affinity for unsaturated linkages and readily forms ozonides. Wulliémoz (1938) has suggested that the mechanism of the disinfecting action of ozone is to be found in the formation of such compounds.

Summary and general conclusion as to the value of ozone as a means of combating air-borne bacterial infection

The question formulated in the introduction to this report can now be answered. Ozone in tolerable concentration is able to inactivate certain bacteria when these are present as unprotected singleton aerosol particles in atmospheres of 60–90 % R.H. When such bacteria are covered with a protective coating of organic matter, however, as in aerosols naturally emitted during a sneeze or cough, then ozone in permissible concentration is without significant effect. Inactivation of such protected organisms can only be achieved by the use of ozone in decidedly higher concentrations, which must be deemed dangerous for prolonged spells of breathing. Bacteria that have settled on surfaces are generally more resistant to ozone than when in newly formed aerosols. Hence experimental evidence leads to the conclusion that ozone, in concentrations that can be breathed over long periods without irritation, cannot be expected to provide any effective protection against air-borne bacterial infection, through direct inactivation of the infectious carrier particles.

Our thanks are expressed to Mr R. E. Glover for his ready co-operation in one section of this investigation.

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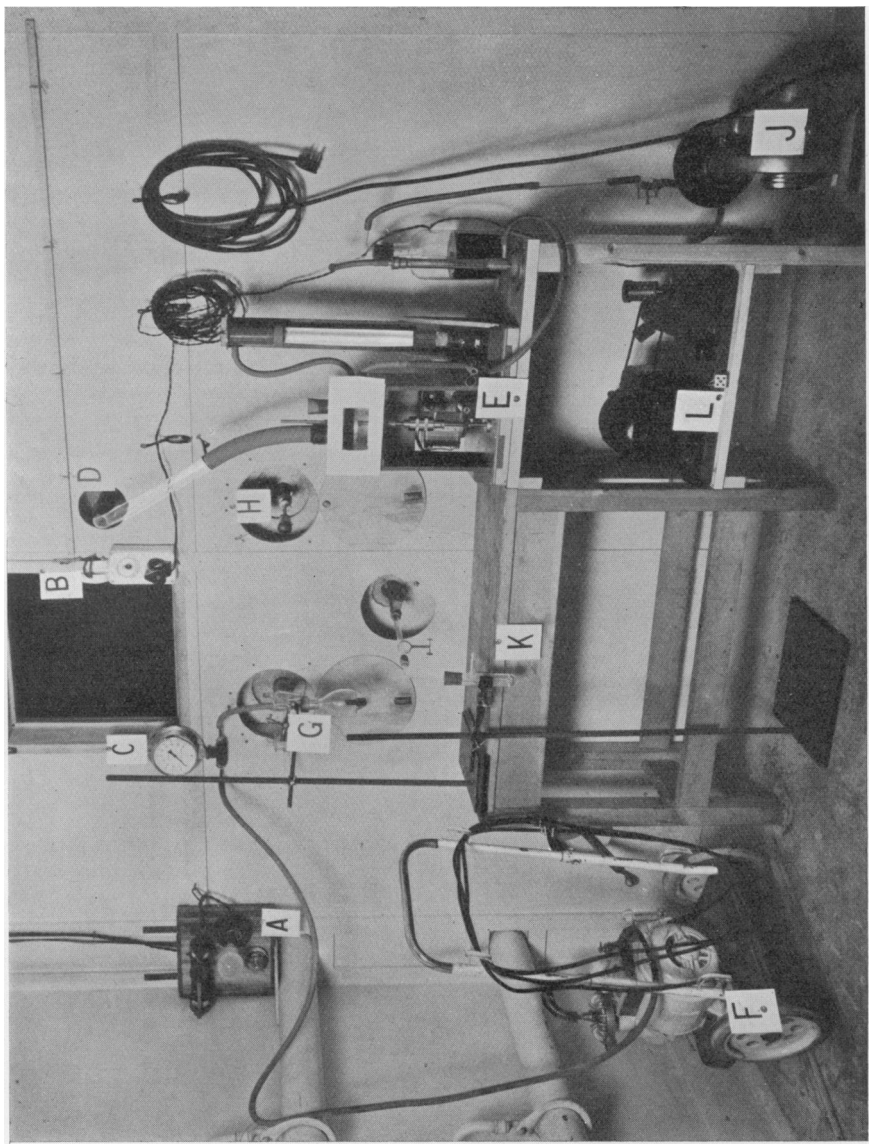
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Fig. 1

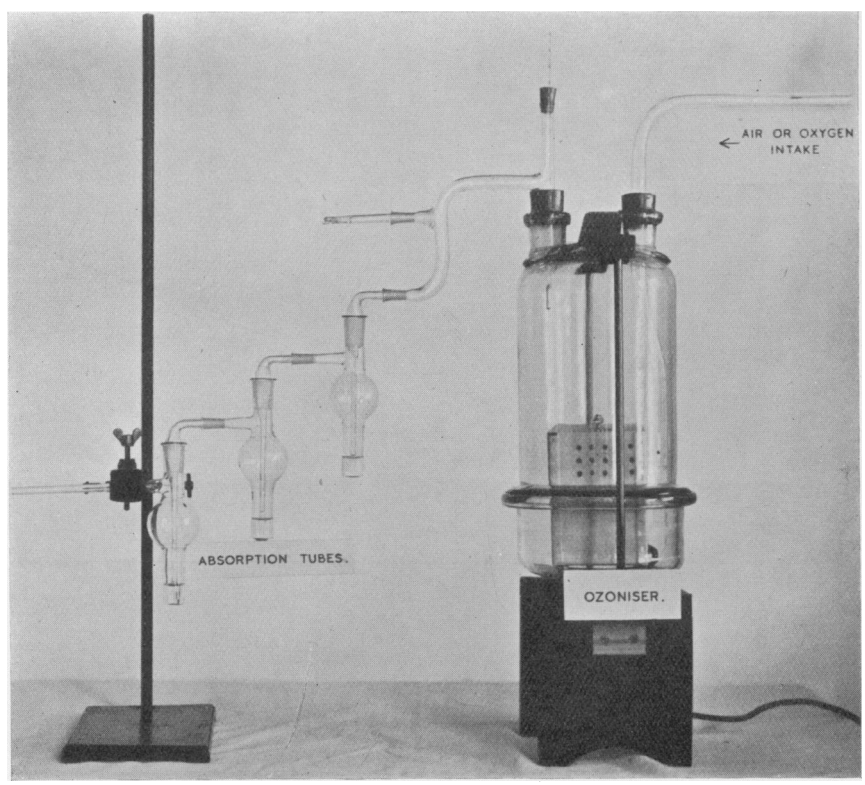

Fig. 2

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EXPLANATION OF PLATES 8 AND 9

PLATE 8

A. Control switch for fan. B. Ozonizer switch and observation window. C. Pressure gauge for spray G. D. Sampling tube to slit machine E. F. Compressor. H. Handle to lever arm for uncovering Petri dish. J. Martindale blower. K. Ozone absorption tube. L. Small suction pump for slit machine.

PLATE 9

Fig. 1. A. Sampling tube leading to slit machine. B. Sampling tube leading to ozone absorption tube. C. Table carrying special dishes for plate exposures. D. Spray inlet tube. E. Lever arm for uncovering Petri dishes. F. Tube from Martindale blower.

Fig. 2. Apparatus used in analysing the ozone produced by the ozonizer tube.

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