BACTERIOLOGICAL OBSERVATIONS ON THE AIR OF OCCUPIED PREMISES

I. AIR DISINFECTION WITH HYPOCHLORITES. A SIMPLE PRACTICAL METHOD OF DISINFECTING THE AIR OF OCCUPIED PREMISES

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(With 4 Figures in the Text)

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INTRODUCTION

The large-scale air attacks on Great Britain which commenced during the later months of 1940 caused large numbers of people to take refuge in air-raid shelters. As the attacks were often of long duration, and many of the shelters were overcrowded, the transmission of infectious disease under these conditions became a serious possible danger. Many of the shelters had been hastily constructed and, consequently, sanitation, ventilation, etc., and hygienic conditions in general, were not always of an adequate standard and, in many cases, were definitely unsatisfactory. Further, for safety purposes, proper ventilation was not always feasible.

Thus, the problem of improving the conditions of the air in occupied shelters with a view to reducing the spread of air-borne disease became a matter of prime importance. An obvious method of tackling this problem was to develop some means of reducing the numbers of pathogenic micro-organisms present in the air of occupied shelters, e.g. by spraying the air with a disinfectant, and the present work was undertaken with this object in view.

While this work was in progress the suggestion was made from the military side that a similar investigation might be carried out in occupied army huts and other premises with the object of establishing a *simple practical* method of reducing the numbers of pathogenic micro-organisms in the air of such quarters, thereby diminishing the possibility of the spread of certain infectious diseases.

The first of these investigations consisted of several experiments carried out in sleeping quarters during the night. In spite of the decrease in ventilation which usually occurs as a result of black-out precautions the ventilation in a Nissen hut used for the first tests was found to be much more efficient than expected and surprisingly few organisms were found in the air—too few to afford reasonable prospects of a successful conclusion to the main investigation. Similar results were obtained even when the number of occupants of the hut was increased above the normal quota.

Accordingly, it was decided to experiment under conditions which could be made better or worse at will, i.e. under controlled conditions. The room chosen for the purpose was of capacity about 1700 cu. ft., and arrangements were made for varying the degree of ventilation in the room. The local military medical authorities provided the personnel, usually twelve or fifteen convalescent surgical patients, who occupied the room for periods of 2–3 hr., and a considerable number (21) of experiments were carried out. Under these conditions the occupied room was overcrowded and the air conditions were considered to simulate those which would usually pertain to overcrowded premises. In all these experiments Bourdillon's 'slit sampler' (see later) was used for the bacteriological tests, and numerous observations of the bacterial content of the air were made during each experiment.

In some of these experiments the occupants of the room were asked to cough or sneeze, and the efficiency of hypochlorite sprayed into the air in reducing the bacterial contamination was studied; in other experiments the conditions were those of normal respiration, i.e. with no experimental coughing or sneezing. In both series of experiments the effect of varying degrees of ventilation was also investigated.

The present communication is concerned solely with the action of hypochlorite on the total bacterial content of the air of an occupied closed room under *fixed* conditions of ventilation. The ventilation was of a low degree of efficiency, and the occupants of the room were under the conditions of normal respiration as mentioned above. It is hoped to publish later the results of the other experiments.

Different hypochlorites were used, and these are described later. The use of hypochlorites for disinfection of the air has received increased attention during the last few years, but there seems to be considerable difference of opinion concerning the efficiency of the process, and the technique of the methods to be employed (e.g. optimum concentration of the hypochlorite solution for atomization; concentration of hypochlorite required in the air; method of atomization of the hypochlorite; duration of persistence of the disinfectant in the air and, therefore, number of atomizations required, etc.). As long ago as 1928, Douglas, Hill & Smith experimented in this way and Masterman made several communications on the subject (for a review of such work see Masterman, 1941). The possible prevention of air-borne infection by hypochlorite spray has been studied by Middleton & Gilliland (1941). They used a suspension of bleaching powder sprayed from a flit gun, and this simple method has been tested in the investigation recorded in this paper. More recently, Bourdillon, Lidwell & Lovelock (1942) have reported upon sneezing in relation to disinfection by hypochlorites, the experiments being carried out in a room which was, however, empty except for the 'producer of the sneezes'.

It appears to be established that hypochlorous acid is the effective germicide when hypochlorites are sprayed into the air, and while Masterman (1941) favours the view that hypochlorous acid gas (set free, for example, by the carbon dioxide of the atmosphere) is the active germicide, other workers incline to the 'aerosol' theory, i.e. that the active agent is hypochlorous acid in solution (e.g. in droplets). Whatever the mode of action, the importance of the method of atomization of the hypochlorite is acknowledged and, for maximum efficiency, a high degree of atomization is said to be necessary.

In these experiments, the hypochlorite was sometimes atomized by means of a Dynalysor (Masterman, 1938, 1941), which is an efficient atomizer and produces a fine 'dry mist'. More often a simple hand-spray (flit gun) giving a coarse spray containing many large droplets was used. This hand-spray was chosen because it is inexpensive and is available in reasonably large quantities, and, as stated above, the main purpose of this investigation eventually became the development of a *simple practical* method of disinfecting the air—in short, a method which could be employed with the minimum of preparation and delay. It is for this reason that bleaching powder (a substance available to all army units) was included among the hypochlorites tested. Owing to the corrosive nature of hypochlorites, certain simple precautions must be taken and these are discussed later.

In view of the object of this work as stated above, investigation of the interesting theoretical considerations involved, e.g. in the mode of action of the disinfectant, could not be pursued as thoroughly as desired. However, it was shown by experiments in which water was sprayed that the effect of the hypochlorite was not merely a mechanical removal (i.e. washing down) of the micro-organisms from the air, as there was no appreciable reduction of the bacterial content of the air after the spraying of water. Also, an attempt was made to correlate the effect of the humidity of the atmosphere and the efficiency of hypochlorite sprays. Baker & Twort (1941) state that hypochlorite sprays are less effective in a dry atmosphere, and Bourdillon *et al.* (1942) finds that the practical lower limit of humidity for effective bactericidal action is below 60% at 70° F. Determinations of the relative humidity were carried out in most of the experiments about to be described and the results are discussed. The advantages and disadvantages of hypochlorites for air disinfection are considered later.

Attempts were made to distinguish potentially pathogenic organisms (those originating from the upper respiratory passages) from saprophytic organisms, and, for this purpose the use of selective media containing inhibitory agents for certain organisms has proved of great value. The medium finally chosen was a blood-agar medium containing crystal violet (in a concentration of 1/1,000,000) and work is proceeding in an attempt to devise a method of ascertaining the degree of contamination of the air of occupied rooms by organisms from the upper respiratory tract. Results to date are sufficiently encouraging to suggest that it may be possible to formulate an index of respiratory tract contamination of the air of occupied rooms, etc.

In the present communication, however, the disinfecting power of hypochlorites has been assessed by observations on the *total* number of organisms found in the air before and after the spraying of the disinfectant, i.e. no attempt has been made to differentiate between pathogenic and saprophytic organisms. It may be stated, however, that of the *total* number of organisms collected from the air in these experiments the number of potentially pathogenic organisms constituted a small percentage only. Unless otherwise stated the medium used for this investigation was blood-agar containing 5% of horse blood.

METHODS OF COLLECTING AND COUNTING AIR-BORNE BACTERIA

Petri has stated that the bacteria in 10 l. of air are deposited on 100 sq. cm. of a gelatine plate during 5 min. exposure of the plate. More recent work has shown that this simple method is open to objections. For example, Bourdillon, Lidwell & Thomas (1941) find that this method collects preferentially those bacteria attached to heavy particles, e.g. dust, etc., as distinct from single bacteria, but agree that the counts obtained by simple plate exposure may be of some value for comparative tests. Methods offering more exact results involve enumeration in a given volume of air (for a brief discussion of some of the available methods see Bourdillon *et al.* 1941).

Probably the most efficient means of enumerating the bacteria present in the air involves the use of the 'slit sampler' designed by Bourdillon *et al.* (1941). In this machine a given volume of air is aspirated through a narrow slit (0·25 mm. wide, 27·5 mm. long and 3 mm. deep) on to a plate of nutrient medium placed close to the slit, the plate being made to rotate slowly in order that the organisms may be distributed evenly over it. The plates are then incubated usually at 37° C. for 24 hr. or longer.

Some of the observations in a few of the early experiments in this investigation were ordinary simple plate exposures made at a height of 3 ft. 4 in. from the floor, but for most of the work, including all the experiments in the occupied room, the slit sampler was available.

THE SLIT SAMPLER

The slit sampler was constructed according to the specification of Bourdillon *et al.* (1941) and was fitted with an electric (a.c.) gramophone motor suitably geared down for rotating the plate. In these experiments, the slit-plate distance was adjusted to 3 mm. (cf. 2 mm. recommended by Bourdillon). This slightly larger slit-plate distance was considered more suitable for work under adverse conditions, e.g. in badly lighted and overcrowded air-raid shelters where exact adjustment of this distance may not always be attained, and this, together with possible irregularities in the surface of the medium and/or of the base of the Petri dishes, might result in the base of the tube carrying the slit making contact with the medium. Furthermore, experiments were carried out at slit-plate distance of 2 and 3 mm. respectively and yielded results differing only slightly from each other, so that, for comparative observations, either distance is satisfactory. The necessary suction was provided by means of an ordinary domestic vacuum cleaner (an Electrolux), this proving more easily procurable, less expensive, probably lighter in weight, and certainly more transportable than the electric motor and special pump recommended by Bourdillon. The vacuum cleaner was covered with several layers of sound-absorbing material in order to reduce noise when used in sleeping quarters, and then fitted with a handle to facilitate transport. Calibration of the slit sampler showed that 1 cu. ft. of air per minute was sucked through the slit when the manometer showed a difference in level of $11\frac{1}{2}$ in. of water. For convenience, the vacuum cleaner was placed on its end and the slit sampler placed on the upper end of the vacuum cleaner. Under these conditions, the top of the short tube carrying the slit was about 40 in. from the floor, this height corresponding approximately with that of the head of a person in a sitting position.

Technique of experiments in an empty room

(1) *Experiments in which no hypochlorite was used*

Experiments were carried out in different rooms but only those experiments performed in the room later used for 'the experiments in the occupied room' will be described here. This room was of medium size (capacity = 1680 cu. ft.) and its small window and ventilator were permanently sealed. Ventilation of the room was further reduced by fixing cardboard along the edges of the door in order to exclude air currents.

Observations of the number of micro-organisms in the air of the room were made by simple exposure, usually of 2 min. duration, of plates of nutrient agar (as already stated, the slit sampler was not available for these early experiments).

Infection of the air of the room was then effected by spraying into the air small amounts (0·15–0·45 c.c.) of a suspension of a 24 hr. culture of the organism. *Staphylococcus albus*, *B. prodigiosus* and a diphtheroid bacillus were each separately used in different experiments.

The medium, in which the bacterial suspension used for infecting the room is prepared, is of importance and failure to appreciate the importance of this factor may account for the different results sometimes obtained in experiments on disinfection of air. The following types of suspension were used: (1) a suspension in sterile saline; (2) a suspension in sterile tap water; (3) a 24 hr. broth culture of the organism. In the latter case, the disinfecting action of the hypochlorite appeared to be lower than when either of the first two types of suspension were used. This result was probably due to the fact that the bacteria, when sprayed into the air as a broth culture, were coated with organic matter (nutrient broth) which inactivated some of the hypochlorite thus leaving a reduced amount of disinfectant available for attacking the bacteria. Such an explanation is in agreement with the fact that hypochlorous acid has a great affinity for organic matter. Accordingly, in most of these experiments, the bacterial suspension used for infecting the room was prepared in sterile saline or in sterile tap water. Suspensions of the organisms used in either of these fluids were tested and it was shown that large numbers of the organisms remained alive after many hours, i.e. for a period many times that of the duration of the experiments.

The fluid (a few c.c.) was added to a 24 hr. agar-plate culture which had been heavily inoculated, the abundant growth carefully removed using a wire loop as sparingly as possible, the resulting suspension adjusted to tube 3 or 4 of Brown's opacity standards, and then transferred to a hand-spray. The spray used was a small hand nasal spray, and it was found that 15–30 compressions of the rubber bulb were sufficient to infect the air of the room to the degree required. A gauze mask of the type used in operating-theatres was worn when infecting the room and for the remainder of the experiment in order to reduce the number of bacteria inhaled during the course of these experiments.

Observations after infection of the room. Plate exposures were made at suitable intervals, duplicate Petri dishes of nutrient agar of a standard size (9·5 cm. in diameter) being used for each observation. The plates were placed side by side on a table or stool and were at a height of 3 ft. 4 in. from the floor.

The exposures were timed with a stop-watch and were usually of 2 min. duration. A few of the exposures, however, were of shorter duration, e.g. the first two or three after infection of the room (1 min. exposure), and others, towards the end of the experiment when the organisms in the air were much reduced in number, were for periods of 4–16 min. or longer. When longer exposures were necessary 2 min. exposures were also simultaneously made. Observations were usually continued for 1–1½ hr. after infection of the room.

The plates were then incubated at 37° C. for 24 hr. after which the colonies on each plate were counted. The usual assumption was made that each colony was formed from a single organism, although it is realized that this is only approximately true. Results showed that, when the rate of deposition of organisms was slow, i.e. towards the end of the experiment, the number of organisms per 4 min. exposure was approximately twice the number of organisms per 2 min. exposure. Occasionally, therefore, the count for a 4 min. exposure was halved and the result averaged with that for a 2 min. exposure, but this procedure was rarely adopted.

At least four control plates, i.e. unexposed plates, were incubated along with the experimental plates in these and all experiments described in this communication. In every case the number of adventitious contaminants on the control plates was very low (usually 0–2 colonies per agar plate and 0–3 colonies per blood-agar plate) and it was unnecessary to correct the counts on the experimental plates by subtracting the average number of contaminants on the control plates.

(2) *Experiments in which hypochlorites were sprayed into the air*

Experiments were then carried out using a similar suspension of the same strain of *B. prodigiosus* or other test organism, but this time hypochlorite was sprayed into the air shortly after infection of the room. Two hypochlorites were tested, (1) calcium chlorohypochlorite in the form of bleaching powder, and (2) sodium hypochlorite in the form of a proprietary antiseptic. The bleaching powder contained 33% of available chlorine and a 1% suspension, which was freshly prepared immediately before each experiment, was used. The proprietary antiseptic (1% sodium hypochlorite, 16·5% sodium chloride and small amounts of calcium chloride and of other salts of Na and Mg) was analysed and the hypochlorite content was in agreement with the manufacturer's specification; this antiseptic is hereinafter called hypochlorite X.

In the experiments in which sodium hypochlorite was used the solution was, in different experiments, introduced into the atmosphere (1) from a Dynalysor which atomized 1 c.c. of the hypochlorite solution per minute, and mention of which has already been made, and (2) from the flit gun used in the experiments in the occupied room and which produces a coarse spray. The bleaching powder used was in the form of a suspension and not a true solution and therefore was not suitable for the Dynalysor; it was tested only in the form of the coarse spray (i.e. using the flit gun).

Varying amounts of hypochlorite were used in different experiments and the concentration (not necessarily the minimum concentration) of the hypochlorite required for

https://doi.org/10.1017/S0022172400035841 Published online by Cambridge University Press

effective reduction of the bacteria in the air of the room was ascertained. During these experiments the concentration of hypochlorite in the air was such that the odour was distinctly perceptible, although the irritant effects were negligible.

(3) *Experiments in which water was sprayed into the air*

Similar experiments were carried out in which water instead of hypochlorite was sprayed into the air and both methods of atomization were used.

Results of experiments in an empty room

The experimental data for a few experiments, typical of the many experiments performed, are summarized in Table 1, in which each of the bacterial counts represents an exposure of 2 min. duration. Five control experiments were carried out, and the period

Table 1. *Variation in the bacterial content of the air of a room infected with a saline suspension of* B. prodigiosus *in a control experiment, and in experiments in which water or hypochlorites were sprayed into the air from a flit gun*

	Period (minutes) after infection of the room														
Exp.	2 min.	5 min.	Disinfectant, etc. sprayed into the air at 6–7 min.	9 min.	10 min.	12 min.	15 min.	20 min.	30 min.	45 min.	60 min.	65 min.	75 min.	90 min.	120 min.
11	—	990	None	—	331	—	—	109	46	18	10	—	4	4	4
42	826	706	Water (13 c.c.)	612	536	475	411	259	115	65	30	23	—	—	—
40	990	842	1% suspension of bleaching powder (12 c.c.)	60	52	18	14	6	1	0	0	0	—	—	—
41	1002	944	1% suspension of bleaching powder (18 c.c.)	18	0	0	1	1	0	0	0	—	—	—	—
45	632	462	1% suspension of bleaching powder (6 c.c.)	258	—	171	158	117	54	20	7	5	—	—	—
47	628	468	('Smoking exp.') 1% suspension of bleaching powder (13 c.c.)	272	285	183	165	115	37	12	4	—	2	2	—
44	956	960	Hypochlorite X (10 c.c.)	86	63	31	4	12	5	1	0	0	—	—	—
34	408	395	Hypochlorite X (4 c.c.)*	—	48	—	18	13	6	2	1	—	3	—	—

All bacterial counts represent the number of colonies of *B. prodigiosus* on an agar plate after exposure for 2 min. — means no reading taken.

* In Exp. 34 the antiseptic was atomized by means of the Dynalysor.

of time which elapsed before the air of the infected room became as free from bacteria as it was before the infection occurred was about 1 hr. (e.g. Exp. 11, Table 1). This time was not reduced when water was sprayed into the air from a flit gun (Exp. 42), and a similar result was obtained in another experiment with water.

When the 1% suspension of bleaching powder was sprayed into the air of the room the air became free from *B. prodigiosus* in a much shorter time than in the control experiments. For example, in Exp. 40, the amount of suspension sprayed into the air by fifty strokes of the flit gun was 12 c.c., and it will be seen that, 3 min. after completion of the spraying (i.e. 10 min. after infection of the room), the bacterial count was as low as 52 and 10 min. later was only 6.

Comparable results were obtained with the proprietary antiseptic containing 1% of sodium hypochlorite (Exp. 44).

The results of Exp. 40 indicate effective disinfection of the air, and the concentration of the 1% suspension of bleaching powder used was exceedingly small (12 c.c. of the suspension in 47,500,000 c.c. of air corresponding to 0·25 c.c. suspension per million c.c. of air), and, it may be added that the odour of the hypochlorite was easily perceptible in the room. At this concentration exceedingly slight irritation of the mucous membranes of the nose and throat was experienced but the eyes were not affected. Although in Exp. 41 the amount of the suspension used was 50% greater than in Exp. 40, the irritant effects were again exceedingly slight, and the reduction of the bacterial count was greater and more rapid than in Exp. 40. The degree of air disinfection achieved in Exp. 41 was very high. When the amount of suspension sprayed into the air (Exp. 45) was reduced to about half of that used in Exp. 40, there was no appreciable disinfection of the air. During each of the experiments so far described the experimental room was occupied only by the writer. In Exp. 47 three other persons were present and all the occupants smoked heavily throughout the experiment, the air becoming thickly clouded with tobacco smoke. The results of this experiment (Table 1) show that the disinfecting action of the hypochlorite was almost entirely inhibited.

The results of a typical experiment (no. 34) in which the Dynalysor was used are included in Table 1, and in this experiment the proprietary antiseptic was used. It will be seen that a degree of disinfection comparable with that of the flit-gun Exps. 40 and 44 was achieved with a concentration of hypochlorite only about one-third of that used in the flit-gun experiments.

The experimental data show that disinfection of air infected with *B. prodigiosus* was effected with a 1% suspension of bleaching powder sprayed into the air by means of a flit gun (concentration = 0·25 c.c. of the suspension per million c.c. of air) and with a similar concentration of a proprietary antiseptic containing 1% of hypochlorite. Comparable results were obtained with a much lower concentration when hypochlorite X was atomized into the air by means of the Dynalysor. It may also be stated that the effect of the hypochlorite was not merely a mechanical removal of bacteria from the air since the spraying of water had no appreciable effect on the bacterial content of the air.

In each of the experiments described above the test organism was *B. prodigiosus*; it may be added that similar results were obtained when the air of the room was infected with each of two strains of *Staphylococcus albus* or a diphtheroid bacillus, each of which had been isolated from the air during the experiments in the occupied room (described later).

TECHNIQUE OF EXPERIMENTS IN AN OCCUPIED ROOM

The room chosen (see p. 20) was a medium-sized room of about 1700 cu. ft. capacity (15 × 14 × 8 ft.). The single small window in the room had been sealed and permanently blacked out (the room was the departmental dark-room) and the one small ventilator situated in the roof of the room was closed. Cardboard was fixed along the top, bottom and side edges of the door in order to exclude air currents as far as possible, and the ventilation of the room was therefore at a minimum. The room, which was electrically lighted, had a wooden floor and contained a water radiator which was turned on when requested by the occupants of the room (this was usually the case). Seats sufficient in number to accommodate the future occupants were arranged around three sides of the room (along the fourth side was a sink and a work-bench) and the centre was occupied by a table. During each of Exps. 1–6 there were fifteen men in the room and this number was reduced to twelve for the subsequent experiments. In addition an assistant and the author were present during each experiment. With this relatively large number of occupants the air of the inadequately ventilated room soon became 'stuffy', and in fact the air conditions in this crowded room resembled those in an air-raid shelter under conditions of moderate overcrowding.

In all these experiments the observations on the number of micro-organisms in the air were made with the slit sampler, and it was found convenient to place the latter on the table and, in this position, the machine was situated in the middle of the room. Wet- and dry-bulb thermometers were hung from a shelf situated along the fourth side of the room, i.e. reasonably far enough away from any of the occupants of the room.

Shortly before occupation of the room, observations of the bacterial content of the air of the empty room were taken with the door closed. For each of these observations the slit sampler was operated for ·3 min., i.e. 3 cu. ft. of air were sampled. At 2 p.m. the men entered the room, took their places on the seats provided, and the door was immediately closed and not opened until the end of the experiment (some 2–2½ hr. later).

On the day before each experiment, the floor of the room was thoroughly washed with water, but, in order that natural conditions might be observed as far as possible, the floor was not treated by any of the methods, e.g. treatment with spindle oil, etc., now available for reducing aerial contamination due to disturbance of bacteria-carrying particles (van den Ende, Lush & Edward, 1940; Thomas, 1941, etc.). It was impractical to allow the men to move about the room during the experiment, since the resulting disturbance of bacteria-carrying particles on the floor would have caused varying degrees of contamination of the air and controlled observations would then have been impossible. In any case, such movement would have been difficult under the relatively overcrowded conditions prevailing in the room.

Accordingly, the occupants of the room were asked to remain in their places during the experiments but were free to talk. Most of them read and there was surprisingly little conversation. Generally, a short account of the object of the experiments was given at the start and proved of great value in interesting the men in this work and thereby obtaining their willing co-operation—an essential requirement in an investigation of this kind.

This co-operation, together with the fact that the men were 'on duty' when taking part in this work, made it possible to ensure that the conditions for each of these experiments were closely similar. This was so although there were frequent changes in the personnel in these experiments. In fact, this degree of controllability of the experimental conditions throughout the course of this investigation was much higher than expected and was an important factor in ensuring that the results obtained were of real value. Also, the combined individual opinions of the men were of value in assessing the degree of irritation (if any) produced in the eyes, nose and throat, by the hypochlorite sprayed into the air, in deciding upon the duration of persistence of the odour of the hypochlorite, and in other ways.

A general description of the course of the experiments will now be given. As already mentioned, observations of the number of micro-organisms in the air of the room were

https://doi.org/10.1017/S0022172400035841 Published online by Cambridge University Press

made just before occupation. Blood-agar plates of standard size were used in the slit sampler and the exposures were each of 3 min. duration. Readings of the wet- and dry-bulb thermometers were taken, and were continued every 15–30 min. throughout the experiments.

Experiments had previously been carried out on different volumes of the air of occupied and unoccupied rooms and, from the numbers of micro-organisms found, it was estimated that 3 cu. ft. of air (i.e. 3 min. operation of the slit-sampler) usually contained a number of organisms sufficiently large for the comparative tests required and did not, even under the worst conditions encountered, overcrowd the plate (counts seldom exceeded 300 colonies per plate). Accordingly, after occupation of the room, slit-sampler observations of 3 min. duration were made and about six or seven plates were thus exposed during each half-hour (see Table 2). Each plate was numbered and its time of exposure was noted.

In control experiments, no hypochlorite was sprayed into the air and exposures were carried out during 2–2½ hr. occupation of the room. In the experiments with hypochlorites, the solution or suspension was sprayed into the air at intervals of half an hour and the first 'spraying' was usually made after half an hour's occupation of the room. Other sprayings of the disinfectant were made at half-hourly intervals, although in one experiment sprayings were more frequently carried out. Except when stated the flit gun was employed for atomizing the hypochlorite and a 1 % solution was used. With bleaching powder, however, a 1·3 % suspension in water was used (see later).

Some idea of the amount of hypochlorite required had already been obtained from consideration of the amounts used for the experiments in the empty room. Thus, it was found that an effective concentration of hypochlorite was introduced into the air of the occupied room (volume = 1680 cu. ft., i.e. 47,500,000 c.c.) by fifty strokes of the flit gun. The average time required for each 'spraying' was about three-quarters of a minute. A somewhat variable quantity of hypochlorite was thus 'atomized'. For example, the volume of 1 % solution or 1·3 % suspension introduced into the air varied from 12 to 24 c.c. per spraying and was usually about 19 c.c., corresponding to a concentration by volume of 0·40 c.c. per million c.c. of air. As a rough guide, spraying was continued *until the odour of the hypochlorite was distinctly perceptible to the majority of the occupants.*

In each experiment usually three or four sprayings were carried out, although in Exps. 13 and 19 the number of sprayings was two and six respectively. Since the results suggested that the bactericidal effect of the spray was of short duration (of the order of 15–20 min), estimation of the concentration of hypochlorite introduced into the air during each spraying was calculated without regard to any residual concentration remaining from a previous spraying (see later).

During each spraying, the occupants of the room were asked to look down at the floor so that all possibility of any droplets of the sprayed hypochlorite solution or suspension falling into their eyes was avoided. At the same time, care was taken to prevent droplets making contact with the clothes of those present. Similar precautions must also be taken by the person operating the 'spray'.

Slit-sampler observations of the bacterial content of the air were made throughout the experiments. The plates were then incubated aerobically at 37° C. for 30 hr. Varying periods of incubation were tried. Thus, an incubation period of 48 hr. sometimes gave rise to unsatisfactory plates, e.g. if many haemolytic organisms were present the whole

https://doi.org/10.1017/S0022172400035841 Published online by Cambridge University Press

plate became discoloured. After 30 hr. incubation slightly higher counts were obtained than after 24 hr. and colonies which were small and difficult to recognize as colonies after 24 hr. incubation were easily observable after a further 6 hr. Practically all the organisms encountered in these experiments grew well during the chosen incubation period of 30 hr. at 37° C.

The colonies on the plates were then enumerated and graphed against the period of occupation of the room. From such comparative data the variation in the numbers of bacteria present in the air could be studied and the effect of the hypochlorite assessed.

Occasionally, an exposure was ruined by organisms (usually sporing bacilli) the growth of which spread over the surface of the plate, but the number of these was small. For example, of the experiments illustrated in Table 2 it will be seen that in Exp. 8 six out of thirty-nine exposures were useless because of the presence of such spreading organisms. The proportion of 'large spreaders' in this experiment was, however, higher than in most other experiments (cf. Exps. 2 and 7 in which no such organisms were present). Also, in a few instances a spreading organism, occupying a relatively small area of the plate, was present. For such plates allowance for the surface area of the medium lost on account of the 'spreader' was made in the following manner: The plate was held to the light and a piece of thin paper of size sufficient to cover the area of the plate occupied by the colonies was held at the back of the plate. The outline of the 'spreader' was then traced on the paper and this area cut out and weighed. From the weight of the paper representing the total area occupied by the colonies and that representing the area occupied by the spreading organism, the relative area of the plate occupied by the latter was determined with reasonable accuracy, and a proportional number of colonies added to the number present on the plate.

Hypochlorites used in these experiments

Two sources of hypochlorite were used: (1) Calcium chlorohypochlorite in the form of bleaching powder as supplied to the Army for sterilization of water, and known as water-sterilizing powder. As this bleaching powder contained only 25% of available chlorine, instead of the 33% present in the commercial product, the strength of the suspension of water-sterilizing powder used in these experiments was 1·3%, corresponding with the 1% suspension of the stronger bleaching powder used for 'the experiments in an empty room'. Suspensions of bleaching powder required for each spraying were always separately prepared, immediately before the spraying was due to take place, by adding 1·3 g. of the powder to 100 c.c. of water, and the whole of this suspension was poured into the flit gun. At the end of the spraying, the remaining suspension was emptied out of the gun and a freshly prepared suspension used for the next spraying. (2) A commercial product consisting essentially of an alkaline solution of sodium hypochlorite. This product contained 10–11% of the hypochlorite, approximately 10% of sodium chloride, 1% of sodium hydroxide and a small amount of potassium permanganate which gives the solution its pink colour. The percentage of hypochlorite in this product was determined volumetrically, and, when used in these experiments, it was diluted with tap water so that the diluted solution contained 1% of hypochlorite. This *diluted* solution is hereinafter called hypochlorite Y, and is chemically equivalent (in available chlorine) to about three times its volume of the bleaching powder suspension.

https://doi.org/10.1017/S0022172400035841 Published online by Cambridge University Press

Concentration of hypochlorite in the air as used in these experiments

The amount of hypochlorite introduced into the air as the result of each spraying of disinfectant has already been stated to be 19 c.c. on the average. Thus, in Exps. 1, 3, 4 and 6 there were thirteen sprayings in all of a 1·3% suspension of bleaching powder, the average volume of bleaching powder suspension per spraying being 20 c.c., and one spraying of hypochlorite Y (18 c.c.). In Exps. 8, 12, 13 and 19 in which hypochlorite Y (that is, approximately 1% sodium hypochlorite) was used, the total number of sprayings was sixteen and the average volume was 18 c.c. per spraying. The total volumes of hypochlorites which were sprayed into the air (and the concentration per spraying) in each of these experiments are summarized later (Table 5). These quantities (20 and 18 c.c.) correspond to a concentration by volume of 0·42 and 0·38 c.c. per *million* c.c. of air, for bleaching powder, and hypochlorites Y respectively. In Exp. 8 an amount of disinfectant smaller than usual (e.g. thirty instead of fifty strokes of the flit gun) was tried for one of the sprayings. The amount of hypochlorite Y thus sprayed into the air was 13 c.c. and, as will be seen later, proved effective in lowering the bacterial content of the air. The concentrations of disinfectant introduced into the air in each of these experiments were, therefore, very minute. Further, the hypochlorite solution Y used contained only 1% of sodium hypochlorite, and the bleaching powder suspensions only the equivalent of about 0·59% of calcium chlorohypochlorite. The concentrations of hypochlorite used in these experiments were *exceedingly minute*.

During the course of these experiments the occupants of the room were repeatedly asked to report any irritation of their eyes, nose or throat and were told that such 'complaints' would, if justified, be welcomed. However, none of the personnel experienced anything more than a very slight tingling in the nose or throat which was regarded by them as of no consequence. It is concluded, therefore, that the concentrations of hypochlorite used in these experiments were below (probably far below) the irritant dose for the human subject. This was to be expected in view of the minuteness of the concentration of disinfectant used.

The odour of the hypochlorite was easily perceptible to almost everyone present in the room although occasionally one of the men was unable to detect the disinfectant by sense of smell. By repeated questioning of all the personnel, it was ascertained that the odour of the hypochlorite was strong immediately after each spraying, remained so for about 5 min., then decreased in intensity, so that 10 min. after spraying only a small proportion of those present were still able to smell the hypochlorite. Fifteen minutes after a spraying, one or two only of the occupants could still detect the odour of the hypochlorite.

Towards the end of an experiment (e.g. after a fourth spraying) the persistence of the odour of the disinfectant appeared to be of rather longer duration, possibly due to the cumulative effect from the previous sprayings of the experiment. This cumulative effect was shown more clearly in an experiment in which the sprayings were made at shorter intervals of time (approximately 15 min. between each spraying). In this experiment (19), the odour of the hypochlorite Y was not detected by any of the fourteen occupants of the room when 12 min. had elapsed after each of the first and second sprayings; 12 min. after the third and fourth sprayings, however, five of the occupants were able to smell the disinfectant; as long as 18 min. after the sixth and final spraying of this experiment, the odour was detected by ten of the occupants.

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At the end of an experiment, on returning to the room after an absence of a few minutes or longer, the odour of the hypochlorite used in the experiment was always noticeable, although the odour had apparently disappeared as judged by observations made during the latter part of the experiment. It is concluded, therefore, that the nose becomes less sensitive to hypochlorites during long exposure to these disinfectants, as under the conditions of these experiments. The duration of persistence of the odour of the disinfectant appeared to be approximately the same for each of the two different hypochlorites tested.

The opacity of the hypochlorite mists persisted only for 1 or 2 min. and the minor inconvenience thus caused was of no importance. It may be mentioned, however, that the opacity of the mists of hypochlorite X, which contained a relatively large proportion of sodium chloride, was of longer duration. (Hypochlorite X was used in the experiments in the empty rooms.)

It is worthy of mention that almost all the numerous personnel taking part in these experiments agreed that the spraying of each of these hypochlorites had a definite 'freshening' effect on the air of the room. Also of importance is the fact that all the personnel were in agreement that the hypochlorites had a marked deodorizing effect upon the atmosphere of the room.

Accumulation of hypochlorite on the surface of the medium when using the slit sampler

During each 3 min. exposure, 3 cu. ft. of air are sucked into the sampler at the rate of 1 cu. ft. per min. and some of the hypochlorite (or possibly hypochlorous acid derived from the hypochlorite due to the action of the CO_2 of the air) will be absorbed into the surface of the medium. In order to make sure that such an accumulation of disinfectant was insufficient to interfere with the growth of the organisms collected, an interval of at least 2–3 min. was allowed to elapse after each spraying and before making an exposure. When this precaution was observed the amount of disinfectant on the surface of the medium was insufficient to inhibit bacterial growth as was shown in the following manner: In each of several experiments a plate was exposed in the slit sampler during the interval (3 min. in each of these cases), i.e. the exposure was started a few seconds after completion of the spraying of the disinfectant. In two of these tests the interval chosen was that after the final spraying, i.e. when the greatest concentration of hypochlorite might be expected. The plate was then inoculated with a loopful of a dilute bacterial suspension. In different experiments the organisms used were *Bact. coli*, *Staphylococcus aureus* and a *Micrococcus*. The two latter organisms were obtained from the air in previous experiments. Control, i.e. unexposed blood-agar, plates were similarly inoculated. There was no visible difference between the growth on the test and control plates after 30 hr. incubation at 37° C. In all, about eight tests of this kind were carried out with results similar to those described above.

It is concluded, therefore, that the amount of disinfectant absorbed into the surface of the nutrient medium of the plates exposed in the slit sampler for 3 min., under the conditions prevailing in these experiments, was of no significance, i.e. was insufficient to inhibit the growth of the organisms collected on the plates. This conclusion is to be expected from consideration of the exceedingly small concentrations of hypochlorite present in the air in these experiments. In this connexion it is of interest to note that, in Exps. 20 and 21, in which the hypochlorite Y was atomized by means of the Dynalysor operated for periods of 6–15 min., test-exposures similar to those described above were carried out during the actual period of atomization and again no inhibition of bacterial growth was observed.

Results of experiments in an occupied room

For each observation of the bacterial content of the air of the occupied room blood-agar plates were used in the slit sampler and the duration of the exposures was 3 min., i.e. 3 cu. ft. of air were sampled for each observation.

Variation in the bacterial content of the air during occupation of a room

(1) *Without spraying of the air*

The variation in the number of bacteria-carrying particles in the air of the room during occupation of 2–2½ hr. duration presented an interesting study and did not follow the course expected. Lack of space prevents publication of the detailed results of each experiment but Table 2 illustrates the course of and data for three experiments—a control experiment in which no disinfectant was used and experiments in which water, or hypochlorite Y were sprayed at half-hourly intervals into the air (see also Fig. 1).

Considering the control experiment (Exp. 2) it is seen that wide variations in the bacterial content of the air took place during occupation of the room. Further, consecutive bacterial counts were, in general, reasonably close to each other, although exposures taken within an interval of 1–2 min. between the end of one and the beginning of the next exposure were sometimes widely different (e.g. exposures 2.46–2.49 and 2.51–2.54 p.m.; exposures 3.36–3.39 and 3.41–3.44 p.m.; exposures 3.45–3.48 and 3.50–3.53 p.m.). The bacterial count was high during the first 20–30 min. of occupation of the room, then fell considerably during the second half-hour and remained near the lower level during the third half-hour of occupation. During the fourth half-hour period there was a rise in the bacterial content of the air which reached a value appreciably in excess of that recorded during the initial half-hour of occupation.

In another control experiment (Exp. 5) similar variations in the bacterial content of the air were observed, and, in this experiment, which lasted for 2½ hr., there was a further rise during the final, i.e. the fifth half-hour; the final bacterial counts were much higher than for the first half-hour of occupation. These deductions are more easily derived from consideration of Table 5 in which the *average* bacterial counts during each half-hour of occupation are tabulated for each of the thirteen experiments considered in this paper (some of the experiments in this series included observations made after sneezing and coughing by the occupants of the room and are not described in this communication). Table 5 contains most of the data appertaining to each of these experiments, e.g. number of occupants of the room, average concentration per spraying of disinfectant, etc., and later will be considered in more detail.

It is probable that the high bacterial content of the air during the first 20 min. of occupation was due to the disturbance caused when the future occupants entered the room, and the relatively lower bacterial counts recorded for the second and third half-hour periods of occupation may thus be explained. Of interest, however, is the conclusion that the bacterial content of the air did not rise appreciably during the second and third

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Table 2. *Consecutive slit-sampler observations (3 min. exposures) on the number of bacteria-carrying particles per 3 cu. ft. of air in experiments in an occupied room*

Control Exp. 2

Time of exposure p.m.	No. of colonies on blood-agar plate
Before occupation of room	
1.54–1.57	72
Men entered room at 2.0 p.m. *After occupation*	
2.09–2.12	304
2.14–	299
2.21–	285
2.29–	181
No spraying	
2.37–2.40	153
2.41–	165
2.46–	178
2.51–	118
No spraying	
3.07–3.10	170
3.12–	193
3.16–	199
3.21–	178
3.26–	161
No spraying	
3.36–3.39	297
3.41–	436
3.45–	418
3.50–	250
3.57–	205

Water sprayed into the air by means of a flit gun, Exp. 7

Time of exposure p.m.	No. of colonies on blood-agar plate
Before occupation of room	
1.41–1.44	l.s.
1.45–	61
1.49–	61
1.54–	92
Men entered room at 2.0 p.m. *After occupation*	
2.00–2.03	328
2.05–	318
2.09–	316*
2.13–	320
2.18–	307
2.22–	285
2.26–	328
First spraying (2.31–2.32 p.m.); 50 strokes of gun; 100 − 84 c.c., i.e. 16 c.c. used	
2.34–2.37	341
2.38–	347
2.42–	352
2.46–	379
2.50–	343
2.54–	337
2.58–	318
Second spraying (3.01–3.02 p.m.); 50 strokes of gun; 100 − 80 c.c., i.e. 20 c.c. used	
3.04–3.07	341
3.08–	307
3.12–	360
3.16–	363
3.20–	375
3.24–	445
Third spraying (3.30–3.31 p.m.); 50 strokes of gun; 100 − 81 c.c., i.e. 19 c.c. used	
3.33–3.36	381
3.37–	412
3.40½–	375
3.44–	382
†	

Hypochlorite Y sprayed into the air by means of a flit gun, Exp. 8

Time of exposure p.m.	No. of colonies on blood-agar plate
Before occupation of room	
1.45–1.48	43
1.50–	69
1.53½–	56
1.57–	80
Men entered room at 2.0 p.m. *After occupation*	
2.01–2.04	277
2.04–	263
2.08–	237
2.14½–	254
2.18–	239
2.22–	197
2.26–	l.s.
First spraying (2.29–2.30 p.m.); 50 strokes of gun; 100 − 85 c.c., i.e. 15 c.c. used	
2.33–2.36	169
2.37½–	168
2.42–	171
2.46–	l.s.
2.50–	201
2.53½–	206*
2.57–	251
Second spraying (3.0–3.01 p.m.); 50 strokes of gun; 100 − 84 c.c., i.e. 16 c.c. used	
3.03–3.06	142*
3.06½–	156
3.10–	160
3.14–	182
3.17–	208
3.21–	196
3.24½–	l.s.
Third spraying (3.30½–3.31½ p.m.); 50 strokes of gun; 100 − 82 c.c., i.e. 18 c.c. used	
3.34–3.37	151
3.37½–	103
3.42–	147
3.46–	136
3.49½–	174
3.53–	149
3.56½–	178
Fourth spraying (3.59½–4.0½ p.m.); 30 strokes of gun; 100 − 87 c.c., i.e. 13 c.c. used	
4.02½–4.05½	99
4.06–	98
4.09–	l.s.
4.12–	70*
4.15½–	91
4.19–	l.s.
4.26–	l.s.

The blood-agar plates were incubated for 30 hr. at 37° C. l.s. = large spreading organism.

* Spreading organism on plate but occupying a relatively small area which was estimated (see text) and for which allowance was made in the final count.

† At this stage hypochlorite Y was atomized into the air.

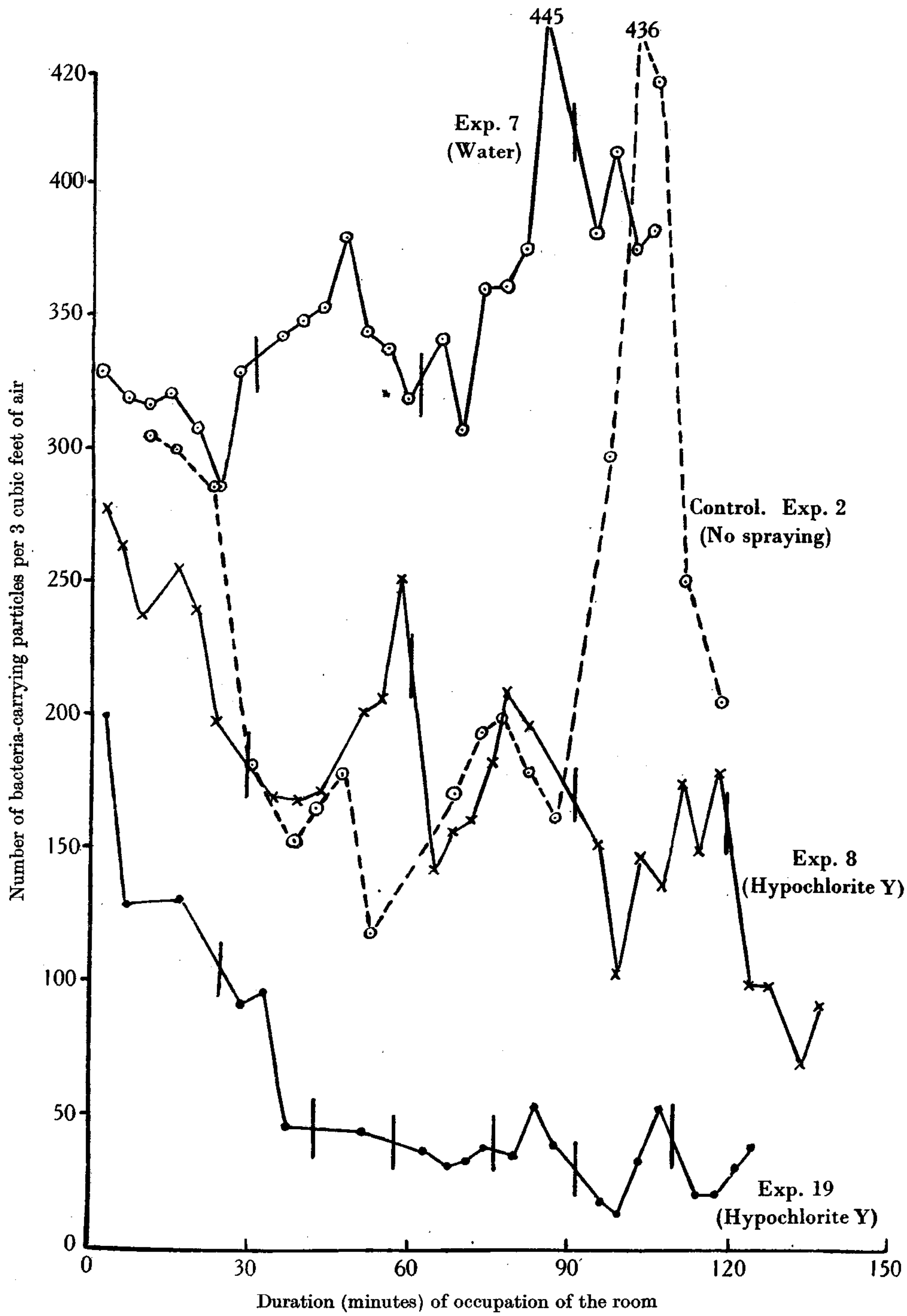

Fig. 1. Variation in the bacterial content (consecutive slit-sampler observations) of the air of the occupied room in a control experiment, and in experiments in which water or hypochlorite Y were sprayed into the air from a flit gun. The small vertical lines indicate the times when the sprayings were carried out.

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periods of occupation of a room containing so many occupants and so poorly ventilated as the room under consideration. The expected change for the worse, bacteriologically speaking, did not therefore take place until after the room had been occupied for 1½ hr.

(2) *Air sprayed with hypochlorites or water*

(*a*) *Duration of the disinfection of the air*

The variations in the bacterial content of the air of the occupied room in experiments in which a disinfectant (hypochlorite) was sprayed into the air by means of a flit gun presented a picture differing greatly from that of the control experiments. Thus, in Exp. 8, in which hypochlorite Y was used, it will be seen from Table 2 that (1) the number of bacteria per 3 cu. ft. of air immediately after each of the four sprayings of disinfectant was lower than before the sprayings; (2) the bacterial content of the air remained at the relatively lower figure during the first half of the 30 min. period after the sprayings (the period between the sprayings was approximately 30 min. in most of these experiments); (3) during the second half of the 30 min. period between sprayings the bacterial content of the air began to rise again. Such a fall and rise in the bacterial content of the air occurred in each of the experiments (after each of the sixteen sprayings) in which hypochlorite Y was sprayed into the air, and after most of the sprayings of bleaching powder suspension.

The interval of time after each spraying during which the bacterial content of the air was lowered was reasonably constant in each of the experiments. Examination of the graphs (bacterial content of air plotted against period of occupation of room of which Fig. 1 is an example) for these experiments shows that this period of time was 15–17 min. in most cases, but occasionally was longer (20 min.). That it was possible to decide upon the duration (and within such narrow limits) of the effect of the disinfectant sprayed into the air on the bacterial content of the air is in itself evidence that the hypochlorites did have a significant lowering effect upon the viable bacterial content of the air.

Since the results of experiments in which the disinfectant was sprayed at half-hourly intervals indicated that the disinfecting action of the hypochlorites persisted during a period of about a quarter of an hour, an experiment in which the sprayings were carried out at 15 min. intervals should have been particularly successful. A very great reduction in the bacterial content of the air was, in fact, achieved in such an experiment. Thus, in Exp. 19, hypochlorite Y was first sprayed after 24 min. occupation of the room, again after an interval of 17 min., then four more times at intervals of approximately 15 min. In view of the importance of this experiment the data are recorded in some detail. The number of occupants in the room was fourteen; the flit gun was used for each spraying and the volumes of hypochlorite Y introduced into the air were 19, 22, 14, 21, 21 and 21 c.c. respectively, an average of 20 c.c. per spraying, corresponding to a concentration of 0·42 c.c. of hypochlorite Y per million c.c. of air. Consecutive slit-sampler observations per 3 cu. ft. of air were as follows: 199, 128 and 130 during the first 24 min. of occupation; 91, 96 and 46 after the first spraying; 44, then a large spreader after the second spraying; 37, 31, 33 and 38 after the third spraying; 35, 53 and 39 after the fourth spraying; 17, 13, 33 and 52 after the fifth spraying; 20, 20, 30 and 38 after the sixth spraying. From these results (see also Fig. 1) it is seen that the bacterial content of the air was reduced after the first spraying, that the second spraying (17 min. after the first)

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brought about a further reduction in the bacterial content to the very low figure of 44, and that this low bacterial content was maintained during the remainder (about $1\frac{1}{2}$ hr.) of the experiment. The average bacterial count per 3 cu. ft. of air during the last 16 min. period of occupation of the room was 28 and this result indicated the bacterial content of the air of the inadequately ventilated room at the *end* of 2 hr. continuous occupation. As the bacterial content of the air of the room before occupation was 20, it may be stated that the number of bacteria present in the air of the occupied room at the *end* of 2 hr. of continuous occupation was almost the same as the number present in the air of the empty room, i.e. before occupation commenced. The degree of disinfection of the air achieved in this experiment was, therefore, of a very high order. Evidence of the accumulation of hypochlorite in the air due to successive sprayings has already been brought forward, but it may be stated that the occupants of the room considered that the slight irritation of the eyes, nose or throat, experienced by some of them (i.e. during Exp. 19), was of no importance. This statement is important since the total quantity of hypochlorite used in this experiment (which was of shorter duration than most of the other experiments) was much greater than in any other (see Table 5). Even in this experiment, however, where the number (6) of sprayings was greater than usual, the *total* quantity of hypochlorite Y sprayed into the air was 118 c.c., corresponding to a concentration by volume of less than 2·5 c.c. per million c.c. of air.

(*b*) *Assessment of the degree of air disinfection* (*method* 1)

The comparison of the disinfecting powers of different hypochlorites and the assessment of the degree of disinfection of the air produced by each individual spraying is a matter of considerable difficulty, and, as no generally accepted method of achieving this object is available, the experimental results were interpreted as follows: an approximately quantitative idea of the disinfecting power of the hypochlorites was obtained by averaging the counts representing the bacterial content of the air during the 16 min. *before* the spraying of the disinfectant, and those for the 16 min. period *after* the spraying, and then comparing the two averages and expressing the reduction (if any) as a percentage. These reductions or increases are then compared with the corresponding changes in the control experiments. Accordingly, in Tables 3 and 4, the averages of the bacterial counts for the first 16 min. period, P. 1, of occupation of the room are followed by the average bacterial counts for the next 16 min. period, P. 2, which is the 16 min. period before the first spraying; P. 3 represents the 16 min. period after the first spraying, and P. 4 the 16 min. period before the second spraying, and so on. Each bacterial count in Tables 3 and 4 is the average of several (usually three or four) taken during each 16 min. period. Table 3 summarizes the results of experiment in which water, or the hypochlorite Y was used, and of the control experiments. The number of occupants in the room was fourteen. In Exp. 8 the average bacterial counts during the period P. 6 and P. 7, i.e. before and after the third spraying of hypochlorite Y, were 202 and 134 respectively, and since P. 7/P. 6×100, i.e. $\frac{134}{202} \times 100 = 66\%$ (i.e. expressing P. 7/P. 6 as a percentage), the 'percentage reduction' provisionally attributed to the third spraying was, therefore, $100 - 66$, i.e. 34. On this basis, the percentage reductions for the four sprayings in this experiment were 27, 27, 34 and 44 respectively, and the average reduction per spraying was 33 %. In Exps. 8, 12 and 13 there were, in all, ten sprayings of hypochlorite Y, and the average percentage reduction for nine of these was 33.

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Table 3. *Variation in the bacterial content of the air of the occupied room (occupation for 2–2½ hr.). Average bacterial counts for each 16 min. period of occupation before and after half-hourly spraying of the air with hypochlorite Y or water by means of a flit gun*

		Average bacterial counts per 3 cu. ft. of air																						
			Room occupied																					
Exp.	Disinfectant, etc., sprayed into the air	Room empty P. 0	P. 1	P. 2	* 1st spraying. Conc. in p.p.m.	P. 3	$\frac{P. 3}{P. 2}$ and % reduction		P. 4	* 2nd spraying. Conc. in p.p.m.	P. 5	$\frac{P. 5}{P. 4}$ and % reduction		P. 6	* 3rd spraying. Conc. in p.p.m.	P. 7	$\frac{P. 7}{P. 6}$ and % reduction		P. 8	* 4th spraying. Conc. in p.p.m.	P. 9	$\frac{P. 9}{P. 8}$ and % reduction		P. 10
2	Control, i.e. no disinfectant	72	302	233	0	165	71	29	118	0	187	158	—	169	0	384	227	—	228	0	—	—	—	—
5	Control, i.e. no disinfectant	84	278	244	0	185	71	29	163	0	193	118	—	181	0	230	127	—	241	0	354	147	—	365
7	Water	71	321	307	0·34	355	116	—	344	0·42	343	100	—	386	0·40	388	100	—	—	—	—	—	—	—
8	Hypochlorite Y	62	259	230	0·32	169	73	27	219	0·34	160	73	27	202	0·38	134	66	34	159	0·27	89	56	44	91
12	,,	22	—	309	0·34	215	70	30	242	0·42	192	79	21	218	0·42	141	65	35	164	0·38	135	82	18†	204
13	,,	48	—	349	0·32	207	59	41	237	0·46	144	61	39	204	—	—	—	—	—	—	—	—	—	—
21	,,	29	210	166	0·21	114	69	31	114	0·17	90	79	21	90	0·15	50	56	44	—	—	—	—	—	—

P. 0 = the 16 min. period before occupation; P. 1 = the first 16 min. period of occupation; P. 2 and P. 3 etc. = 16 min. periods before and after spraying.

* These columns indicate the number of the spraying and the concentration (c.c. of disinfectant, etc., per million c.c. of air) of disinfectant introduced into the air by the spraying.

† The occupants were allowed to smoke during the period P. 9, i.e. after the fourth spraying, and the corresponding percentage reduction in the bacterial count was lower for the other sprayings of this experiment (12).

In each of Exps. 12 and 13 there was only one 16 min. period between the commencement of occupation and the first spraying.

In Exp. 21 hypochlorite Y was atomized into the air by means of the Dynalysor.

In Exp. 19 hypochlorite Y was sprayed six times at intervals of approximately 15 min. and the experimental data cannot conveniently be included in this table.

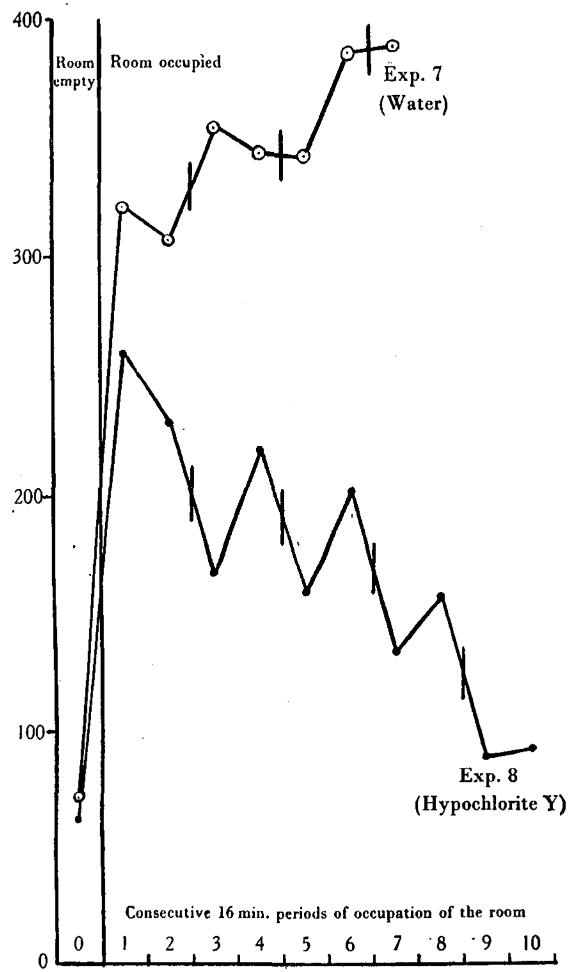
The result for the tenth spraying of the hypochlorite (percentage reduction = 18) has not been included, because the occupants were allowed to smoke after this spraying, in order that some idea of the effect of the smoke on the disinfecting action of the hypochlorite might be obtained. In Exp. 6 (Table 4) hypochlorite Y was sprayed once (third spraying of experiment), and the reduction was 32%. Therefore, according to this method of interpretation of results, ten out of ten sprayings of hypochlorite Y have each effected an appreciable reduction in the bacterial content of the air.

However, it will be observed from Table 3 that, in the control Exps. 2 and 5, an appreciable reduction (29%) occurs at the stage where, in comparison with the experiment in which hypochlorites were used, the first spraying would have taken place, i.e. after the first (imaginary in Exps. 2 and 5) spraying. Accordingly, the reductions 27, 30 and 41% which occurred after the first sprayings in each of Exps. 8, 12 and 13 need reviewing with special care. It is probable that only one of these three reductions (i.e. the reduction of 41% in Exp. 13) is large enough to indicate significant disinfecting action, and the *degree* of disinfection is difficult to assess. It may be stated that the relative humidity of the air was much higher at the time of the first spraying of Exp. 13 than in Exps. 8 and 12 and most other experiments of this series.

In order, therefore, to arrive at some figure which indicates (if only approximately) the average reduction in bacterial content of the air per spraying, it is necessary to omit the results obtained after each of the first sprayings of Exps. 8, 12 and 13, as well as the result of the fourth spraying (smoking) of Exp. 12. The average reductions for the remaining six sprayings of these three experiments and including the single spraying of hypochlorite Y in Exp. 6 is 33%. The quantities of the hypochlorite used in these seven sprayings were such that the concentrations by volume ranged from 0·27 to 0·46 c.c. (average 0·38 c.c.) of diluted hypochlorite Y per million c.c. of air.

The effect on the bacterial content of the air of the occupied room produced by the hypochlorites is clearly seen by plotting the average bacterial counts per 16 min. period of occupation, against consecutive 16 min. periods of occupation, and such graphs have been drawn for each experiment. In Fig. 2 A, B, however, for the sake of clearness, only typical experiments are represented (hypochlorite Y, Exp. 8, bleaching powder, Exp. 3, water, Exp. 7 and a control, Exp. 2, in which there was no spraying). The graphs show clearly (1) the reduction in the bacterial content during the 16 min. period after the spraying, (2) the rise which takes place during the next 16 min. period, (3) the *considerable* reduction in the bacterial content of the air brought about as the result of several (three or more) sprayings of hypochlorite; this reduction due to several sprayings is demonstrated much more clearly by the graphs than by Tables 3 and 4.

The method of assessing the results as just described probably underestimates the degree of disinfection effected, since the magnitude of the 'percentage reduction' in the bacterial content of the air per spraying is dependent, to some extent, upon the reductions brought about by previous sprayings (see Discussion). This factor is illustrated clearly by the results of Exp. 19, in which the six sprayings were separated by intervals of approximately 15 min. only. In this experiment the reduction effected by the first two sprayings was considerable, and the bacterial content of the air was brought to such a low level (as already shown in Fig. 1) that further large reductions were impossible. The subsequent four sprayings were, however, obviously effective inasmuch as the bacterial content of the air was maintained at the low level reached after the first two sprayings.



2A. Variation in the bacterial content of the air of the room during consecutive 16 min. periods of occupation, in experiments in which water or hypochlorite Y was sprayed into the air by means of a flit gun. The small vertical lines indicate the times when the sprayings were carried out.

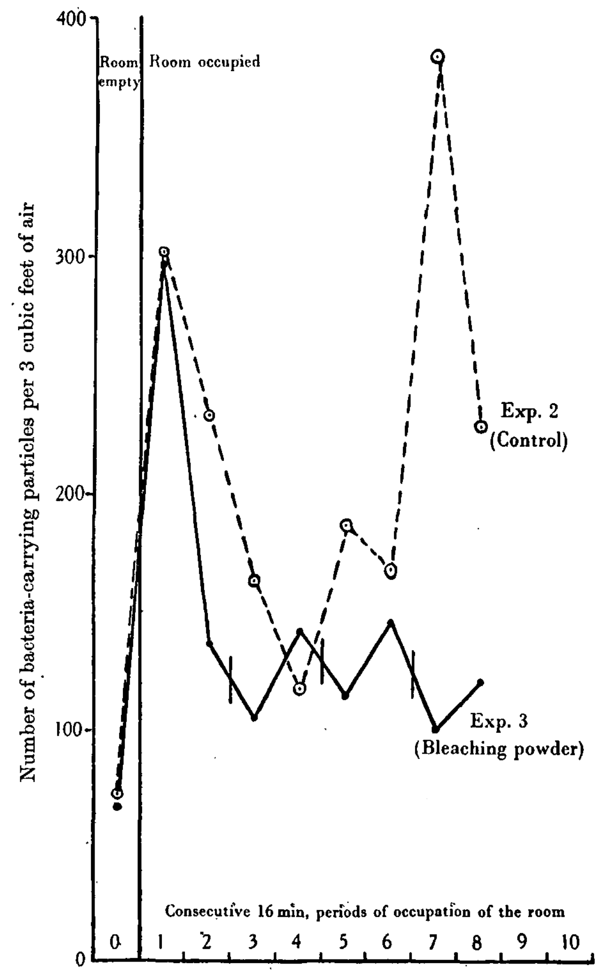


Fig. 2B. Variation in the bacterial content of the air of the room during consecutive 16 min. periods of occupation in an experiment in which a 1·3% suspension of bleaching powder was sprayed into the air by means of a flit gun, and in a control experiment (i.e. no spraying). The small vertical lines indicate the times when the sprayings were carried out.

It may, therefore, be claimed that 7+6, i.e. 13 out of 16 (see, however, section on relative humidity), sprayings of hypochlorite Y from a flit gun into the air of the occupied room were effective in appreciably reducing the bacterial content of the air of the room; the average reduction for seven of these sprayings was of the order of 33%.

Similar results were obtained in two experiments (20 and 21) in which hypochlorite Y was atomized into the air by means of the Dynalysor, and the results of one of these are included in Table 3. Failure of the first atomization is indicated, and the degree of disinfection effected by the second and third atomizations was of the same order as in the flit-gun experiments. It should be emphasized, however, that the quantities of hypochlorite used in the 'Dynalysor experiments' were much smaller (see Table 3) than in the flit-gun experiments.

When the control Exps. 2 and 5 (sixteen and seventeen occupants respectively in the room), in which there were no sprayings, are examined in this manner, it is seen (Table 3) that a percentage reduction in the bacterial content of the air occurs after two only of the seven 'imaginary sprayings' and a substantial *increase* is recorded after each of the five other 'imaginary sprayings'. The two reductions occurred after the 'first spraying' of each experiment and were to be expected from the variation in bacterial content which has been shown to take place in these 'no spraying' experiments. For Exp. 2, the pertinent figures (Table 3) are 29% reduction, 58% increase and 127% increase (average equals 52% *increase*).

The results obtained in Exp. 7 (fourteen occupants in the room), in which sterile tap water was sprayed into the air by means of the flit gun, show no percentage reduction in the bacterial counts for the 16 min. periods of occupation after two of the three sprayings of water; after the remaining (the first) spraying of water there was an *increase* of 16%. The quantities of water sprayed were 16, 20 and 19 c.c. respectively. A fourth 'spraying' was made in this experiment; hypochlorite Y (7·5 c.c.) was atomized into the air by means of the Dynalysor and a significant reduction in the bacterial content of the air was effected. It is concluded, therefore, that the three sprayings of water in this experiment produced no measurable effect upon the bacterial content of the air, although a single spraying of hypochlorite Y had an appreciable disinfecting action.

The results for bleaching powder will now be considered (Table 4). In these experiments (Nos. 1, 3, 4 and 6) the number of occupants in the room was seventeen, i.e. three more than for the later experiments in which hypochlorite Y was used. This small difference in the number of occupants in the room is considered later (see Discussion). Omitting, for the time being, the results of Exp. 6, the total number of sprayings in Exps. 1, 3 and 4 was ten, and the volumes of the 1·3% suspension used were 19, 19, 24, 20, 19, 14, 20, 21, 20 and 22 c.c. respectively, and these quantities are strictly comparable with those used in the experiments with hypochlorite Y. The percentage 'reductions' in the bacterial content of the air were as follows: 7% *increase*, 22%, 7% *increase* (Exp. 1), 24, 20 and 32% (Exp. 3), 26% *increase*, 10, 22 and 16% (Exp. 4), i.e. for the ten sprayings a reduction in the bacterial counts greater than 27% occurred after one spraying only; reductions of 20% or more occurred after five of the ten sprayings. Further, one of these reductions occurred after the *first* spraying (Exp. 3), at which stage of the experiment similar reductions occurred in each of the control Exps. 2 and 5. Interpretation of the *degree* of disinfecting power of bleaching powder from these results is difficult. The results of Exp. 3 appear to be fairly conclusive, and although, in Exp. 1,

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Table 4. *Variation in the bacterial content of the air of the occupied room (occupation for 2–2½ hr.). Average bacterial counts for each 16 min. period of occupation before and after half-hourly spraying of the air with a suspension of bleaching powder or water by means of a flit gun*

Exp.	Disinfectant, etc.	Room empty P. 0	P. 1	P. 2	* 1st spraying. Conc. in p.p.m.	P. 3	P. 3/P. 2 and % reduction		P. 4	* 2nd spraying. Conc. in p.p.m.	P. 5	P. 5/P. 4 and % reduction		P. 6	* 3rd spraying. Conc. in p.p.m.	P. 7	P. 7/P. 6 and % reduction		P. 8	* 4th spraying. Conc. in p.p.m.	P. 9	P. 9/P. 8 and % reduction		P. 10
			Average bacterial counts per 3 cu. ft. of air — Room occupied																					
2	No disinfectant, i.e. control	72	302	233	0	165	71	29	118	0	187	158	—	169	0	384	227	—	228	0	—	—	—	—
5	,,	84	278	244	0	185	71	29	163	0	193	118	—	181	0	230	127	—	241	0	354	147	—	365
7	Water	71	321	307	0·34	355	116	—	344	0·42	343	100	—	386	0·40	388	100	—	—	—	—	—	—	—
1	Bleaching powder 1·3%	82	309	199	0·40	214	107	—	161	0·40	125	78	22	129	0·51	138	107	—	167	—	—	—	—	—
3	,,	67	297	138	0·42	105	76	24	143	0·40	115	80	20	147	0·29	100	68	32	121	—	—	—	—	—
4	,,	67	186	161	0·42	203	126	—	225	0·44	204	90	10	211	0·42	165	78	22	166	0·46	140	84	16	229
6	,,	44	271	275	0·40	283	104	—	265	0·44	300	113	—	349	0·38†	238	68	32†	354	0·38	357	101	—	356

P. 0 = the 16 min. period before occupation; P. 1 = the first 16 min. period of occupation; P. 2 and P. 3, etc. = 16 min. periods before and after spraying.

* These columns indicate the number of the sprayings and the concentration (by volume) in parts per million of the disinfectant, etc. sprayed into the air.

† For this third spraying hypochlorite Y was used instead of bleaching powder.

small increases in the bacterial counts occur after two of the three sprayings, it should be borne in mind that, in the control experiments, as already mentioned, large increases (not decreases) in the bacterial counts occurred after five of the seven imaginary sprayings. For example, the small increase of 7% associated with the third spraying of Exp. 1 almost certainly indicated appreciable disinfection of the air as a result of this spraying since the corresponding changes in the control experiments were large increases (127 and 27% respectively). It was concluded therefore, that, for each of these three experiments (1, 3 and 4) the first sprayings were ineffective; the second and third sprayings were effective (there is, however, some doubt of the effectiveness of the second spraying of Exp. 4) as was the fourth spraying of Exp. 4, i.e. seven of the ten sprayings were effective. The degree of disinfection achieved in Exp. 4 was lower than in the other two experiments (see section on relative humidity).

The graphs for Exps. 1, 3 and 4 (similar to those in Fig. 2 A, B) show that, when the complete experiments (i.e. not individual sprayings) are considered the degree of disinfection effected was high in Exp. 3, rather lower in Exp. 1, and much lower in Exp. 4 than in either of the other two experiments.

Although, therefore, by this method of assessment bleaching powder suspension does not appear to be as good a disinfecting agent as hypochlorite Y the above results do indicate a moderate degree of disinfecting power for bleaching powder.

The results of Exp. 6 in which there were three sprayings of bleaching powder and one (the third) of hypochlorite Y do not indicate appreciable disinfecting action for any of the bleaching powder sprayings; the spraying of hypochlorite Y produced a reduction in the bacterial count of 32%. This latter reduction is comparable with that observed on the average, for the sprayings in the other experiments in which hypochlorite Y was used, but, taking into account the fact that the previous two sprayings of the experiment were ineffective, it is reasonable to postulate that this reduction of 32% was lower than expected from comparison with the results of the other experiments in which hypochlorite Y was used. In support of this tentative suggestion, it may be added that, as will be shown by the method of assessment of the degree of disinfection described in the next section, this particular spraying of hypochlorite Y did not appear to be as effective as the other twelve sprayings of the hypochlorite. It is suggested, therefore, that there was some factor present in Exp. 6 which was not present in the other experiments, and it was considered that, in assessing the value of bleaching powder as an air disinfectant, a more accurate result would be obtained by disregarding the results of Exp. 6 (see Discussion).

Note. As the period of time between the commencement of occupation of the room and the first spraying, and between this and subsequent sprayings was, in most of these experiments, about 30 min., this means that the period P. 1 represents the first 16 min. of occupation and P. 2 the period 14–30 min. from the start of occupation which is the 16 min. period before spraying, i.e. there is a slight overlap. This overlap occasionally results in a bacterial count being included in the counts from which the average bacterial count for each of two consecutive 16 min. periods (e.g. P. 1 and P. 2) are calculated, but this is of no significance.

(c) *Assessment of the degree of air disinfection* (*method* 2)

According to this method the average bacterial content of the air per consecutive half-hour periods of occupation are compared with the average bacterial content for the first *hour* of occupation (Table 5; see also 'Discussion', where the reasons for choosing

Table 5. *Average bacterial counts (per 3 cu. ft. of air) during consecutive half-hourly periods of occupation of the room in 'spraying' and in control experiments. Comparison of the half-hourly counts with the average bacterial count for the first* hour *of occupation*

Exp.	Disinfectant, etc., used	Total (c.c.) of disinfectant used in exps.	Av. conc. (p.p.m.) per spraying	No. of occupants of the room	*Average* bacterial counts per 3 cu. ft. of air: 1st ½ hr.	2nd ½ hr.	3rd ½ hr.	4th ½ hr.	5th ½ hr.	Av. for 1st *hr.*	Comparison of average bacterial counts per consecutive ½ hr. periods of occupation: Av. for 1st ½ hr. / Av. for 1st hr.	and % change A	Av. for 2nd ½ hr. / Av. for 1st hr.	and % change B	Av. for 3rd ½ hr. / Av. for 1st hr.	and % change C	Av. for 4th ½ hr. / Av. for 1st hr.	and % change D	Av. for 5th ½ hr. / Av. for 1st hr.	and % change E
2	None (control)	None	None	16	267	154	180	321	—	211	267/211	+27	154/211	−27	180/211	−15	321/211	+52	—	
5	None (control)	None	None	17	259	185	180	224	359	222	259/222	+17	185/222	−17	180/222	−19	224/222	+ 1	359/222	+62
7	Water (3 sprayings)	55	0·38	14	315	345	365	388	—	330	315/330	− 5	345/330	+ 5	365/330	+11	388/330	+18	—	
1	Bleaching powder (3 sprayings)	62	0·44	17	254	203	120	129	—	229	254/229	+11	203/229	−11	120/229	−48	129/229	−44	—	
3	Bleaching powder (3 sprayings)	53	0·37	17	244	125	131	107	—	185	244/185	+32	125/185	−32	131/185	−29	107/185	−42	—	
4	Bleaching powder (4 sprayings)	83	0·43	17	175	223	204	165	184	199	175/199	−12	223/199	+12	204/199	+ 3	165/199	−17	184/199	− 8
6	Bleaching powder* (4 sprayings)	76	0·40	17	272	270	322	308	362	271	272/271	0	270/271	0	322/271	+19	308/271	+14*	362/271	+37
8	Hypochlorite Y (4 sprayings)	62	0·33	14	265	194	174	148	90	230	265/230	+15	194/230	−16	174/230	−34	148/230	−36	90/230	−60
12	Hypochlorite Y (4 sprayings)	74	0·39	14	309	231	201	152	162	270	309/270	+14	231/270	−14	201/270	−26	152/270	−44	162/270	−40
13	Hypochlorite Y (2 sprayings)	37	0·39	14	349	224	164	—	—	287	349/287	+21	224/287	−22	164/287	−43	—		—	
19	Hypochlorite Y (6 sprayings)	118	0·42	14	137	62	26	—	—	100	137/100	+37	62/100	−38	26/100	−74	—		—	
20	Hypochlorite Y from Dynalysor (3 sprayings)	13	0·09	14	283	168	144	155	—	226	283/226	+25	168/226	−26	144/226	−36	155/226	−31	—	
21	Hypochlorite Y from Dynalysor (3 sprayings)	25	0·18	14	192	134	106	67	—	163	192/163	+18	134/163	−18	106/163	−35	67/163	−59	—	

The first hour of occupation included the initial half-hour before the first spraying and the half-hour period of occupation between the first and second sprayings.

* Hypochlorite Y (not bleaching powder) was used for the third spraying of Exp. 6.

In Exps. 12 and 13 the period of occupation before the first spraying was 16 min. only and, in these two experiments, the average bacterial count for the first hour was more correctly the average bacterial count during the 16 min. and 30 min. periods before and after the first spraying.

As described elsewhere, the course of Exp. 19 was different from that of the other experiments.

this method of comparison are explained). For example, in Exp. 8, in which hypochlorite Y was used, the average bacterial count (90) during the fifth half-hour of occupation divided by the average bacterial count (230) for the first hour of occupation and expressed as a percentage equals 40%, i.e. the percentage difference is 60%. The percentage differences A, B, C, D and E for each of the half-hour periods of occupation in this experiment were +15, −16, −34, −36 and −60% respectively. As will be seen from Table 5, a similar sequence of changes, i.e. first an increase, then successive decreases, usually of increasing magnitude, occurred in each of the 'flit-gun experiments' 12, 13 and 19. Bearing in mind that in the spraying experiments the first spraying of hypochlorite took place after the room had been occupied for half an hour, and that other sprayings were made at half-hourly intervals, the percentage changes in the bacterial counts for the second, third, fourth and fifth half-hour periods of occupation (see columns B, C, D and E in Table 5) will indicate the effect (if any) of the individual sprayings, i.e. when these changes are compared with the corresponding changes in the control experiments.

In the control experiments the changes B, C, D and E were as follows: B and C were decreases ranging from 27 to 17% and from 15 to 19% respectively; the 'D and E' changes were *increases*, and two of them were considerable (52 and 62%). Comparing these changes with those of Exps. 8, 12 and 13 it will be seen that, in the latter experiments (1) the 'B' changes are of similar sign and order of magnitude; (2) the 'C' changes (−34, −26 and −43% respectively) are significantly greater than the corresponding changes in the control experiments; (3) the 'D' changes (−36 and −44%) and the 'E' change (−60% in Exp. 8) are large decreases in contrast to the increases in the control experiments. The above data indicate (1) considerable disinfecting action for the third and fourth sprayings ('D' and 'E' changes) of the experiments; (2) some disinfecting action for the second sprayings ('C' changes) of each experiment; (3) little or no disinfecting action for the first sprayings of these experiments. These findings are in full agreement with those recorded in the preceding section (method 1).

Interpretation of the *degree* of disinfection per spraying from the results in Table 5 is facilitated by examination of Fig. 3, in which the average bacterial counts for each half-hour of occupation are plotted against consecutive half-hour periods of occupation of the room. The graphs for Exps. 8, 12 and 13 when compared with those for the control Exps. 2 and 5 show that: (1) the decrease in the bacterial content which occurred after the first spraying of each of the Exps. 8, 12 and 13 was similar to but not greater than the corresponding decreases in the control experiments, indicating little or no disinfection as a result of a first spraying; (2) the decrease, as indicated by the slope of the graphs, after the second spraying of each experiment was greater, and much greater in Exps. 12 and 13, than in the control Exp. 5 (the bacterial count for the control Exp. 2 shows an *increase* at this stage), thus indicating appreciable disinfecting action for each of the second sprayings; (3) there was a sharp fall in the bacterial content after the third spraying of Exps. 8 and 12 in contrast to the steep rises in each of the control experiments indicating considerable disinfecting action for each of the third sprayings; (4) the considerable fall in the bacterial content which took place during the fifth half-hour of Exp. 8 contrasted sharply with the considerable rise of the control Exp. 5, and indicated a high degree of disinfection as a result of the fourth spraying of Exp. 8, while, in Exp. 12, the corresponding change was a small rise, and the smaller disinfecting action

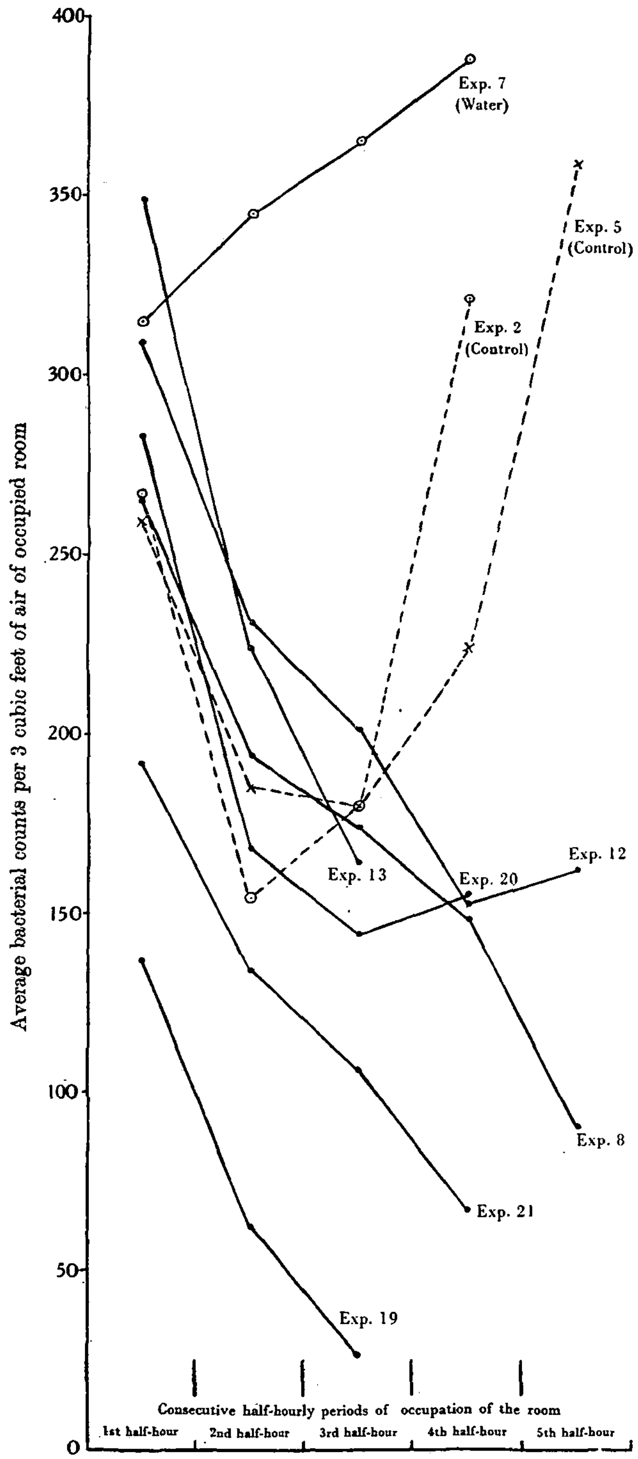

Fig. 3. Variation in the bacterial content of the air of the room during consecutive half-hour periods of occupation. Continuous lines show the effect of spraying hypochlorite Y, or water (Exp. 7). Discontinuous lines show the result of the control Exps. 2 and 5 (i.e. for no spraying). The small vertical lines indicate the times when the sprayings were carried out.

thus indicated may have been due to the fact that, as already stated, the occupants of the room were smoking during the half-hour period after the fourth spraying.

Further, it will be observed that the *differences* between the average bacterial counts at the start and finish of each of these three experiments indicate considerable *reductions* whereas in the control experiments these differences are *increases*. It follows, therefore, that the degree of disinfection achieved in each *complete* experiment was of a high order.

Although, as already stated, the sprayings in Exp. 19 were not made at half-hourly intervals, the results of this experiment are included in Table 5 and graphed in Fig. 3, both of which demonstrate the effectiveness of the sprayings and the high degree of air disinfection achieved in this experiment.

The results of the two experiments (20 and 21) in which hypochlorite Y was atomized into the air by means of the Dynalysor may be interpreted as follows: in both experiments the first sprayings produced changes in the bacterial count which were similar to but not greater than the corresponding changes in the control experiments, i.e. the sprayings had little effect; the second and third sprayings were effective. From Fig. 3 it will be seen that the second and third sprayings were more effective in Exp. 21 than in Exp. 20, this difference being more marked for the third sprayings of the two experiments. In Exp. 20, however, the total amount of hypochlorite Y atomized (three atomizations) was 13 c.c. only (compare 25 c.c. in Exp. 21, which quantity is much smaller than the amount used in the 'flit-gun experiments'), and the quantity of hypochlorite Y atomized during each of the second and third atomizations corresponded with a concentration of only 0·08 c.c. per million c.c. of air. It is possible that this minute concentration of hypochlorite Y is near the minimum concentration required for effective disinfection of the air under the conditions of these experiments.

This method of assessment of the degree of disinfection has yielded conclusions in full agreement with those of method 1, except for the first spraying of Exp. 13 which is here assessed as negative whereas method 1 indicated a probable slight degree of disinfection. Both methods indicated some lowering of the degree of disinfection after the fourth spraying of Exp. 12 (e.g. in comparison with the fourth spraying of Exp. 8), i.e. during the period when the occupants of the room were smoking.

When the bleaching powder Exps. 1, 3, 4 and 6 are considered the findings are similar to those of method 1. For example, Fig. 4 indicates: (1) the considerable reduction in the bacterial counts which was effected in each of the complete Exps. 1 and 3; (2) the relatively poorer result of Exp. 4; and (3) the failure of Exp. 6. Of the individual sprayings only the second of Exp. 4 and the third spraying of Exp. 6 require detailed attention. Thus, after the second spraying of Exp. 4 the 'C' change (Table 5) was a 3% increase which compares rather unfavourably with the corresponding changes in the control experiments (compare method 1 according to which this spraying was effective although less so than the second sprayings of Exps. 1 and 3). The third spraying of Exp. 6 was a spraying of hypochlorite Y which was assessed (method 1) as effective although not so effective as was expected, a conclusion which is confirmed by the present method, since the 'D' change (+14%) associated with this spraying does not compare favourably with the corresponding changes (+52 and +1%) of the control experiments.

Note. The blood-agar plates used in each separate experiment were all of one batch and three or four (sometimes more) were used as control plates, i.e. they were not exposed, but were incubated for 30 hr. at 37° C. along with the experimental plates. On these control plates the number of contaminating

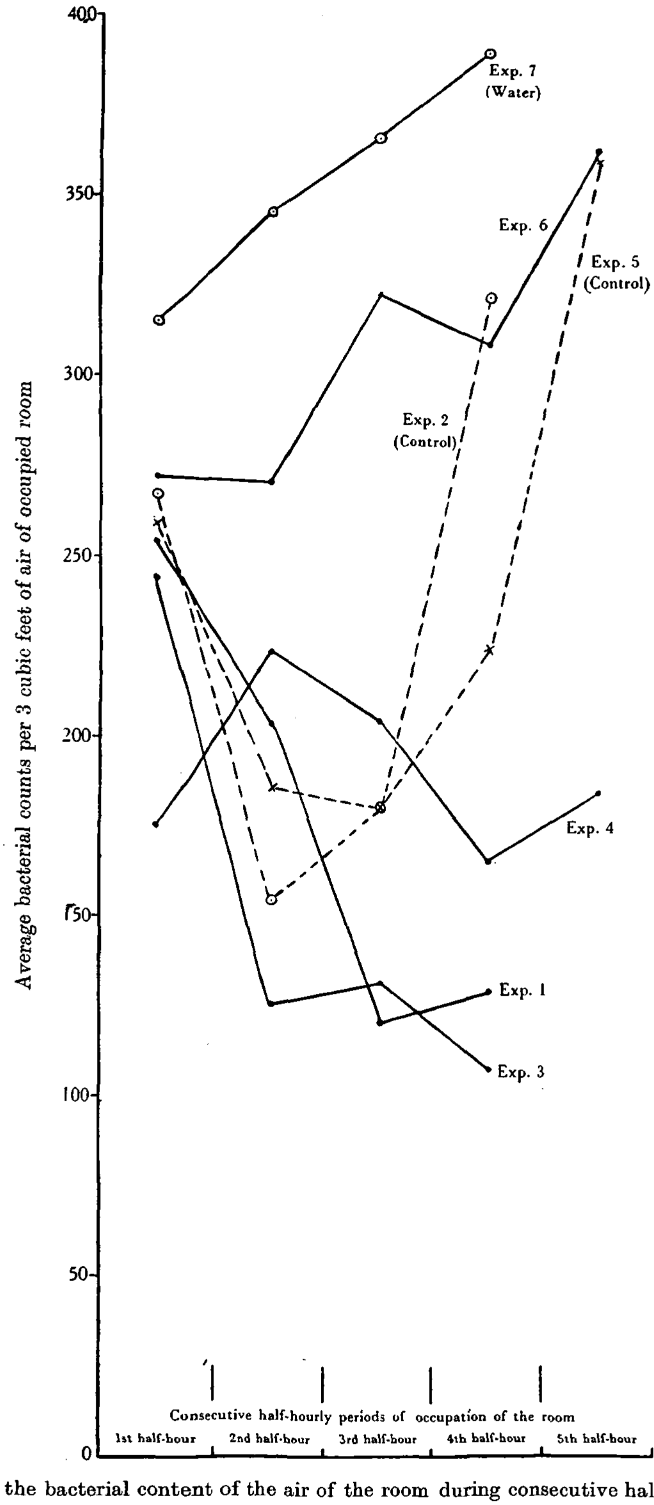

Fig. 4. Variation in the bacterial content of the air of the room during consecutive half-hour periods of occupation. Continuous lines show the effect of spraying water (Exp. 7) or a 1·3% suspension of bleaching powder into the air by means of a flit gun. Discontinuous lines show the result of the control Exps. 2 and 5 (i.e. for no spraying). The small vertical lines indicate the times when the sprayings were carried out.

organisms was small, e.g. 0, 2, 0 and 2 for Exp. 2; 1, 1, 0, 3, 1, 3, 4 and 1 for Exp. 6. The total number of colonies on the seventy-four control plates used in the thirteen experiments was 156, i.e. an average of two organisms per plate.

(*d*) *Relative humidity and air disinfection*

The relative humidity of the air of the occupied room during these experiments was calculated from the dry-bulb thermometer temperatures and the differences between the wet- and dry-bulb thermometer readings. These temperatures were plotted against the period of occupation of the room and the wet- and dry-bulb temperatures were interpolated from the graphs, thus minimizing the errors unavoidably associated with such observations. Calculations of the relative humidity were then made with the aid of the Hygrometric Tables issued by the Meteorological Office. Numerous calculations were made for each experiment and the relative humidities were then plotted against the period of occupation of the room. From such graphs it was possible to determine the relative humidity of the air at any given moment during the experiment, e.g. at the time of the sprayings, etc. It should, however, be emphasized that the calculated relative humidities are subject to error inasmuch as adequate motion of the air (i.e. an air current in excess of 5 m.p.h.) over the wet bulb was not ensured. The figures quoted for relative humidity, however, are of comparative value (see later).

The quantities of aqueous fluids introduced into the air of the poorly ventilated room in these experiments were far too small to produce any appreciable effect on the relative humidity of the air of the room. At the start of the experiments (dry-bulb temperature 51–66° F.) the relative humidity ranged from 48 to 71% but usually was between 59 and 64%. During the experiments there was a considerable rise in the relative humidity, and the increases which took place during the first, second, third and fourth half-hour periods of occupation were of the order of 8–10, 5, 1–3 and 1–2%, respectively. The relative humidities and dry-bulb temperature at the time of the sprayings in the different experiments are summarized in Table 6 and, for comparison, the control, i.e. 'no spraying' experiments, are included.

Omitting the control Exps. 2 and 5 and the 'water experiment' 7, it will be observed that, at the time of the first spraying of each experiment, the relative humidity was 71% or lower except in Exps. 13 and 19 when the relative humidity was 73%. There were ten spraying experiments and the only two first sprayings which were effective were those of Exps. 13 and 19. At the time of the second sprayings of each of the eleven experiments, the relative humidity was 71% or higher, except in Exp. 4 (relative humidity = 67%), the second spraying of which has been shown to be less effective than the second sprayings of the other 'bleaching powder experiments' 1 and 3. It is true that there was one other failure among the second sprayings but this was in Exp. 6 (relative humidity = 72%) which has been shown to differ considerably from the other experiments. All the third sprayings were successful (the third spraying of Exp. 6 was less effective than expected): the only fourth spraying which failed was in Exp. 6. The relative humidity at the time of the third and fourth sprayings was never below 72% and usually was much higher. Further, the relative humidity in Exp. 4 was appreciably lower than in the other bleaching powder Exps. 1 and 3 and may possibly be correlated with the comparatively poorer results of the former experiment.

It has not escaped notice that the average of the relative humidities prevailing at the time of the sprayings of the hypochlorite Y experiments were a little greater than the corresponding average humidities of the bleaching powder experiments. The above reasoning, however, does not suggest that this difference in humidity *per se* could account for the relatively higher degree of air disinfection obtained with hypochlorite Y. In connexion with the rather higher relative humidity of the air during the experiments with the latter hypochlorite it may be added that these experiments were carried out during the period February to April 1941, while the 'bleaching powder experiments' were performed during the preceding months of December and January.

In these experiments the hypochlorite sprayings were unsuccessful when the relative humidity of the air was below a certain value and at higher relative humidities the

Table 6. *Relative humidities* and dry-bulb temperatures at time of the sprayings*

Exp.	First spraying	Second spraying	Third spraying	Fourth spraying
		Bleaching powder experiments		
1	71% at 72° F.	77% at 73° F.	79% at 73° F.	—
3	66% at 68° F.	72% at 69° F.	74% at 70° F.	—
4	62% at 65° F.	67% at 67° F.	72% at 68° F.	72% at 68° F.
6	68% at 67° F.	72% at 69° F.	75% at 70° F.	77% at 70° F.
		Experiments with hypochlorite Y		
8	66% at 65° F.	71% at 67° F.	74% at 68° F.	79% at 67° F.
12	70% at 54° F.	76% at 56° F.	78% at 58° F.	79% at 59° F.
13	73% at 58° F.	79% at 59° F.	—	—
19	73% at 66° F.	77% at 67° F.	78% at 68° F.	79% at 69° F.
20	70% at 61° F.	75% at 63° F.	76% at 64° F.	—
21	71% at 63° F.	75% at 64° F.	75% at 65° F.	—
		Water experiment		
7	66% at 65° F.	71% at 67° F.	77% at 67° F.	77% at 67° F.
		Control experiments		
2	71% at 68° F.	73% at 70° F.	72% at 72° F.	81% at 71° F.
5	68% at 67° F.	73% at 68° F.	75% at 68° F.	77% at 69° F.

There were no sprayings in the control experiments, and the values of relative humidity and temperature recorded for these experiments (2 and 5) relate to the times at which sprayings were carried out in most of the other experiments.

For calculation of relative humidity the temperatures were interpolated from the graphed temperature readings and were recorded to the nearest one-fifth of a degree but are given to the nearest degree in above table.

* All relative humidities in this table have been calculated from 'still-air' thermometer readings and are, therefore, mainly of comparative value.

hypochlorites were effective air disinfectants. This critical value of the relative humidity, below which the hypochlorite sprayings were ineffective, was of the order of 71%, but, since the relative humidities were calculated from readings of the wet- and dry-bulb thermometers taken in still air, the above figure requires correction. It may be stated that an adequate current of air at room temperature over the thermometers was not provided because such air movement in the restricted space of the experimental, occupied room would have interfered with the bacteriological observations. However, the results of preliminary experiments in an occupied room in which a current of air (greater than 5 m.p.h.) was directed on to the thermometers have shown that the relative humidities calculated from the 'still-air' thermometer readings are too high.

https://doi.org/10.1017/S0022172400035841 Published online by Cambridge University Press

Discussion

The ventilation of the rather small (cubic content = 1680 cu. ft.) room used in these experiments was of a low order and, as the number of occupants was fourteen or seventeen, the room was overcrowded and the conditions were similar to those which prevail in many air-raid shelters. It has been shown that spraying the air of the occupied room with hypochlorite solution (or suspension) from a flit gun exerts a partial disinfectant action on the air of the room. This was achieved with minute quantities of the hypochlorite, e.g. approximately 0·4 c.c. of a 1 % solution of hypochlorite per million c.c. of air (i.e. about 11 c.c. per 1000 cu. ft.). Of the hypochlorites tested, the 1·3 % suspension of water-sterilizing powder is not chemically equivalent to a 1 % solution of sodium hypochlorite, but reasons for this choice have already been given. The *amount* of hypochlorite sprayed into the air was controlled to some extent by the personnel present in the room, i.e. spraying was continued until the odour of hypochlorite was distinctly perceptible.

That such a coarse spray as that produced by a flit gun was effective is of great interest since much stress on the need for sprays consisting of exceedingly fine droplets has been made by previous workers who have used expensive nebulizers such as, for example, the Dynalysor (Masterman, 1938), the Atmozon and the Aerograph (Baker, Finn & Twort, 1940). It is worthy of note that the latter authors often obtained better results with the Aerograph than with the Atmozon, although the mist of the Aerograph consisted of larger droplets than that of the Atmozon. As suggested by the latter authors, the superiority of the larger droplet mist may have been due to their slower evaporation—thus allowing longer time for contact between the bacteria and the still moist mist, or, to the possibility that too small a droplet may not contain a lethal dose of the germicide. Even the finest of the droplets of the flit-gun sprays used in these experiments were many times larger than those of the mists from the nebulizers mentioned above. In 1941, Masterman suggested that the active agent, when hypochlorites are used for air disinfection, is hypochlorous acid gas. Evidence that the molecularly dispersed form of another air disinfectant (propylene glycol) is an effective air disinfectant was brought forward (1941) by Robertson and his co-workers, who showed that the glycol in vapour form was ten times more effective against air-suspended bacteria and air-borne influenza virus than the glycol in mist form. If Masterman's postulate is correct, it seems feasible to the writer that efficient disinfection might be obtained with droplets of hypochlorite varying in size to a greater extent than would be possible if the 'Aerosol' theory (favoured by Baker *et al.* 1940) were applicable. For example, in the series of experiments described in this paper, even the droplets large enough to be seen by the naked eye, and which reached the floor, etc., before evaporation was complete, would continue to evolve hypochlorous acid gas (although part of such droplets would be absorbed). The results of the experiments in which a relatively fine droplet mist was produced by the Dynalysor were comparable with the results of the flit-gun experiments, but much smaller quantities of hypochlorite were atomized when the Dynalysor was used than in the latter experiments.

No reduction of the bacterial count resulted when water was sprayed into the air (three sprayings), and the action of the hypochlorites was not, therefore, merely a mechanical removal of bacteria from the air. Although sodium chloride solutions are known to produce a small bactericidal effect, this effect is much smaller than that of hypochlorites (Baker *et al.* 1940). The sodium chloride content of the hypochlorite Y used in these experiments was only 1%.

Baker *et al.* (1940) have shown that the addition of alkali (1% of sodium hydroxide) lowers the efficiency of hypochlorite as an air disinfectant, and the modification of hypochlorite Y, in order to reduce its alkalinity, was considered but was not carried out. However, the hypochlorite Y used in these experiments contained only one-tenth of 1% of sodium hydroxide and it is improbable that this amount of alkali was sufficient to cause any serious interference with the action of the hypochlorite.

In these experiments the hypochlorite solutions used contained 1% of hypochlorite and, in view of the fact that Masterman, and Baker *et al.* have obtained improved results by diluting the 1% hypochlorite to give a one-fifth of a 1% solution, the advisability of testing the more dilute solutions was apparent. However, this series of experiments was limited in number by the availability of the occupants and, as it was not possible to investigate thoroughly hypochlorite solutions of both strengths, the 1% solution was chosen. Furthermore, the time required to spray the 1% solution is shorter than the time required to spray the correspondingly larger amounts of the weaker solution, an advantage which is of practical value. This advantage becomes of more importance if spraying is carried out by means of an improved hand-spray, such as the No. 8 hand-spray for hypochlorite spraying of shelters, recommended by the Medical Research Council in 1941. This hand-spray is somewhat similar to the flit gun but has several advantages over the latter, one of the most important being the provision of a removable baffle plate to trap the very large droplets, thus reducing the risk of damage to articles (clothing of occupants, bedding, etc.). When used in this way the output of the spray is 4 c.c. per 50 strokes of the hand-pump, i.e. only about one-fifth of the output of the flit gun, and, since the time required to spray the necessary quantity of a very dilute hypochlorite solution would be excessive, the use of the 1% solution is preferable.

That partial disinfection of the air of the occupied room was accomplished in these experiments is not open to question, but the methods developed for assessing the degree of disinfection produced need some explanation. In this connexion, it may be mentioned that no published accounts are available of similar experiments in occupied rooms under conditions as carefully controlled as in the present experiments and in which an efficient method of air sampling has been used. Assessment of the degree of disinfection in experiments of the kind described here is difficult inasmuch as the occupants of the room are continually emitting bacteria into the air at a rate which is not necessarily constant. Simultaneously with the reduction of the bacterial content of the air due to the germicidal action of the disinfectant there is, therefore, a tendency for the bacterial content to increase at a variable rate.

The bacteria present in the air of an occupied room may be roughly divided into two groups: (1) bacteria liberated into the air from the respiratory tract and skin, and (2) organisms passing into the air from the clothes of the occupants, and bacteria from dust on the floor, walls, etc., stirred by the movements of the occupants. Most of the potentially pathogenic bacteria present in the air of the occupied room during these experiments would belong to the first of these two groups, and qualitative examination of the bacteria isolated during this investigation (an account of which will, it is hoped, be published later) has shown that potentially pathogenic bacteria were present in very small numbers, and constituted probably less than 2% of the total bacterial content of the air. The fate of the potentially pathogenic bacteria is obviously of major importance in the present investigation, and a knowledge of the relative susceptibility of such bacteria to the action of air disinfectants, as compared with that of the saprophytic bacteria which constituted the bulk of the bacteria present, would be of considerable value. Unfortunately, such knowledge is very incomplete. However, one factor which may have a bearing on this problem is the condition (i.e. with regard to moisture) of the bacteria in the air. Thus, bacteria emitted into the air from the respiratory passages are in a moist condition whereas a large proportion of the dust bacteria are dry, and it is possible that bacteria in a moist condition (e.g. the potentially pathogenic bacteria in these experiments) are more susceptible to air disinfectants, such as hypochlorites, than the dry bacteria, but the source and nature of the 'moisture' must also be considered. It is well known that dry bacteria are very resistant to adverse influences. For example, ultra-violet light has a greater bactericidal action on moist than on dry bacteria. Also, bacteria from the respiratory tract, such as streptococci, staphylococci and pneumococci, etc., when attached to particles of dry dust, have been shown to survive for relatively long periods of time in an unfavourable environment such as air. The suggestion that moist bacteria may be more susceptible than relatively dry bacteria to the action of hypochlorites is, therefore, worthy of consideration; and of interest in this connexion, is the fact that, as shown by the results of the present investigation, hypochlorite sprays were ineffective at low relative humidities, and, while it is not suggested that the rôle of the water vapour of the air is solely that of maintaining the bacteria in a moist condition, such action of the water vapour may be of importance in the disinfection process. The resistance to hypochlorites of dry organisms found in dust has been observed by Bourdillon, Lidwell & Lovelock (1942), who used hypochlorite mists generated by a hand-spray for air disinfection after sneezing, and they state that many of the dry organisms found in dust are not quickly killed by the hypochlorite in the concentrations they used, although the spray appeared to kill all the oral and nasal organisms liberated into the air by the sneezes. During the sneezing experiments of Bourdillon *et al.* (1942), large numbers of bacteria from the respiratory tract were present, and the authors state that about 100,000 bacteria-carrying droplets are emitted into the air as the result of a single vigorous sneeze. There was no sneezing during the experiments described in this paper, and apart from the question of the relative susceptibilities of moist and dry bacteria, it cannot be too strongly emphasized that the air samples taken in these experiments consisted chiefly of saprophytic bacteria (micrococci, staphylococci, diphtheroids, sporing aerobes, etc.), some of which are very resistant to disinfectants (e.g. the sporing aerobes), and others (possibly a large proportion) are relatively resistant to disinfection. When this, together with the other considerations outlined above, are taken into account, it will be realized that the degree of air disinfection by hypochlorites recorded in these experiments was as high as could be expected, and that the percentage reduction of the potentially pathogenic bacteria present was probably greater than that recorded for the total bacterial content of the air of the occupied room.

In these experiments, reduced bacterial counts were observed during the 15–20 min. period after a hypochlorite spraying. The relatively short period during which effective disinfection was observed is of practical importance, and it seems reasonable to postulate that this period would be shorter, or the degree of disinfection smaller, in well-ventilated rooms because of more rapid disappearance of the hypochlorite from the air of the room. In any case, the need for repeated sprayings of hypochlorite is obvious whatever the efficiency of the ventilation may be.

Development of method 1 for assessing the degree of disinfection per individual spraying is logical in view of the extent of the duration of air disinfection after a spraying. As already mentioned, this method suffers from the disadvantage that the results thus obtained for a single spraying may be influenced to some extent by the effect of previous sprayings. For example, in Exp. 19, the bacterial content rapidly became very low (about 50 per 3 cu. ft. of air), and, with such low counts, small changes become increasingly difficult to measure. The bacterial content of 50 is not much above that of the room before occupation, i.e. of the empty room, the bacterial content for which was never lower than 20. It would not have been unreasonable to have subtracted the average bacterial count for the empty room from the counts obtained during the experiments, in order to calculate the bacterial content *due to occupation*. This procedure would have yielded greater percentage reductions in the bacterial counts but was not adopted. Although method 1 is, therefore, not above criticism, it yielded results for individual sprayings of a disinfectant which, in general, were in reasonably close agreement with each other (e.g. for the sprayings of hypochlorite Y).

According to method 2, the average bacterial counts for half-hourly periods of occupation of the room were compared with the average counts for the first *hour* of occupation. This method, which at first sight may seem somewhat arbitrary, was chosen only after much consideration. Greater percentage reductions in the bacterial counts would have been obtained if the comparison had been made against the average count for the first half-hour, but the major disturbance of air, dust, etc., caused when the men enter the room, together with the somewhat variable 'settling-in period', affect the bacterial count during this initial period to a pronounced and variable degree. Furthermore, the effect on the bacterial content of the air of the 'settling-in', etc., did not appear to be of short duration, and in each of the two control experiments, for example, the bacterial count continued to fall appreciably for at least 1 hr. Accordingly, in comparing the bacterial counts with the average count for the first hour of occupation (usually the average of 12 or more counts), errors due to initial disturbance, etc., are minimized. In the majority of these experiments, the first spraying was made after half an hour's occupation but, as first sprayings of an experiment were almost always unsuccessful, their effect on the average count for the first hour of an experiment is of no significance.

Sufficient has been said to indicate the difficulties involved in the measurement of the degree of air disinfection in experiments of this kind, and it is again emphasized that only comparative value is claimed for the methods adopted in this work. Each individual spraying has been assessed by both of these methods. The bacterial counts for corresponding periods in most of these experiments are, as will be seen from Table 5, of the same order of magnitude, and indicate the similarity in conditions which prevailed throughout this series of experiments. The graphs of Figs. 3 and 4 show clearly that a considerable degree of disinfection was effected in most of these experiments and, in particular, in Exp. 19, when the sprayings of hypochlorite were more frequent than in the other experiments. It may also be stated that the results obtained with hypochlorite Y were consistently good and were superior to those of the bleaching powder experiments.

https://doi.org/10.1017/S0022172400035841 Published online by Cambridge University Press

The number of occupants in the room during the first six of these experiments (Exps. 1–6, i.e. including the 'bleaching powder experiments' 1, 3, 4 and 6, and the two control Exps. 2 and 5) was seventeen, but for the remaining eight experiments fewer men were available and the number of occupants was fourteen. During the bleaching powder and control experiments there was available for each occupant about 100 cu. ft. of air as against the 120 cu. ft. in all other experiments; it seems improbable that this difference alone could account for the inferior results obtained with bleaching powder as compared with hypochlorite Y, but the possibility cannot be disregarded.

The reduced efficiency recorded for the fourth spraying of Exp. 12 when the occupants of the room were smoking, together with the results of the 'smoking experiment' in the empty room, suggest that tobacco smoke interferes with the disinfecting action of hypochlorites, but the experimental data are too meagre for any conclusion to be drawn. Although the air was heavy with tobacco smoke during the half-hour period after the spraying (experiment in occupied room) the efficiency of the spraying was only reduced, i.e. not entirely eliminated. Tobacco smoke has been shown to inhibit completely the action of a mist of hexyl-resorsinol dissolved in propylene glycol by Twort & Baker (1940), who also showed that the smoke itself was devoid of bactericidal action.

Hypochlorous acid gas is extremely reactive towards organic compounds, and Bourdillon *et al.* (1942) have on two occasions attributed the failure of hypochlorite sprays to the presence of cresol vapour in the air of the experimental room. It is possible that the almost complete failure of Exp. 6 may have been caused in a similar way, but no positive evidence is available and the suggestion is brought forward with reserve.

The conclusion reached concerning the dependence of the disinfecting action of hypochlorites on the relative humidity of the air is extremely interesting. Baker & Twort (1941) have indicated the importance of relative humidity in air disinfection, and Bourdillon *et al.* (1942) have stated that the practical lower limit of humidity for effective killing of the organisms introduced into the air during sneezing is below 60% at 70° F. In these experiments in which, as already indicated, bacteria from various sources were present in the air, effective disinfection was not obtained when the relative humidity was below about 71%* at temperatures ranging from 54 to 72° F. The need for more accurate determination of this critical relative humidity has already been stated.

Almost all the personnel who took part in these experiments agreed that the hypochlorite sprayings had a pronounced 'freshening' effect upon the air of the occupied room, and it is, therefore, suggested that this effect *per se* is of some value. The effect might be increased by the use of perfumed hypochlorite solutions as used by Lovelock & Lidwell (1941). The marked deodorizing effect of the hypochlorite sprays on the atmosphere of the room is also of importance and the effect has been observed by Pulvertaft & Walker (1939).

Substances suitable as air disinfectants have been studied by Twort, Baker, Finn & Powell (1940), and by other workers. Robertson and his co-workers (1941) claim that propylene glycol is an effective air disinfectant and their tests were made against air-suspended bacteria and air-borne influenza virus. Pulvertaft & Walker (1939) have reported favourably upon the suitability of hypochlorites for air disinfection, and Masterman (1941) has, for many years, advocated the use of hypochlorites for this purpose.

* The relative humidities were calculated from thermometer readings in still air and are therefore too high (see section 'Relative humidity and air disinfection').

Hypochlorites have many properties which render them suitable for large-scale air disinfection. Thus, (1) they are easily obtained as large stocks are always available, (2) they are inexpensive when purchased in bulk, (3) as shown in the present work and elsewhere, they act as efficient air disinfectants even when used in exceedingly small concentrations, (4) no elaborate method of introduction into the air is necessary. Hypochlorite solutions are corrosive liquids and this and other disadvantages attendant upon the use of hypochlorite mists in combating aerial infections are discussed by Baker *et al.* (1940) and Masterman (1941). In the present work the need for observing certain simple and obvious precautions when spraying hypochlorite solutions from a flit gun has been mentioned. Concerning the possible disadvantages of such hypochlorite sprays, the following conclusions are based upon the observations of the numerous personnel who took part in these experiments: (1) the irritant effects were exceedingly small; (2) the odour was not objectionable; (3) the slight opacity produced in the air by the mist from the flit gun disappeared within about 1 min. With regard to the corrosive action of hypochlorite mists on metals, it may be stated that the metal parts of the slit sampler and vacuum cleaner were little affected during this series of experiments, extending over several months, and that the same flit gun was used and is still in good working order. Masterman (1941) points out that the corrosion of metals consequent upon the use of hypochlorite sprays is due mainly to deposition of sodium chloride, and that, for the proprietary hypochlorite solution which he was using, such deposition was exceedingly minute. The hypochlorite Y used in these experiments contained much less sodium chloride than the hypochlorite used by Masterman.

Therefore, it is concluded that intermittent spraying of small quantities of a 1% hypochlorite solution containing only a small percentage (about 1%) of sodium chloride, such as hypochlorite Y, from a flit gun is a practical method of air disinfection in poorly ventilated premises and that there are no serious objections to such a procedure. A suspension of bleaching powder may be used instead of hypochlorite solution although results obtained with the former substance (not chemically equivalent to hypochlorite Y) were less satisfactory than with the hypochlorite solution. The investigation demonstrates the need for repeated sprayings at intervals not longer than half an hour and preferably at intervals of 15–20 min. Such a procedure has been shown to reduce materially the number of viable bacteria in the air of an occupied room and the extent of the reduction was satisfactory in view of the fact that the air of the room contained a large proportion of resistant saprophytic bacteria.

It is concluded that the application of this method for air disinfection in crowded places such as air-raid shelters, some military quarters, and even in places of entertainment under epidemic conditions, would reduce the danger of the spread of infectious disease by transmission of air-borne bacteria. As this method of air disinfection is recommended for air disinfection in air-raid shelters, the relative humidity of the air of such occupied shelters is a factor which must be considered. Numerous observations of the relative humidities of the air of occupied shelters in Edinburgh have been made during several months in collaboration with Dr H. E. Seiler and Dr R. P. Jack of the Public Health Department, City of Edinburgh. Owing to the many factors which influence the humidity in such premises, e.g. meteorological conditions, especially the wind, and the temperature of the outside air, the temperature and condition of the walls, floors and roofs of the shelters, numbers of occupants, etc., all that can be said is that the relative humidity of the air of occupied shelters was high and was usually of the order of 70–80%

(thermometer readings in still air) shortly after occupation commenced. The relative humidity of the air of occupied shelters is therefore usually above the minimum required for effective air disinfection by hypochlorites.

Thanks are due to Officers of the Scottish Command (Brigadier F. A. E. Crew, former A.D.M.S., Edinburgh; Colonel T. F. Kennedy, O.B.E., D.D.H., Scottish Command, Major R. N. Hunter, R.A.M.C., and others) for their interest and assistance in providing the men who occupied the room in these experiments, and without whom this work could not have been performed. The author wishes to thank Dr H. E. Seiler of the Public Health Department, City of Edinburgh, for his interest and valuable co-operation which has made possible the taking of numerous bacteriological observations of the air in shelters, the results of which indicated the need for air disinfection and added interest to the work described in this paper. The slit sampler used in this investigation was constructed by Mr N. E. Condon, of the Department of Pharmacology, Edinburgh University, and the assistance of Dr S. A. Levvy, of the latter department, in calibrating the air-flow through the slit, is also gratefully acknowledged. I wish to express my gratitude to Prof. T. J. Mackie, C.B.E., for his constant interest and valuable advice.

SUMMARY

Substantial reduction in the bacterial content of the air of an empty room after infection with *B. prodigiosus* (or with *Staphylococcus albus* or a diphtheroid bacillus) has been effected by means of hypochlorites introduced into the air by atomization from a Dynalysor or by spraying from a flit gun.

The 'normal' variation of the bacterial content of the air of an occupied (crowded) room has been described.

Repeated spraying of a solution containing 1% of sodium hypochlorite or of a 1·3% suspension of water-sterilizing powder (bleaching powder) from a flit gun has been shown to reduce materially the bacterial content of the air of the occupied room.

Similar sprayings of water have been shown to have no appreciable effect on the bacterial content of the air of the occupied room and the action of the hypochlorite was, therefore, not merely a mechanical removal of bacteria from the air.

Methods have been devised for assessing the degree of disinfection per individual spraying of hypochlorite, and, although the methods are mainly of comparative value, it may be stated that the reduction in the *total* bacterial content of the air of the occupied room for a single spraying of hypochlorite Y ($\equiv$1% sodium hypochlorite) was *of the order of* 33%, or more. The percentage reduction of potentially pathogenic bacteria which may have been present was almost certainly greater than the reduction of the *total* bacterial content, which included resistant saprophytic bacteria. The average concentration of hypochlorite Y used in these experiments was 0·38 c.c. per million c.c. of air (i.e. less than 11·0 c.c. per 1000 cu. ft. of air) per spraying, i.e. was exceedingly small, and even smaller concentrations were equally effective when the hypochlorite was atomized into the air by means of the Dynalysor.

Rather less consistent results were obtained with the chemically less active bleaching powder suspension than with hypochlorite Y in the flit-gun experiments.

The relative humidity of the air has been shown to be a factor of great importance, and in the experiments described in this paper effective air disinfection was not obtained

https://doi.org/10.1017/S0022172400035841 Published online by Cambridge University Press

at low relative humidities at temperatures ranging from 54 to 74° F. The need for accurate determination of the critical lower limit of relative humidity is stressed.

Tobacco smoke has been shown to reduce the efficiency of hypochlorite sprays but the experimental evidence was insufficient for any definite conclusion to be drawn.

The advantages and disadvantages of hypochlorites as air disinfectants have been discussed and the conclusion reached that repeated (every 20 or 30 min.) spraying of the air of an occupied room with a 1% solution of hypochlorite (e.g. hypochlorite Y) constitutes a simple, practical, inexpensive and efficient method of air disinfection, and, provided simple precautions are observed, there are no serious objections to such a procedure.

General conclusions

The investigations have shown that repeated spraying of the air of an occupied room with 1% hypochlorite solution from a flit gun materially reduces the bacterial content of the air, and it is suggested therefore that application of this simple, practical method for air disinfection of occupied air-raid shelters, military quarters, etc. would reduce the spread of air-borne infectious diseases.

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(*MS. received for publication* 9. ix. 42.—Ed.)