HEXYLRESORCINOL AS AN AERIAL DISINFECTANT

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(With Plates 10–12 and 6 Figures in the Text)

There are several efficient methods of diminishing the bacterial content of indoor air but none has attained popularity in this country; at any rate, none so far accepted as suitable for large-scale use. An editorial article in the *British Medical Journal* (1949), in reviewing the Medical Research Council Report entitled *Studies in Air Hygiene*, stated: 'The keynote to the general conclusions of this report is that air disinfection is practicable only in special circumstances. There is no prospect whatever of the widespread adoption of any of these methods in public buildings and conveyances; such problems are almost insuperable, owing either to the great size of the task or to the variable conditions for which provision would have to be made. It is also emphasized that appliances used for this purpose are apt to be neglected: "people lose interest" in them, as is perhaps natural when the result of their use is at best only the negative condition of absence of infection.' It would appear pertinent, therefore, to draw attention to the potentialities, in this field, of chemicals such as hexylresorcinol when disseminated by heat in the form of an aerosol. We have been able to demonstrate that, using *Chromobacterium prodigiosum* as a test organism, concentrations in the air of between 6 and 7 μg./cu.ft. of this reagent will reduce in a very short time the bacterial content of the air from over one million bacteria per cu.ft. of air to fewer than ten.

A considerable amount of research on the chemical sterilization of air has been carried out in this country by teams of workers, notably, Pulvertaft, Lemon & Walker (1939); Twort, Baker, Finn & Powell (1940); Andrewes (1940) and Bourdillon *et al.* (1948). Of the extensive range of chemicals investigated by these workers, perhaps the most promising would appear to be hexylresorcinol, the study of which was interrupted during the war because of the shortage of material.

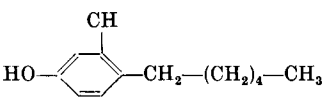
It is important to differentiate between the action of fumigants such as formaldehyde and hypochlorous acid, the sterilizing concentrations of which are of the same order whether they are acting in air or in liquid media, and the actions of mists or vapours of substances such as glycols and phenolic compounds. As Trillat (1938) showed, disinfectants of this class with a bactericidal titre in broth culture of 1/200 may kill the same organism when suspended in air in a dilution of 1/5,000,000.

Chemical aerosols may be generated in various ways. If liquid, the chemical may be sprayed directly into the air from an atomizer of the scent-spray type. If solid, the chemical may be dissolved in an appropriate solvent such as spirit, glycerin or propylene glycol and atomized, in which case if the spray is sufficiently fine the solvent evaporates almost instantaneously leaving a dry mist or vapour. The use

of atomizers for large scale or for continuous generation of aerosols is troublesome. The very fine jets required readily block, the method tends to be wasteful of material and if solvents are required they may be expensive. A much simpler method is to vaporize by heat using a device such as a hotplate with thermostatic control. A number of heat stable disinfectants such as glycols and phenolic compounds including hexylresorcinol lend themselves admirably to this form of dissemination.

The fate of the chemical aerosol depends on various factors such as the atmospheric temperature and relative humidity, the rate of oxidation or of decomposition of the chemicals and their volatility and vapour pressure. If hexylresorcinol is vaporized at between 125 and 130° C. the bulk of the vapour is in the form of microscopic particles mostly less than 1 μ in diameter. Particles of this size remain suspended in the air until completely evaporated, which for 1 μ particles takes somewhat over 1 hr. at normal room temperatures. The mist from hexylresorcinol may therefore be considered a relatively persistent one.

HEXYLRESORCINOL



Hexylresorcinol is a pinkish white crystalline powder with a sharp and astringent taste. It is slightly soluble in water (1 in 2000); readily soluble in alcohol, ether, glycerin and propylene glycol. The melting-point is from 66 to 68° C. and the boiling-point at about 180° C. The therapeutic dose (British Pharmaceutical Codex, 1949) is 0·12–1 g. (2–15 grains) and it is described as an antiseptic and anthelminthic. It is a constituent of proprietary throat pastilles, tooth-pastes, etc. It has been used in the treatment of genito-urinary infections where it is more effective against Gram-positive organisms such as staphylococci and streptococci than against Gram-negative organisms such as *Bacterium coli* and gonococci. Its toxicity to animals is low.

BACTERIAL AEROSOLS

The list of diseases transmissible through the air is a formidable one. We have much to learn about the mechanisms of the transmission of these diseases, but a certain amount is known of the behaviour of bacteria-carrying droplets emitted during talking, coughing and sneezing, this being a subject which has long been studied by bacteriological and physical methods and more recently by means of high-speed photography (Jennison, 1942). In some diseases gross aerial dissemination of the causal organism may be produced by the patient, the fate of the emitted bacteria-carrying particles depending on their size. Large droplets travel only a short distance, falling out rapidly by gravity, being entrapped in dust or collected by fomites. Such on drying out may produce secondary aerosols as happens when floors are swept, beds made, etc. Smaller droplets up to 25 μ in diameter evaporate to dryness in a fraction of a second while still airborne, forming droplet nuclei. The size of these droplet nuclei corresponds roughly to that of the contained bacteria and the larger ones gradually fall out of the air by gravity. Small particles of diameter 1 μ and less show active Brownian movement, and under ordinary

atmospheric conditions remain suspended almost indefinitely. Pathogens which resist drying, such as the staphylococcus, streptococcus, or the tubercle bacillus, may therefore be carried considerable distances while still viable. Their detection is not easy because they are not deposited readily on exposed agar plates, and complicated air sampling apparatus is needed to assess the true extent of the aerial contamination which they produce.

To produce artificial bacterial aerosols under laboratory conditions is a very simple matter. It can be done by disseminating from an atomizer of the scent-spray type either a broth culture or a saline suspension of the organism being studied. To do this on a large scale, however, or to produce clouds composed of particles of a uniform predetermined size requires more complicated apparatus, examples of which are mentioned below.

THE TEST ORGANISM

The use of pathogenic organisms in conducting bacterial cloud experiments is obviously very hazardous not only to the laboratory workers concerned but also to everyone in the vicinity. In view of the magnitude of the experiments contemplated in this case, the use of a test organism even slightly pathogenic to man was considered unjustifiable. Perusal of the literature seemed to indicate that where chemical sterilization of the air was effective, it was within fairly narrow limits effective against all the vegetative bacteria tested. Pulvertaft, Lemon & Walker (1939) used *Chromobacterium prodigiosum*, *Staphylococcus albus*, *Streptococcus haemolyticus*, pneumococcus, and *Corynebacterium diphtheriae*. Twort, Baker, Finn & Powell (1940), in the very extensive work they carried out in this field, used a variety of test organisms which included *Bact. coli*, *Bact. lactis aerogenes*, *C. xerosis*, *Strep. agalacteae*, 'F' coccus (a white micrococcus isolated from a foodstuff), *Pasteurella aviseptica* and *Mycobacterium phlei*. These were chosen because *in vitro* tests showed them to have medium resistance to phenolic compounds.

The organism selected for our experiments was a highly pigmented strain of *Chr. prodigiosum* (labelled in this Department M 1/48) non-pathogenic to mice in a dose of 100,000 organisms intraperitoneally. *In vitro* tests showed this strain to be highly resistant to hexylresorcinol, a concentration in solid medium of 1/4000 being required to inhibit growth as compared with an inhibitory dose for *Staph. aureus*, *Bacillus anthracis*, and *B. subtilis* of 1/40,000.

For making up the spray suspension the organism was grown in Roux bottles on 1 % Evans peptone agar with 1 % mannitol and 1/400,000 methyl violet. The growth was suspended in phosphate buffer (pH 7·6), 2 % glycerin was added and the suspension standardized at 5×10^{10} organisms/ml. The reason for the addition of glycerin is that it increases the time of viability of vegetative organisms when suspended in air as a particulate cloud.

The above culture medium was used throughout the air-sampling tests. The dye inhibited almost all casual airborne contaminants including those of the *B. subtilis* type and so specific for *Chr. prodigiosum* did this medium prove that in over ten thousand plates used in these experiments, none had to be discarded on account of contamination.

TECHNIQUE

The test chamber was of 16,500 cu.ft. capacity of irregular rectangular shape with concrete floors and cement-rendered walls. The air was conditioned to a temperature within 1° of 68° F. with a relative humidity of 54 %. It was supplied to the chamber through plenum ducts by an input fan balanced against an extractor recirculating fan operating through a second set of air ducts. The air was thus in continuous recirculation with a constant proportion of fresh (conditioned) air being constantly admitted. The total airflow was 51,000 cu.ft./hr. (approx. 3·0 air changes) of which 8600 cu.ft. (0·5 air change) was fresh air. This rate of flow produced in the chamber no sensation of draught, but the air movement could be felt by the hand at the louvres of the input ducts as a gentle breeze. Smoke tests demonstrated that diffusion throughout the chamber was even and rapid and that there were no dead spaces.

Two kinds of apparatus were used for generation of bacterial aerosols. The first one, which was used in most of the experiments was the Collison sprayer, as modified by Henderson (1951), adjusted to produce a cloud of dried particles of 1 μ size* (single organism particles) when operating with an air pressure of 20 lb./sq.in. supplied by a pair of Edwards compressor pumps (Mark IV) linked in parallel. The sprayer was housed in a box built into the input air duct just before its entry into the chamber, the ducting extending some 40 ft. beyond this point. The capacity of the spray bottle was 50 ml. of bacterial suspension and with an airflow of 70 l./min. its output was approximately 1 ml. of suspension per min. For dissemination of uniform clouds of dried particle size greater than 1 μ the apparatus used was the spinning top of Walton & Prewett (1949), as modified by May (1949). This apparatus could not conveniently be housed in the ventilation ducting and was therefore located centrally inside the test chamber.

In air sampling for bacterial content three vacuum-operated air-sampling devices were used, no reliance being placed on gravity plates for quantitative estimation. These were the impinger (Henderson, 1951), the Bourdillon slit sampler, and a modification of the latter designed by Schuster (1948) for prolonged recording. The impinger is a glass vessel containing a measured amount (10 ml.) of phosphate buffer solution. Air to be sampled is drawn by vacuum pump through a glass tube constricted at the lower end to capillary bore, the orifice of which reaches to within 2–4 mm. from the bottom of the vessel. On the size of this capillary depends the rate of airflow. Air impinges at high speed on the bottom of the vessel and any particles present are trapped in the fluid. Because of the violent agitation particles made up of more than one bacterium are disrupted, and the count obtained is not one of the number of particles collected but is a total bacterial count. Sampling was at the rate of 10 l./min. The fluid was then diluted in phosphate buffer according to the expected bacterial content and plated out, using a modification of the technique described by Miles & Misra (1938). The impinger used gave recordings down to 1000 organisms/cu.ft. of air, but at this level of sampling there tends to be a fall in efficiency.

* Wherever mention is made of clouds of 1 μ particle size (single organism particles), the size 1 μ is an approximation only.

For lower aerial concentrations the Bourdillon slit sampler was used. In this the air is impinged at high speed through a slit direct on to a revolving Petri plate of culture medium. This type of sampler, therefore, gives a count not of the total number of bacteria present but of the total number of bacteria-carrying particles. It was estimated to give reliable counts in the zone of 0–500 particles/cu.ft. of air.

To bridge the sampling gap between impingers and Bourdillon slit samplers, use was made of the Schuster sampler modified for this purpose. This is a slit sampler with an agar tray 15 by 10 in. in size. The slit is held in a mobile carriage 2 mm. from the agar surface and traverses the agar from end to end at a pre-set speed. After a total of fourteen traverses the instrument switches off automatically. The tray is incubated and photographed life size. Counting of colonies is done from the photograph. This method allows continuous air sampling over a period which may be varied at will from 45 min. to 8 hr. and has the further advantages that counting may be done at leisure and that a permanent record of the experiment is produced. Pls. 10 and 11 show photographs of exposed trays.

For dissemination of the hexylresorcinol vapour the apparatus used was the 'Aerovap' (Shepherd's Aerosols Ltd.). Two 'K' types, slightly modified for these experiments, were installed in the test chamber. They consist of electrically heated cups holding approximately 300 g. of hexylresorcinol, operated from mains voltage, with a thermostat set at 126° C. The concentration maintained in the chamber was between 6 and 7 μg./cu.ft. of air. The cloud issuing from the vaporizer consisted of particles having a number median diameter of 0·75 μ; the diameter of 70 % of them lay between 0·4 and 1·4 μ (Powell, E. O., personal communication).

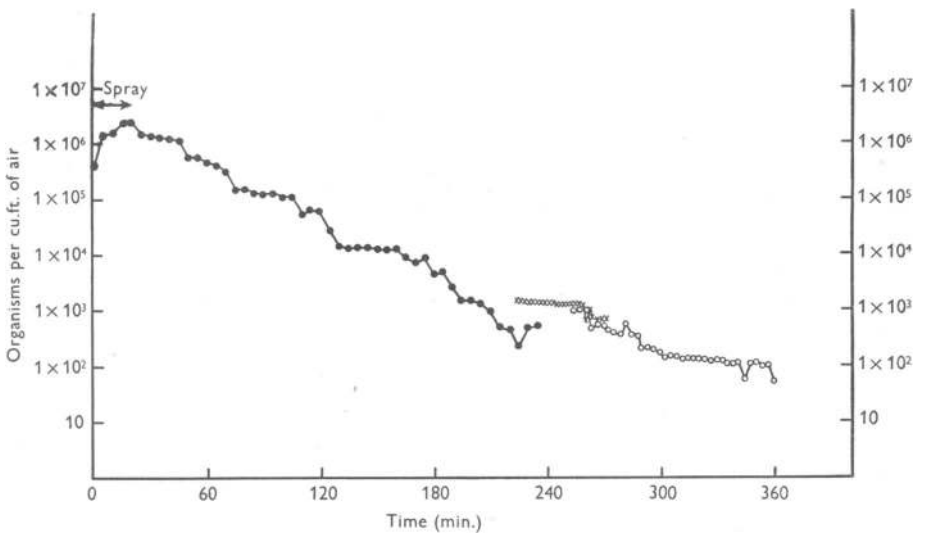
RESULTS

Many experiments were carried out to study the behaviour of bacterial clouds under the conditions of temperature, humidity and airflow provided in the test chamber. In most of the experiments the bacterial aerosol was of 1 μ dried particle size, i.e. single organism particles, nebulized from the Collison sprayer run for 15 min. The output in this time varied between 9 and 12 ml. when the organism used was *Chr. prodigiosum* in a suspension of viable count 5×10^{10} per ml. The time of 15 min. was initially chosen because preliminary information from the ventilation engineers suggested an airflow rate of four changes per hour and the intention was to saturate one total volume of air. The time proved to be convenient at the air changes actually used, and was adhered to for most experiments.

Sampling with impingers was begun synchronously with the start of the spray. They were used in pairs and a 1 min. air sample was taken every 5 min. The impingers were passed out of the chamber through an air-locked hatch and the fluid was plated out without any delay. The timing of sampling by the tray sampler and the Bourdillon slit sampler had to be chosen by trial and error until sufficient experience of cloud density in relation to time was gained. Text-fig. 1 shows the result of a typical experiment when 11·3 ml. of a suspension of *Chr. prodigiosum* of strength $4{\cdot}50 \times 10^{10}$ bacteria/ml. were sprayed. The peak concentration in the chamber which was attained in 15 min. was $4{\cdot}26 \times 10^{6}$ viable organisms/cu.ft. of air, as single organism particles. This corresponds to 13·8 % of

the total bacteria sprayed. Of the balance it is believed that a proportion are killed in the process of spraying, but most are deposited on the internal surfaces of the ducting and do not reach the chamber.

It is seen that on charting the total bacterial count on a logarithmic scale against time, the fall in the count is a steady one, almost in a straight line. This was the invariable experience with 1μ particles. In the experiment illustrated the count had dropped to around 100 organisms/cu.ft. at the end of 6 hr., at which time sampling ceased. Impingers gave reliable counts from zero time to 3 hr. 55 min. It can be seen that towards the end of this time the impinger curve shows a dip reflecting the fall off in efficiency of this method of assessment in its lower range. The tray sampler was started at 3 hr. 45 min. and set to run for 45 min. It was countable throughout, the highest count being 2280 colonies/cu.ft. (a 2 min. sample)



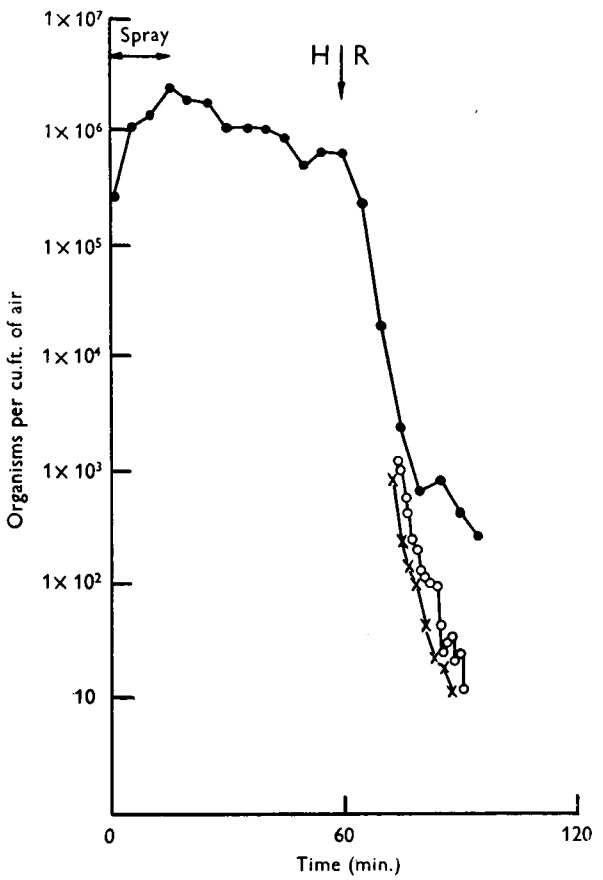
Text-fig. 1. The bacterial content of the air (1μ particles) on spraying *Chr. prodigiosum* into the air ducting for 15 min. Sampling for 6 hr. —•—•—, impinger count; xxxxx, tray sampler count; —o—o—, slit sampler count.

and the lowest 768 colonies. This tray is illustrated in Pl. 10. From 4 to 6 hr. the Bourdillon slit sampler gave countable plates, although the earlier plates were rather crowded and were difficult to count.

This experiment was repeated and recorded many times, and the results in every case were in close agreement. Differences which occurred were due to slight variations between experiments in the viable count of the spray fluid, and to uncontrollable factors such as voltage drop in the mains operating the compressor pumps, with resulting fall in the spray output.

In the first experiment with hexylresorcinol the procedure was as follows. The Aerovaps were fitted with close-fitting lids which effectively prevented the escape of any vapour. This allowed them to be left *in situ* while warming up and release of the vapour could then be arranged for a pre-set time, simply by removing the lids. 12·5 ml. of *Chr. prodigiosum* of strength $5{\cdot}04 \times 10^{10}$ per ml. were sprayed over 15 min.

Sampling with impingers at 5 min. intervals was done as before, the peak count in the chamber being $6{\cdot}06 \times 10^6$ organisms/cu.ft. The curve during the first hour was similar to that of the control experiments. At the end of 60 min. the lids were removed from the Aerovaps. In 20 min. the impinger count had dropped to $2{\cdot}76 \times 10^3$ organisms/cu.ft., and 37 min. after the release of the chemical aerosol the slit sampler gave a count of 30 organisms/cu.ft. The succeeding slit sampler

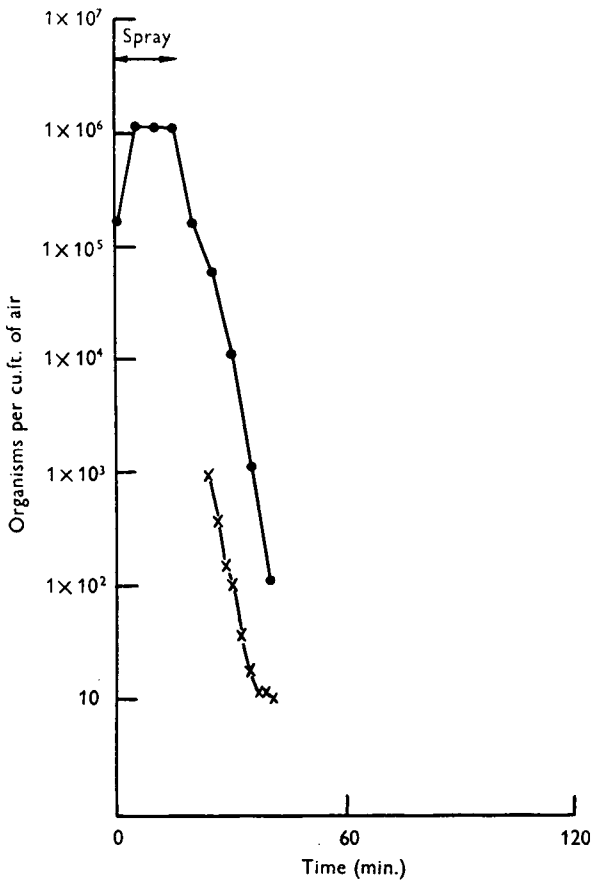


Text-fig. 2. Repeat of experiment recorded in Text-fig. 1. At point marked by arrow (zero + 60 min.) hexylresorcinol vapour released into atmosphere. —•—•—, impinger count; xxxxx, tray sampler count; —o—o—, slit sampler count.

plates showed occasional colonies only, but all the plates which were exposed during the third hour of the experiment were found after incubation to be sterile.

This experiment was repeated a week later, when in view of experience gained in the previous test better use could be made of the tray sampler. The results which were much as before are shown in Text-fig. 2. In 39 min. from the release of the hexylresorcinol aerosol the bacterial content of the air in the chamber dropped from nearly one million organisms per cu.ft. to ten organisms. Pl. 12 shows photographs of six slit sampler plates, each taken over $\frac{1}{2}$ min. and showing the equivalent count for $\frac{1}{2}$ cu.ft. of air. The first plate was exposed 2 min. after the release of hexylresorcinol and the remainder at 5 min. intervals after this.

The next series of experiments was arranged to assess the result when the bacterial aerosol is disseminated into an atmosphere with hexylresorcinol already present in effective concentration, i.e. at a level of between 6 and 7 μg./cu.ft. of air. The Aerovaps were switched on 1 hr. before the bacterial spray was due to be released, the lids having been discarded. In the first trial the spray fluid count was



Text-fig. 3. *Chr. prodigiosum* sprayed into atmosphere containing hexylresorcinol aerosol. —•—•—, impinger count; xxxxx, tray sampler count.

$5{\cdot}00 \times 10^{10}$ organisms/ml. and 11·2 ml. were sprayed in 15 min. Impinger sampling results showed the bacterial air content to be as follows (see Text-fig. 3):

		Organisms/cu.ft. of air
Spray started	Zero time (1 min. sample)	$3{\cdot}00 \times 10^{5}$
	Zero + 5 min.	$1{\cdot}50 \times 10^{6}$
	+ 10 min.	$1{\cdot}53 \times 10^{6}$
	+ 15 min.	$1{\cdot}50 \times 10^{6}$
Spray stopped	+ 20 min.	$2{\cdot}90 \times 10^{5}$
	+ 25 min.	$8{\cdot}32 \times 10^{4}$
	+ 30 min.	$1{\cdot}12 \times 10^{4}$
	+ 35 min.	$1{\cdot}69 \times 10^{3}$
	+ 40 min.	$1{\cdot}69 \times 10^{2}$

The peak concentration of *Chr. prodigiosum* in the atmosphere is thus only a quarter of that attained in the absence of hexylresorcinol. Likewise, after 30 min. from the time of stopping of the bacterial spray, the impingers failed to recover any viable organisms. The tray sampler count showed a comparable rate of fall with a count at 24 min. of 983 particles/cu.ft. of air, dropping at 40 min. to 10 particles. Inspection of Text-fig. 3 thus shows that for the first time in 1μ particle experiments we have an apparent marked discrepancy between impinger and slit sampler results. In samples taken simultaneously the impinger result is greater by one-thousand-fold than the slit sampler counts. There are two possible explanations for this phenomenon. One is that hexylresorcinol produces gross agglutination of the bacterial aerosol with the formation of particles containing an average of 1000 bacteria, which would be about 20μ in size, with subsequent breaking down in the impingers of the large particles, as indeed happens in sampling a 20μ particle size cloud. The other possible explanation is that the violent agitation and aeration in the buffering fluid in the impinger resuscitates large numbers of moribund bacteria. Further investigation of this question is needed.

The above experiment was repeated several times with similar results, and Pl. 11 illustrates in a typical experiment the tray, after incubation, from the Schuster sampler. It will be noted how the track of the slit rapidly fades out after the time that the Collison sprayer has stopped. The background of scattered colonies present on the agar even where it has not been traversed by the slit results from particles which have not adhered on primary impingement. This appearance is seen in all trays when, during the experiment, the vacuum box is flooded with a high bacterial concentration.

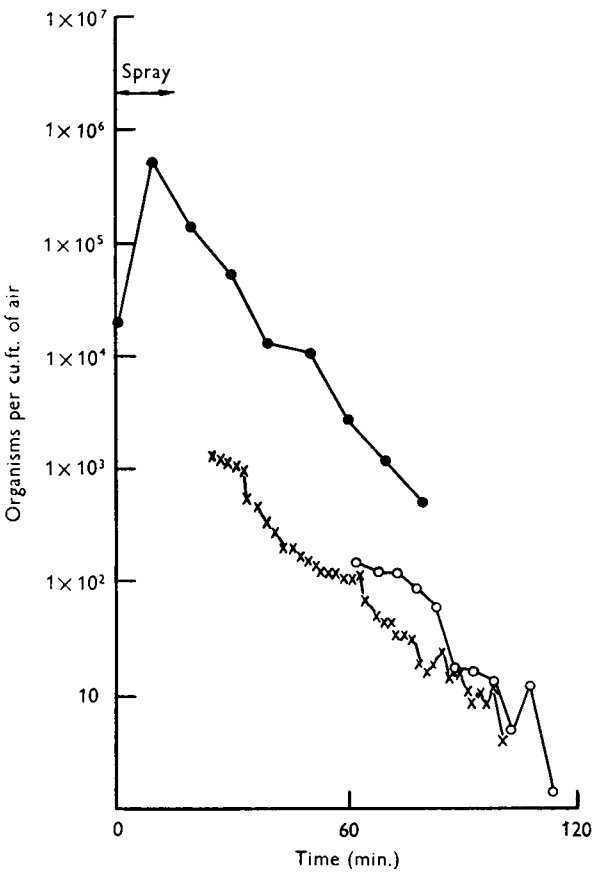
To investigate whether the dosage of bacterial cloud introduced into the chamber materially affected the time taken for the sterilizing action to result, another group of experiments was done. Making use of the tray sampler as the mode of assessment and using the same spray bottle, the Collison was run for periods of 15, 5 and 1 min. respectively, leaving enough time between runs for the test chamber to attain sterility. The times from stopping of the spray until the bacterial count dropped to below 10 organisms/cu.ft. of air were respectively 20, 24 and 19 min. That is, when the bacterial cloud intensity sprayed into the chamber with an effective dose of hexylresorcinol present is lowered to one-fifteenth, there is no apparent reduction in the time lag before sterility is reached. This is another point for further investigation.

EFFECT ON LARGE DROPLETS

In all the experiments done up to this point the bacterial aerosol was of 1μ dried particle size. It seemed necessary to determine whether the particle size of the bacterial cloud had any material effect on the disinfecting action of hexylresorcinol. The nebulizing apparatus used was the spinning top previously mentioned, operated centrally inside the test chamber. It was found that a uniform cloud of dried particle size between 6 and 7μ, i.e. each particle comprising about 100 bacteria, would remain detectable in the atmosphere of the chamber for 2 hr. For physical reasons the bacterial suspension used had to be diluted to a strength of

2×10^{10} organisms/ml., which meant that the same saturation of the air could not be attained as with 1 μ particles. The spray apparatus was run as before for 15 min. with an output of 1 ml./min., an indeterminate proportion of which was converted into effective droplet cloud.

The results of a typical control experiment are shown in Text-fig. 4. The impingers gave a peak count of $7{\cdot}15 \times 10^5$ organisms/cu.ft. of air, with a fall to $7{\cdot}05 \times 10^2$ in 1 hr. 20 min. The slit sampling devices in the countable range gave



Text-fig. 4. Control experiment, *Chr. prodigiosum* disseminated as 6–7 μ particle cloud (approx. 100 organisms/particle). —•—•—, impinger count; xxxxx, tray sampler count; —o—o—, slit sampler count.

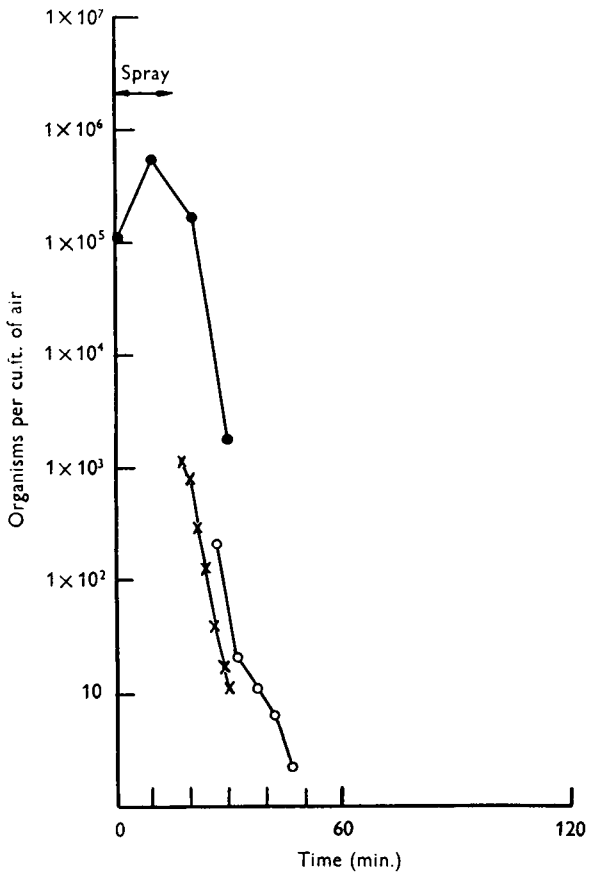
a count of 1612 particles/cu.ft. at 25 min., falling steadily to 10 particles in 1½ hr. A few particles were still recoverable up to 2 hr.

Text-fig. 5 shows the course of events when the same suspension was sprayed into the hexylresorcinol-containing atmosphere. The peak concentration was of a similar order, $7{\cdot}73 \times 10^5$ organisms/cu.ft., but the drop in the impinger count was more rapid, at 30 min. being $3{\cdot}47 \times 10^3$, the 40 min. sample recovering no viable bacteria. The fall in the total particle counts corresponded to this, the tray sampler count being 10 particles/cu.ft. at 30 min. Repetition of this experiment gave similar results.

An attempt to study the behaviour of a cloud of particles of 20μ size (approximately 1000 organisms/particle) failed since this cloud did not remain suspended in the air long enough to give a fair assessment of the effect of hexylresorcinol.

EFFECT ON *BACILLUS SUBTILIS* SPORES

It was known that hexylresorcinol had some sterilizing action on a broth culture of *B. subtilis* when sprayed into the air, and likewise that a titre of 1/40,000 in solid medium would inhibit growth of the organism. It seemed interesting to establish



Text-fig. 5. *Chr. prodigiosum*, 6–7μ particle cloud sprayed into atmosphere containing hexylresorcinol. —•—•—, impinger count; xxxxx, tray sampler count; —o—o—, slit sampler count.

what effect an aerosol of hexylresorcinol would have on a cloud of *B. subtilis* spores. A suspension of a strain of *B. subtilis*, whose spore behaviour had been extensively studied in this laboratory, was prepared and heated for 1 hr. at 60° C. to kill vegetative forms. Of this suspension 17·3 ml. containing $5{\cdot}74 \times 10^9$ spores/ml. were sprayed into the chamber over 15 min., giving a cloud of 1μ particles. The peak concentration attained was $1{\cdot}31 \times 10^6$ spores/cu.ft. of air. This dropped steadily, again almost by straight line on a logarithmic scale until, at the end of 6 hr. when sampling was stopped, the count was $4{\cdot}85 \times 10^3$ spores/cu.ft.

After a lapse of a week, by which time the air of the chamber had cleared itself of *B. subtilis* spores, the experiment was repeated with hexylresorcinol aerosol in the atmosphere. It was presumed that any effect would be observed within 3 hr. Impinger sampling was carried out at 10 min. intervals for this time and the tray sampler was run for 94 min. The results of this experiment were almost identical with those of the control one. That is, in a period of 3 hr., hexylresorcinol in a concentration of 6–7 μg./cu.ft. of air produced no appreciable effect on a *B. subtilis* spore suspension of 1 μ particle size.

DISCUSSION

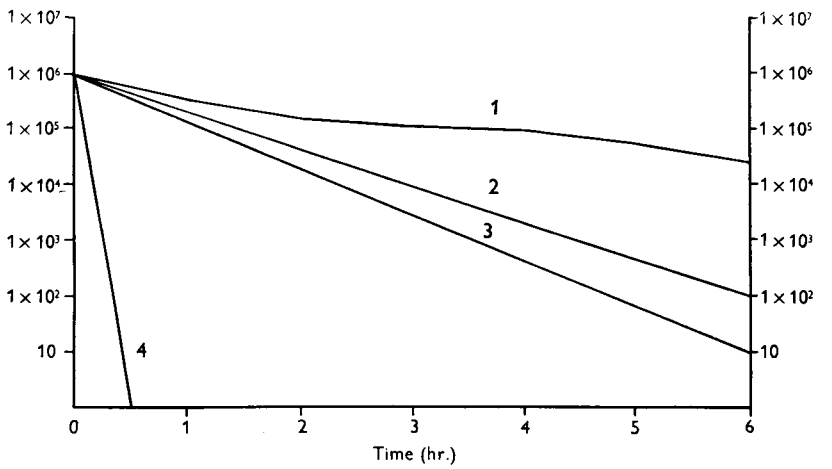
Certain interesting points have come to light in the course of these studies. One is the remarkable speed of diffusion of 1 μ particle clouds. Trillat made the observation that such a cloud of *Chr. prodigiosum* in still air travels at the same speed as the odour of musk. In all our 1 μ cloud experiments the first 1 min. air sample was taken beginning simultaneously with the start of the spray in the air duct outside the chamber. In every case, in that first minute, the bacterial concentration at the sampling points inside the chamber was not much below the peak concentration reached after 15 min. spraying. Trillat ascribed this rapidity of diffusion to the mutual repulsion of particles carrying similar electrical charge. This would explain the observation, which we were able to confirm, that a 1 μ particle cloud is capable of travelling upwind against a gentle breeze.

The speed of diffusion of the 6–7 μ particle cloud is less. This was obvious on comparing the appearance of the tray sampler plates when the sampler was running at the time of starting of the spray. With 1 μ particles the start is indicated by an abrupt solid streak of culture, but in the case of the larger particles the build up was more gradual.

The persistence in the air of the 1 μ particle cloud was likewise greater than might be expected of what is generally regarded as a fairly labile vegetative organism. The die-away rate for this organism is compared in Text-fig. 6 with that of *B. subtilis* for the conditions prevailing in our test chamber. The upper line shows the theoretical die-away rate for a cloud of inanimate particles provided the only operative factor is dilution from admitted fresh air (0·5 change/hr.). It may be assumed that the *B. subtilis* spores suffered little or no mortality while suspended in the air. The increase in the observed die-away rate is due in part to leakage from the lengths of air duct outside the chamber, and to the centrifugal effect of the recirculating fan, producing agglutination and throw-out of the particles. The difference in the slope of the *Chr. prodigiosum* curve reflects the mortality rate of the vegetative organism. Also for comparison is shown the die-away rate of *Chr. prodigiosum* when sprayed into the hexylresorcinol-containing atmosphere, this rate corresponding to about twenty-eight air changes per hour.

It is not proposed to discuss the mode of action of hexylresorcinol, since work on this still goes on. But it must be made clear that this is essentially an aerial reaction. As has been mentioned, incorporated in solid medium the inhibitory dose for *Chr. prodigiosum* is 1/4000 compared with the aerial lethal dose of 1/5,000,000,000. At the latter concentration there is no sterilizing action on

surfaces. This is obvious from inspection of culture plates exposed during experiments since bacterial particles, once impinged on culture medium, appear to be protected from the action of the chemical. In preliminary tests it was demonstrated that exposure in the slit sampler in the hexylresorcinol atmosphere for 6 min. instead of the ½ min. exposure given in actual experiments, did not in any way affect the behaviour of the culture medium when seeded with the test organism. It was also found that after the air of the chamber had attained sterility, *Chr. prodigiosum* could still be recovered by swabbing floors, walls and bench surfaces. This is probably only true of a chamber exposed to small intermittent dosage with hexylresorcinol. Where vaporization is continuous for any length of time there is condensation on exposed surfaces which then act as a reservoir for further re-vaporization. Such surfaces are self-sterilizing for some time.



Text-fig. 6. Die-away rates in chamber with total airflow of three changes per hour of which 0·5 change is fresh air: 1, theoretical curve from air dilution only of inanimate particles; 2, observed die-away of a 1μ cloud of *B. subtilis* spores; 3, die-away of 1μ cloud of *Chr. prodigiosum*; 4, die-away of 1μ cloud of *Chr. prodigiosum* in an atmosphere containing 6–7μg./cu.ft. of hexylresorcinol.

The range of bacteria against which hexylresorcinol has been demonstrated to be effective is considerable. It is true that most of the test organisms have been non-pathogenic, but a strong case has been made for testing pathogenic organisms as well. It seems likely that hexylresorcinol will prove to be equally effective in clearing the air of them. That bacterial spores are resistant is not surprising, especially spores of a strain of *B. subtilis* which withstand boiling for many minutes.

What is important is to demonstrate whether or not hexylresorcinol in dosages effective in the laboratory is equally effective against naturally occurring airborne organisms. What little evidence we have is promising. In laboratories in this department, where work was being done with hexylresorcinol vapour, routine air samples taken with the Bourdillon slit sampler showed the air to be free of vegetative organisms. Cruickshank & Muir (1940), using a chemical aerosol the chief constituent of which was resorcinol, observed a sharp reduction in the streptococcal content of the air, and in the nose and throat carrier rates among the patients

in a small ward in which a post-influenzal airborne outbreak of streptococcal pharyngitis had occurred. Resorcinol has similar properties to hexylresorcinol, but ten times the dosage is required. J. McGrath (personal communication) states that in the Dublin Zoo very favourable results have been obtained in the control of respiratory infections in monkeys following the installation in the monkey house of two Aerovaps charged with resorcinol.

The assessment of the value of this method of air hygiene for the control of aerially-spread infections in man must await the results of large-scale field trials. Some of these are already under way and others are contemplated. Of the practicability of the method in buildings where there is any control of the airflow, there is no doubt. Apparatus for the generation of aerosols by heat requires a minimum of attention. In the Aerovaps used in our experiments one charging of hexylresorcinol (300 g.) was enough to maintain effective concentration in the chamber, running continuously, for a period of 3 months. The vapour is odourless and its presence can be detected only by the more sensitive observers by a slight feeling of dryness at the back of the throat. There is nothing to indicate that even prolonged inhalation of the vapour will be toxic to man. G. McCoull (personal communication) states that he has had some forty patients inhaling the vapour continuously, day and night, for over 6 months without observing any ill effects.

Even should chemical air hygiene prove ineffective in the control of common ailments there may be a place for it in some specialized branches of medical practice. Places that immediately come to mind are operating theatres, the wards of infectious diseases hospitals and sanatoria, bacteriological laboratories and practitioners' waiting rooms during epidemic times.

SUMMARY

Experiments were carried out in a test chamber of 16,500 cu.ft. capacity with air conditioned at 68° F. and 54 % relative humidity. Ventilation was controlled at 3·0 changes/hr., air being recirculated with 0·5 air change/hr. of fresh air admitted. By spraying into the air ducting a concentrated suspension of *Chr. prodigiosum* a bacterial cloud was introduced into the chamber of over 5×10^6 organisms/cu.ft. of air as 1 μ particles. Viable organisms were still present in the atmosphere after 6 hr.

When a heat-generated vapour of hexylresorcinol was introduced in a concentration of between 6 and 7 μg./cu.ft. of air the bacterial count dropped from nearly one million organisms per cu.ft. to fewer than ten in 35 min.

When the bacterial suspension was sprayed into the chamber with hexylresorcinol vapour present, the peak concentration reached was about one-quarter and on stopping the spray, the bacterial count fell from one million per cu.ft. to fewer than ten in less than 25 min.

Hexylresorcinol is also effective when the bacterial aerosol is in particles of 6–7 μ in size (approx. 100 bacteria per particle).

Hexylresorcinol has no effect against a bacterial aerosol of *B. subtilis* spores in 1 μ particles.

No attempt has been made to analyse the mode of action of hexylresorcinol in

diminishing the bacterial content of the air. The evidence collected clearly indicates the necessity for further study of this problem.

The following took part in these experiments and are to be commended not only for the technical skill shown but for the physical endurance which was at times required: Experimental Officers W. R. Bale, W. A. Gillett, E. J. Morris, Ministry of Supply, and F./Sgt. R. Taylor, R.A.F. Air estimates of hexylresorcinol were made by A. H. Baker and G. F. H. Whitney of Avebury Research Laboratories. Nebulizing apparatus was assembled by H. A. Druett and K. R. May, who also advised on the physical aspects of bacterial cloud formation and behaviour. We are indebted to Shepherd's Aerosols Limited for the generous loan of equipment. Thanks are also due to Dr D. W. Henderson for advice and active co-operation. The paper is published with the permission of the Chief Scientist, Ministry of Supply, and the Director General, R.A.F. Medical Services.

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EXPLANATION OF PLATES 10–12

PLATE 10

Photograph of tray from Schuster sampler after 18 hr. incubation showing colonies of *Chr. prodigiosum*. Sampler started at zero + 3 hr. 45 min. and run for 45 min. Each traverse 3¼ min. sampling at ½ cu.ft./min. The count is recorded on Text-fig. 1 as line XXXX.

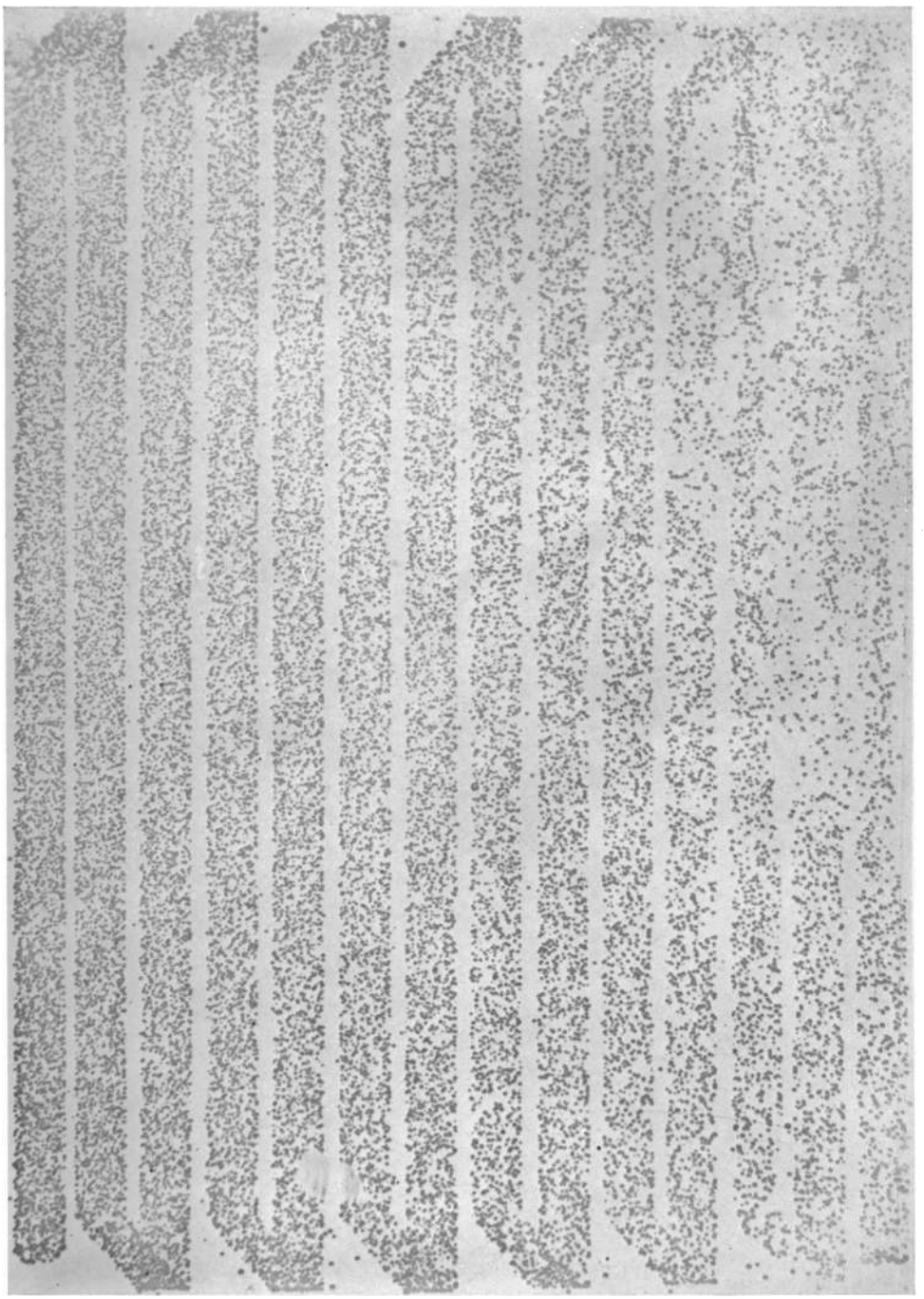
PLATE 11

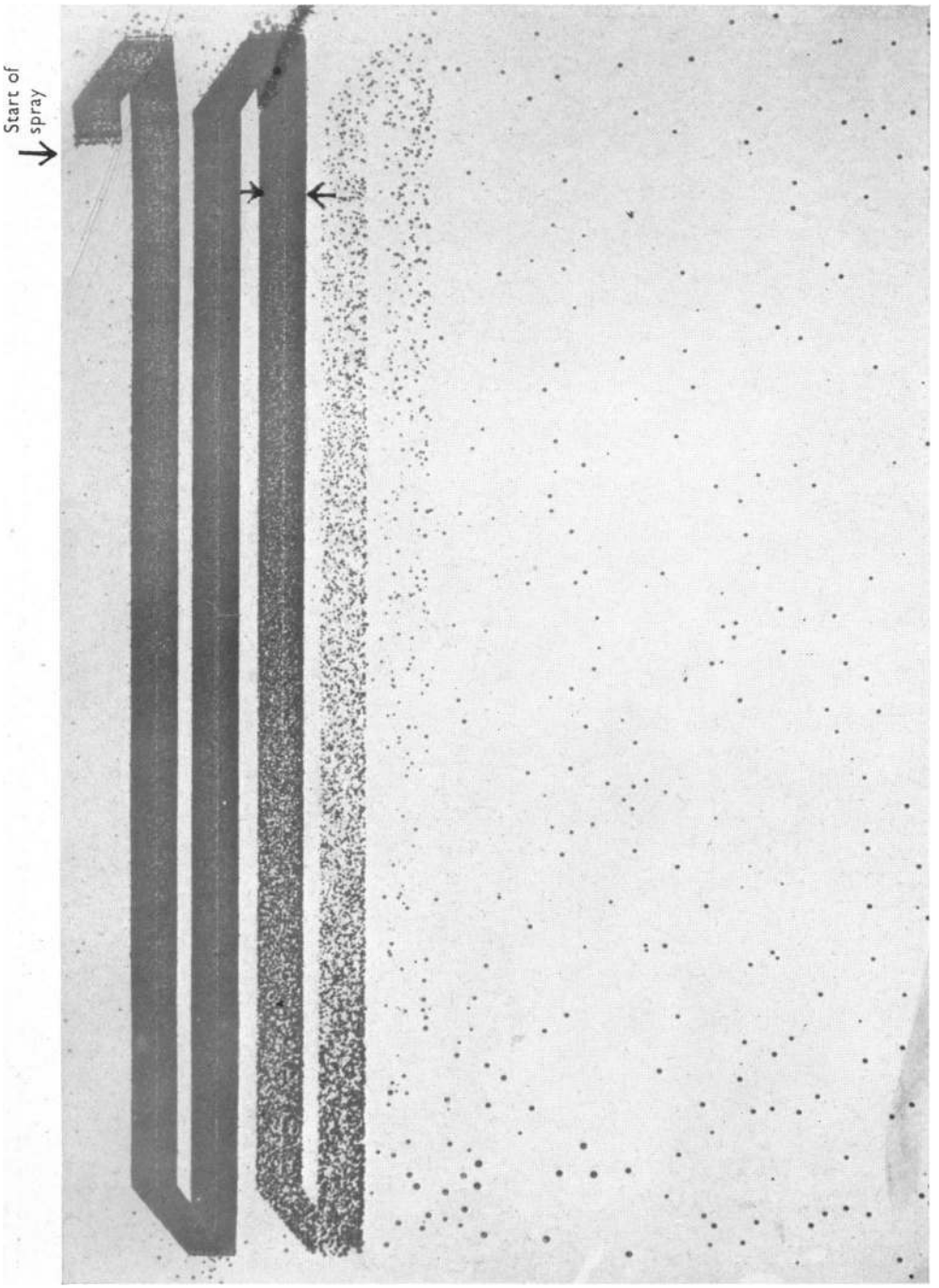
Tray from sampler after incubation. *Chr. prodigiosum* sprayed as 1 μ particles for 15 min. into atmosphere containing hexylresorcinol. Tray started simultaneously with spray. Each traverse 6½ min. Arrows indicate start and stopping of spray.

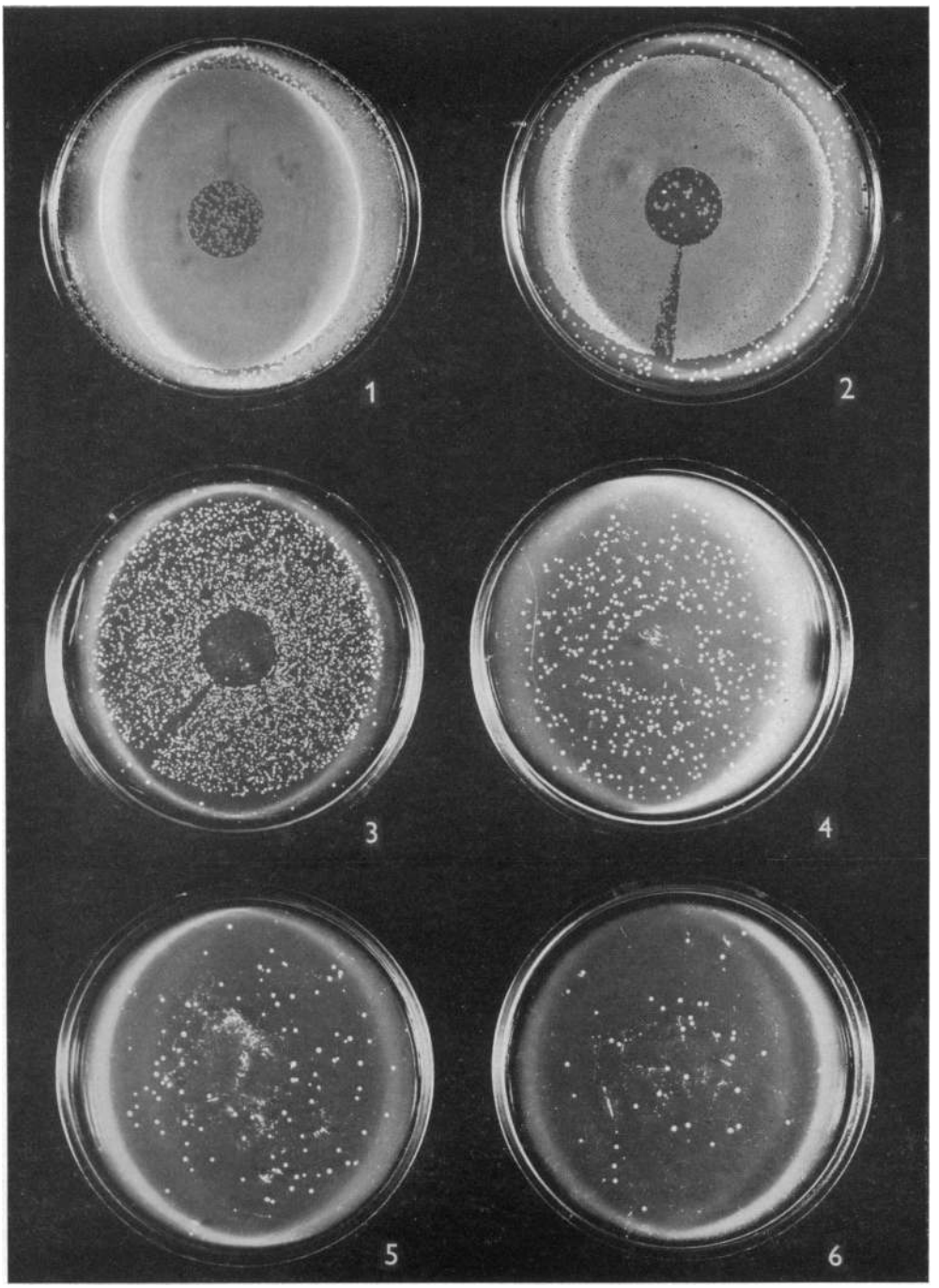
PLATE 12

Petri plates exposed in Bourdillon slit sampler for ½ min. (½ cu.ft. of air). No. 1 exposed for 2 min. after the release of hexylresorcinol vapour into the atmosphere. The others at 5 min. intervals subsequently.

(*MS. received for publication* 21. VIII. 51.)




Start of spray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