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G.O., No. 1527 P Mis ✓

DATED 6-12-98

[Despatch Abstract.]

Dr. Haffkine's report on disinfectants.

Recording letter from the Govt. of Bombay forwarding ten additional Copies of ——.

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Local & Munl. (1898) Department.

CURRENT No. 2564AP

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INDIA OFFICE
28 NOV 98
POLITICAL DEPARTMENT

No. 6569/P. of 1898.

GENERAL DEPARTMENT (PLAGUE).

Bombay Castle, 26th November 1898.

FROM

THE HONOURABLE MR. A. WINGATE, C.I.E., I.C.S.,
Acting Chief Secretary to Government.

To

The Secretary to the Government of Madras, Local and Municipal Department.

SIR,

With reference to your letter No. 14764-P. this, dated the 23rd November 1898, I am directed to forward herewith 10 additional copies of Doctor Haffkine's Report on disinfectants.

I have the honour to be,

Sir,

Your most obedient Servant.

A Wingate

Acting Chief Secretary to Government.

for

BACK PAPERS.

G.O.	No.	Department.	Date.

FURTHER PAPERS.

G.O.	No.	Department.	Date.

Report of experiments carried out to ascertain the efficacy or otherwise of Sulphur Dioxide as prepared in a liquid form by Messrs. A. Boake, Roberts & Co., Stratford, London, as a disinfectant for rooms in which infectious disease has occurred.

The sulphur dioxide gas is compressed into a liquid and sent out in small tin cylinders which on being opened give off dense fumes of gas which quickly fills the apartment.

In the paper of instructions it is ordered that the room be closed for 8 hours, so that the gas may have time to act on the objects to be disinfected; so the method is only applicable to those rooms which it is possible to render airtight by pasting up cracks in doors and windows. It is quite evident, therefore, that the method of gaseous fumigation is not applicable to the majority of Indian houses, for owing to the construction of houses and doors, &c., it is impossible to close them in such a manner that the required percentage of the gaseous disinfectant can be maintained for the necessary length of time. The prevailing practice of building houses and huts with tiled roofs, without providing any ceiling for instance, at once precludes this method. It is quite necessary to keep this in mind when recommending any method of disinfection by means of gas, and it would be advisable to make this known to manufacturers in England, so that they may turn their attention in other directions, which may prove more universally applicable.

Two series of experiments were carried out, but as they practically point to the same conclusions, only the second series will be detailed here.

Two similar rooms in the second storey of a chawl in Gilder Street were chosen for the experiment. The doors and windows fitted fairly well and were rendered airtight by pasting up the joins and cracks with strips of paper in the usual way, the floors were of rammed earth resting on boarding, the roof was of wood with rammed earth above, and the walls were of brick and mortar plastered and whitewashed. The room measured 1,684·6 cubic feet. For testing the efficacy of the process, cultures of plague microbes were chosen, and of these three test-tubes were hung in each room, as also a cloth soaked in a broth culture and hung on a cord stretched across from wall to wall. The cloth had been carefully sterilised and kept in sterilised coverings till hung up by means of sterilised strings to the supporting cord. The tubes were hung in the vertical position with the mouth downwards, so that no floating microbes might find their way in, and were placed at the level of the roof, the floor and midway between these positions.

The tubes being opened, the cloth in position, and all openings save one closed, the cylinder was opened at 8-30 A.M. and the room at once began to be filled with dense sulphur fumes. The door was closed and pasted up and the room left closed till 5-30 P.M., that is for 9 hours. It will, therefore, be manifest that the full time specified was given, and as each cylinder is certified to be sufficient for 1,728 cubic feet, it is certain that all the necessary conditions were complied with, as laid down by the makers.

On opening the room in the evening, a distinct smell of sulphur was perceptible. The tubes were at once plugged. The cloth being held up by the corners had then some sterile broth poured into the hollow thus formed, a platinum needle being used to ensure the thorough application of the broth to the cloth. The needle was then used to inoculate two agar tubes. An agar tube, similar to those used above, was placed in the second room along with a cloth and similar procedures were adopted, the only difference being that no sulphur was evaporated in this room, so that it acted as a control. The agar cultivations used had been prepared only 40 hours previous to the experiment, so that very little growth was to be seen, and if the sulphur dioxide had done its work, there should have been no subsequent development; the following table will, however, make it plain that the disinfectant had not killed the bacillus:—

	Floor.	Midway.	Roof.
Agar tubes	?	+	+
Microscopic appearance... ...	?	+	+
Broth culture (stalactites) ...	+	+	?

In the above table the "+" sign means that the reaction characteristic of the plague microbe was obtained, the "?" means that the appearance was faint or doubtful.

The tube hung up in the open room gave the characteristic reactions when examined in the same manner as the above.

The tubes inoculated from the cloths, both, in the fumigated and in the control rooms, remained sterile for the most part, or at most in a few instances showed the presence of foreign organisms, and no plague colonies.

As it is not believed that the mere hanging up of a cloth infected with plague microbes for a few hours in the air would serve to render it sterile, there must in this case have been something wrong with the technique of the method; but as the tubes gave no uncertain sound, it was not thought necessary to repeat the experiment, as time was limited.

Report of experiments carried out to ascertain the efficacy or otherwise of the vapor of Carbolic Acid as a disinfectant, as generated by the patent apparatus of Messrs. Scott.

The experiment was carried out in a similar room and at the same time as the sulphur dioxide series.

The apparatus consists of an iron bottle which is heated red-hot, placed in the centre of the room and filled with pure carbolic acid. At once dense irritating fumes arise, which appear to be deadly to insects, as on one occasion several dead cockroaches were found on the floor when the room was opened.

The following table gives the result:—

	Floor.	Midway.	Roof.
Agar tubes	?	+	+
Microscopic appearance	+	+	+
Broth cultures (stalactites) ...	+	+	+

It will, therefore, be seen that here also the method of disinfection employed did not kill the plague microbe, and cannot in consequence be recommended for employment during an epidemic.

W. B. BANNERMAN, M.D.,
Surgeon-Major, I. M. S.

Bombay, 1st September 1897.

Note summarising certain experiments with Sulphur Dioxide on the Bacillus of Bubonic Plague.

In continuation of the experiments* on the efficacy or otherwise of sulphur dioxide as a disinfectant in plague-infected houses, cultures of the bacillus of plague, which had been isolated from the body of a native dead of plague in Bombay, were submitted to the action of the gas. The bacillus cultures used in the experiments were examined microscopically by means of subcultures and found to yield typical pure growths on various suitable media.

The experiments to which this statement relates were commenced on March 9th, under the ordinary conditions of house disinfection, in a room next to one in which a plague patient had died. The capacity of this room was 1,152 cubic feet, the measurements being, 18 feet × 8 feet × 8 feet, for length, height and breadth, respectively. The construction of the room allowed of it being hermetically sealed to light and air during the time of disinfection. A sample of the

* Report on experiments, *vide supra*, carried out to ascertain the efficacy or otherwise of sulphur dioxide as prepared in a liquid form by Messrs. A. Boake, Roberts & Co., Stratford, London, as a disinfectant for rooms in which infectious disease has occurred. By Surgeon-Major W. B. Bannerman, M.D. September 1897.

contained air gave a neutral reaction on testing. The moisture in the atmosphere was slightly increased by the sprinkling of water on the floor—a procedure that would materially increase the potency of a disinfectant such as sulphur dioxide.

On March 18th, at 11 A.M., a cylinder containing sulphur dioxide under pressure was opened in the above room when the temperature of the room was 84°F. The following details relate to the effect of the gas on cultures of the plague microbe exposed to its action during twenty-four hours.

It is worthy of note that the atmosphere of the room when opened at the end of this interval gave a faint smell of sulphurous acid: also that strips of blue litmus paper moistened in water and scattered about the room at the commencement of the treatment were completely reddened.

On admitting fresh air and light into the room, large numbers of live bugs were seen in motion on the walls. Several small lizards were found dead on the floor.

Experiments made with reference to the action of sulphur dioxide on growths of the plague bacillus.

Series a.—Experiment on the action of sulphur dioxide on broth cultures of the plague bacillus exposed in capillary glass tubes of from 5—10 centimetres in length and from 1—2 mm. in diameter. The ends of these tubes were opened in the experimental room and

9 of them (from 2—3½ inches long) exposed on the floor level.

3 „ (from 2—4 inches long) exposed in sconces at midheight.

5 „ (from 2—4 inches long) exposed under the ceiling.

Subsequent examination by subculture showed that in none of the tubes had the bacilli been killed by the disinfectant.

Series b.—Experiment on the action of sulphur-dioxide on cultures of the plague bacillus growing on agar in test tubes.

Cultures in test tubes with the plugs removed were exposed in duplicate at the floor level and under the ceiling during the 24 hours of the experiment. As in Series *a*, subsequent examination by subcultures showed that the microbes had not been killed by the disinfectant.

Series c.—Experiment on the action of sulphur dioxide on growths of the plague bacillus on agar plates.

Cultivations of the plague bacillus on agar plates were freely exposed—two at the floor level (one face downwards, the other face upwards), two in sconces between the floor and ceiling (one face downwards, the other face upwards), and two immediately under the ceiling (both face upwards). When removed at the end of 24 hours' exposure, the agar culture media had become opalescent in appearance. Attempts to make subcultures from the colonies on the plates gave negative results, showing the bacilli were dead. Control experiments on plates not exposed to the action of the disinfectant yielded healthy bacilli on subculture.

Series d.—Experiment on the action of sulphur dioxide on living cultures of the plague bacillus in broth approaching desiccation.

As in Series *c*, the specimens were in duplicate and exposed (face upwards) at the three levels—floor, sconce at midheight, and ceiling.

Attempts to obtain subcultures at the end of 24 hours' exposure gave negative results, but a control experiment on specimens kept under favourable conditions yielded active growths.

The experiments in Series *c* and *d* demonstrate the efficacy of the disinfectant when cultures of the plague bacillus are placed under conditions allowing of *free* exposure to the action of the gas.

An important factor in aiding the germicidal action of sulphur dioxide is the presence of moisture, and the moisture that exists in association with bacilli living on artificial culture media must have materially assisted the results obtained in the experiments *c* and *d*.

In order to test further the potency or otherwise of sulphur dioxide as a disinfectant under circumstances where the bacillus of plague is not directly exposed to its action, and where some power of penetration is necessary, a number of experiments are being carried out under similar conditions of cubic space, temperature and moisture of the atmosphere as the above. In these experiments, cultures of the plague bacillus are protected by various thicknesses of sterile earth, sterile cotton-wool and rags. The results are still under investigation.

Laboratory, Nesbit Lane,
Bombay, March 31st, 1898.

ERNEST L. MARSH, M.B., D.P.H. (Oxon.)

Report on the efficiency of vaporised Carbolic Acid as a disinfectant with special reference to the Bacillus of Bubonic Plague.

The present report has to do with experiments made with an apparatus for vaporising large quantities of carbolic acid, and is a record of certain tests applied to carbolic acid vaporised by this method to prove its efficiency or otherwise as a house disinfectant in case of plague. The apparatus used in the experiments is known as "Scott's Patent Disinfector," and consists of (1) a stout cast-iron vessel—the 'disinfector' proper—capable of holding about half-a-pint of liquid and (2) an iron stand on which the above apparatus rests when in use. The 'disinfector' is shaped somewhat like a short stout bottle the mouth of which is large and expanded funnel-wise, and the interior of which presents incomplete partitions of iron running from the walls towards the centre. To facilitate the carrying of the 'disinfector,' an iron rod is supplied which screws into the shoulder of the vessel. In the table of instructions issued with the apparatus, the directions for disinfecting a room are as follows :—

(1) That all windows, chimneys and apertures in the room be sealed up preparatory to disinfection.

(2) That the stand be placed on any tray, tile or brick on the floor of the room.

(3) That the 'disinfector' be heated in a fire until it is red-hot.

(4) That the 'disinfector' be placed on the stand in the centre of the room and a little pure clear carbolic acid from any strooped vessel poured at intervals into the vessel until about half-a-pint of carbolic acid has been vaporised and the room is filled with dense fumes of carbolic vapour.

(5) That the room under treatment be kept closed for at least twelve hours.

The above instructions were carefully observed in the following experiments which were carried out on May 14th, 1898, in the same room (measuring 18 × 8′ × 8′, or 1,152 cubic feet capacity) as that used in the experiments upon compressed sulphur dioxide. The more important of the observations have to do with the action of the disinfecting fumes on cultures of the bacillus of bubonic plague.

The patent 'disinfector' having been heated to a dull red heat was placed upon its iron stand in the centre of the experimental room. Half-a-pint of pure liquid carbolic acid was then gradually poured into the hot vessel. It was only possible to pour in small quantities at one time. Immediately the liquid touched the hot iron it boiled and fumed with such great violence that the face and hands of the operator were splashed with the disinfectant. This rendered the process of volatilising the carbolic acid lengthy and somewhat risky. When the last of the carbolic acid had been poured into the apparatus, the further requirements of the test were completed by the closure and sealing of the door of the room.

The tests arranged in the room were allowed to remain under the influence of the carbolic fumes for twenty-four hours.

The temperature of the room when first sealed up was 86° F. and was the same when opened : in the interval there had been a rise and fall of 3°F.

A faint smell of carbolic acid remained in the room at the conclusion of the experiment. The floor and walls of the apartment presented numerous dead mosquitoes, bugs and cockroaches. Two small lizards were also found dead. The only living insect discovered in the room was found in a hole in the wall the entrance to which had been covered with paper.

The first experiments to be recorded demonstrate the powers of penetration and diffusion possessed by carbolic fumes without any reference to their germicidal action. With sulphur dioxide, blue litmus was used to indicate its presence in an active form : with carbolic fumes, advantage has been taken of the precipitation of tribromphenol, which occurs in bromine water when the vapor of carbolic acid acts upon it. This is so delicate a test that even the faintest trace of carbolic vapor can be detected by it.

Experiments to show the powers of diffusion and penetration possessed by volatilised carbolic acid.

Experiment 1.—Six shallow watch glasses containing bromine water were distributed in different parts of the room—

a. Two on the floor.
b. Two in sconces in the wall.
c. Two on a shelf close to ceiling.

} *All showed complete precipitation of tribromphenol.*

Experiment 2.—Four test-tubes (15 cm.×1·5 cm.) containing bromine water to a height of 4 cm. were suspended with open mouths at different levels in the room—

a. One at ceiling level.—*Showed a thin film of tribromphenol on the surface.*

b. One at midheight.—*Showed a film as in a.*

c. Two at floor level.—*Showed films of less depth than the above.*

Experiment 3.—Three test-tubes (15 cm.×2·5 cm.) containing bromine water and having their mouths securely closed by dry Swedish filter paper were stood upright at floor level—

a. With mouth closed by one layer of paper.—*Slight film of tribromphenol.*

b. With mouth closed by two layers of paper.— *Do. do.*

c. With mouth closed by three layers of paper.—*Very slight film of tribromphenol.*

An experiment to discover whether a moistened medium, such as wet filter paper, will transmit carbolic fumes with greater readiness than dry filter paper had been undertaken in the laboratory beforehand, under the following conditions :—Two small beakers of equal dimensions and containing bromine water to the same height were closed by single sheets of Swedish filter paper tied over their mouths. The paper covering one was moistened. The two beakers were then placed under a bell-jar together with a hot iron vessel containing carbolic acid. The bromine water in the beaker closed by dry filter paper was rapidly precipitated, that in the beaker closed by moist paper much less rapidly.

Experiment 4.—Three test-tubes (15 cm.×1·75 cm.) containing bromine water and having dry loin cloth material stretched across their orifices were stood upright at floor level—

(*a*) With mouth closed by one layer of loin cloth.
(*b*) ,, ,, ,, two layers.
(*c*) ,, ,, ,, three layers.

} *Slight film of tribromphenol on the surface of each : that in c being a little less marked than in a.*

Experiment 5.—Two test-tubes (14 cm. × 2 cm.) containing bromine water and having their mouths plugged with dry absorbent cotton-wool—

(*a*) Closed by plug 1·5 cm. thick ... *Slight film of tribromphenol.*
(*b*) „ 2·5 „ ... *Very slight film of tribromphenol.*

Experiment 6.—A wide-mouthed ointment jar containing bromine water and having its mouth closed by one layer of dry loin cloth material stretched over it was placed upon the floor.—*Well marked precipitate of tribromphenol.*

Experiment 7.—A large beaker (two-litre capacity) containing a small quantity of bromine water and having its mouth filled with dry hay—the hay extending down the beaker to a depth of 4½ inches—was placed upon the floor.—*Slight film of tribromphenol upon the surface.*

It will be observed that in every one of the foregoing experiments the vapor of carbolic acid rendered its presence evident. The vapor proved itself capable of penetrating three thicknesses of unglazed paper, three thicknesses of loin-cloth material, or one inch of fairly compact cotton-wool. In each case, however, in which some obstacle had been placed in the way of its free access to the bromine water—such as requiring it to traverse the narrow lumen of a test-tube or pass through cotton material—its effects were comparatively feeble. In those instances, such as the unprotected watch glasses, where no serious impediment existed, its action was well marked and vigorous. It is to be very much doubted whether in those instances where the vapor showed its presence by only a film upon the surface of the bromine water, a sufficient quantity of carbolic acid to have any appreciable germicidal action could have penetrated.

The following experiment illustrates the amount of the germicidal effect of the fumes *upon varying quantities of liquids*:—

Experiment 8.—Five small glass beakers containing different depths of pure broth culture of the plague bacillus were exposed upon the floor and carefully screened from dust. The summary of the experiment is as follows:—

Broth culture ·3	cm. deep	...	*Vitality of plague bacillus destroyed.*
„ ·625	cm. „	...	*Vitality of plague bacillus not destroyed.*
„ 1·25	cm. „	...	„
„ 2·5	cm. „	...	„
„ 5·	cm. „	...	„ „
			„ „

Thus it will be noted that plague bacilli were recovered in a living active state from all the beakers except the shallowest. The vapor had not been sufficiently active to destroy the germs of plague in any but the shallowest layer (⅛th inch) of fluid. Moreover, the inefficiency of the fumes as an aërial disinfectant was shown by the fact that putrefactive germs had gained access to the broth in one or two of the beakers during the time of their exposure. It may also be recorded that a Petri plate agar growth of the hay bacillus freely exposed in the room retained its vitality unaffected by the vapor: similarly, a fragment of the hay plug in the beaker mentioned in experiment 7 yielded a growth of hay bacillus when inoculated in broth.

The remaining experiments have as their object a demonstration of the germicidal power of the fumes *upon agar cultures of the plague bacillus, these cultures being exposed freely as well as protected in various ways.*

Experiment 9.—Three agar plate cultivations of the plague bacillus were freely exposed at different levels to the carbolised atmosphere of the room—

a. At floor level ... ... *Sterilised by the fumes.*
b. In sconce at midheight ... ... *Do. do.*
c. At ceiling level ... ... *Not sterilised.*

Experiment 10.—Six unplugged test-tube cultures of plague bacilli growing on agar (dimensions of test-tubes 15 cm. × 1·5 cm.) suspended in different situations—

a. At floor level with mouth up ... *Not sterilised.*
b. „ „ down ... *Do.*
c. At midheight with mouth up ... *Do.*
d. „ „ down ... *Do.*
e. At ceiling level with mouth up ... *Do.*
f. „ „ down ... *Do.*

Experiment 11.—An agar plate cultivation of plague bacilli covered with a thin layer of sterile absorbent cotton-wool and laid upon the floor.—*Sterilised.*

Experiment 12.—A similar cultivation of plague bacilli covered by a single layer of sterile loin-cloth material and hung face downwards one foot above the floor.—*Sterilised.*

Experiment 13.—A similar cultivation of plague bacilli covered with dry sterile earth to a depth of ⅙th of an inch and placed upon the floor.—*Not sterilised.*

Experiment 14.—A similar cultivation of plague bacilli upon the surface of which a sterile leaf had been allowed to fall, placed upon the floor.—*Part not covered by leaf sterilised, that protected by leaf not sterilised.*

Experiment 15.—A similar cultivation of plague bacilli upon the surface of which a sterilised feather had been allowed to fall, placed upon the floor.—*Protected as well as unprotected portion of growth sterilised.*

From these experiments it will be seen that the fumes do not enter an ordinary unplugged test-tube in sufficient quantity to destroy the vitality of plague bacilli lying at the bottom, whereas a plate cultivation of the bacillus is, as a rule, sterilised. It is also evident that, when a plate cultivation is covered by cotton-wool or cotton material, the fumes can successfully penetrate this impediment and sterilise the growth, whereas a protection of ⅛th of an inch of dry earth saves the bacillus from destruction.

Conclusions:—

1. That the fumes of carbolic acid evolved by the method and under the circumstances above described are very destructive to insect life.
2. That although carbolic fumes under such circumstances can penetrate through considerable obstruction, yet they do so in such small quantities that their germicidal efficiency must be very slight.
3. That a damp material does not permit the transmission of carbolic vapor so readily as a dry one does.
4. That the vitality of the bacillus of plague is destroyed by such fumes, provided that the bacilliary growth be more or less freely exposed to their action.

A comparison of the results given in this report with those recorded in the last report upon compressed sulphur dioxide gas will indicate the superiority of that preparation as an aërial disinfectant.

ERNEST L. MARSH, M.B., D.P.H. (Oxon.)
WILFRED WATKINS-PITCHFORD, M.B. (Lon.),
F.R.C.S. (Eng.)

Plague Research Laboratory,
7th June 1898.

Further report on the action of compressed Sulphur Dioxide as a disinfectant with special reference to the Bacillus of Bubonic Plague.

In the former reports [a] on compressed sulphur dioxide it was shown that under certain conditions of free exposure to the action of this disinfectant, cultures of the plague bacillus were killed, but when similar cultures were not so openly exposed, the gas failed to destroy the vitality of the bacillus.

The present report has to do chiefly with experiments in which cultures of the plague bacillus were introduced to the action of the gas under conditions more closely resembling those likely to exist in infected houses.

The tests about to be detailed were carried out on April 13th, in the same room as was used on the occasion of the experiments referred to in the footnote. The dimensions of this room in length, breadth and height were, 18′×8′×8′, respectively, so that a capacity of 1,152 cubic feet was made available for the trial of the disinfectant. Precautions to exclude all light and communication with the exterior were adopted as formerly, and every care was taken to prevent unnecessary disturbance of the air of the room during the initial distribution of the test articles in the room and their subsequent collection after the interval allowed for disinfection, namely, twenty hours. The cannister containing the sulphur dioxide under pressure was opened at 6-20 P.M. on the 13th of April and was allowed to empty itself of its contents from a position in the centre of the floor. The experimental room was kept closed, as already mentioned, for twenty hours, and the success with which it was hermetically sealed is indicated by the fact that a thermometer suspended in the room during the twenty hours of the test registered a difference of only half-a-degree Fahrenheit between its maximum (86° F.) and minimum (85·5° F.).

On first entering the room after disinfection, the contained air gave a faint, but obvious, smell of sulphurous acid. A complete absence of all insect life was noted.

Before proceeding to an account of the experiments with cultivations of the plague bacillus, certain experiments will be described in which the property of blue litmus to turn red under the influence of an acid was utilized to demonstrate in what degree sulphur dioxide possesses powers of diffusion and penetration. In a former report, *viz.*, that referred to in footnote (*b*), it was stated that moisture was of material importance for the efficient action of sulphur dioxide as a disinfectant, and in proof of this it was shown how pieces of blue litmus paper *moistened in water* evidenced the action of the gas when scattered about the experimental room at various levels. In the present instance a similar trial was made with pieces of *dry* litmus paper in order to ascertain if there were a sufficiency of moisture in the atmosphere of the room to enable the disinfectant to affect the unmoistened test-paper. The result showed that most of the dry litmus papers had been reddened, but that some of them, especially those lying in a sconce about five feet from the floor, had been only partially affected by the gas.

The further power of the gas to penetrate through different substances was investigated by enclosing pieces of moistened litmus paper in test-tubes, the mouths of which were plugged or covered by different materials. That the gas could enter an ordinary open test-tube and affect a strip of litmus placed therein was proved by hanging four such tubes containing strips of moistened litmus paper at different levels in the room. Examination of the tubes at the end of the interval allowed for disinfection discovered each strip of litmus to be uniformly reddened.

The following are the details of the experiments with compressed sulphur dioxide on moist litmus paper to show its power of diffusion and penetration :—

[a] *a*. Report by Surgeon-Major W. B Bannerman, I. M. S., on experiments carried out to ascertain the efficiency or otherwise of sulphur dioxide as prepared in a liquid form for use as a disinfectant for rooms in which infectious disease has occurred.

b. Note by Ernest L. Marsh, M.B., D.P.H., summarising certain experiments with sulphur dioxide on the bacillus of bubonic plague.

1. Two test-tubes (15·5 cm.×2·5 cm.), each containing a strip of moistened blue litmus paper, had their mouths covered by a layer of dry Swedish filter paper and were laid horizontally on a shelf immediately under the ceiling, remaining there during the twenty hours of the test.—*Examination of the tubes at the end of this interval discovered each piece of litmus paper to be distinctly reddened.*

2. Two test-tubes of the same description and having the same contents as the above were exposed exactly as in the first experiment, except that the paper closing the mouths of the tubes was moistened.—*The litmus was vividly reddened in both tubes.*

3. Two test-tubes (15·5 cm.×1·75 cm.) containing strips of moistened litmus paper had their mouths covered by dry cotton fabric (native loin cloth), the one by a single layer, the other by two layers, and were laid upon the floor.—*Each showed the same degree of reddening, but in both where the litmus was in contact with the bottom of the tube it had remained unaffected.*

4. Two test-tubes (14 cm.×2·5 cm.), each containing a strip of moistened blue litmus paper, had their mouths closed by plugs of cotton-wool, the one of 2·5 mm. and the other of 1·5 mm. in length, and were laid upon the floor.—*Litmus completely reddened in both tubes.*

5. Two strips of moistened blue litmus paper placed at the bottom of a large beaker stood on the floor and holding in its upper part dry hay to the depth of four inches.—*The litmus was distinctly, but not vividly, reddened.*

The above results make it evident that the gas is able to reach and redden litmus paper protected in various ways, though its power to affect the paper varies somewhat with the different methods of such protection. The importance of noting this latter circumstance is connected with the observation that an acid germicide requires to be of a certain definite strength to destroy the vitality of a given culture of the plague bacillus. This will partly account for the failure of the gas to destroy the cultures of the plague bacillus forming experiments *c* and *d* in the report referred to in footnote *b*. That the germicidal action of the gas makes it an efficient aërial disinfectant is evidenced by the fact that plates of sterile agar exposed in the room throughout the above experiments proved to be perfectly sterile when subsequently incubated.

Experiments partly supplementary to the above were undertaken with plague bacilli growing in broth and exposed to the action of the gas in the experimental room. Five small beakers of the same diameter were filled with a broth culture of the bacillus to heights varying from 3—50 millemetres, or from $\frac{1}{8}$th—2 inches. These beakers were arranged at the floor level and screened from dust. After exposure for 20 hours in the disinfectant atmosphere it was found that the fluid in all the beakers had a distinct acid reaction, the degree of acidity varying inversely with the depth of the broth, but that the vitality of the bacillus had been destroyed only in the cultures of a depth of twelve millimetres and under. From the beaker containing broth of a depth of 25 mm., bacilli were recovered on sub-culture, though this sub-culture did not appear so vigorous as that obtained from the beaker containing 50 mm. of broth. This reduction of vitality short of actual destruction is comparable with that observed in the action of other germicidal agents.

The remaining experiments have to do with the action of the gas on cultures of the plague bacillus protected in such a way that the vapor would have had to exert some action of penetration before reaching the growth. These experiments were made to conform, as far as possible, to the conditions that would exist in a room requiring treatment by disinfectants. For convenience of description the experiments and their results are detailed in series as follows:—

Series I.—Experiments with compressed sulphur dioxide as a plague germicide after penetration through cotton-wool.

(*a*) A Petri capsule containing an agar growth of the bacillus of plague covered by a layer of sterile cotton-wool about $\frac{1}{8}$th of

an inch thick was placed on the floor.—*Attempts to sub-culture the plague bacillus were without result, proving that the original culture had been killed.* In control experiments carried out in the laboratory the bacillus was easily recovered on sub-culture.

(*b*) A small block of agar with plague growth upon its surface was wrapped in sterile cotton-wool and hung in the room about four feet from the floor and half-a-foot from the wall.—*All attempts to sub-culture failed.* The control experiments carried out in the laboratory yielded positive results.

Series II.—Experiments with compressed sulphur dioxide as a plague germicide after penetration through cotton fabric (native loin cloth).

(*a*) A Petri capsule containing an agar growth of plague was covered with a layer of coarse cotton material and placed upon the floor in the centre of the room.—*The plague growth was completely sterilised.*

(*b*) Agar blocks with colonies of the plague bacillus were placed in three small sterile bags made of cotton material. These bags were made of one, two and three thicknesses of the material respectively. They were suspended about eight inches above the floor.—*Attempts to sub-culture failed in each instance.* Control experiments conducted in the laboratory yielded characteristic sub-cultures.

(*c*) Agar blocks with colonies of plague bacilli were placed within the folds of a handkerchief made of coarse cotton material and exposed on a shelf six feet from the floor.—*Subsequent examination showed the bacilli to be dead.*

Series III.—Experiments with compressed sulphur dioxide as a plague germicide after penetration through a covering of sterile earth.

(*a*) Two Petri capsules containing agar growths of plague were covered with layers of loose dry sterile earth, $\frac{1}{16}$th and $\frac{1}{4}$th of an inch in depth respectively.—*Attempts to recover the bacilli by sub-culture failed in both.* Control experiments in the laboratory showed that plague growths can be recovered from a similar capsule covered for twenty-four hours with $\frac{1}{16}$th of an inch of sterile earth.

(*b*) Experiment similar to (*a*), except that the covering of earth was moist and not dry.—*The results were as in* (*a*).

(*c*) A quantity of broth in which the bacillus of plague was growing was used to saturate sterile cotton material which was afterwards covered with a layer of loose dry sterile earth to the depth of $\frac{1}{6}$th of an inch. This experiment was duplicated.—*Subsequent examination failed in both cases to recover the bacillus from the cotton material.*

Series IV.—Experiments with compressed sulphur dioxide as a plague germicide after penetration through pieces of paper, feather, &c.

(*a*) Pieces of sterile filter paper lying face to face in pairs were saturated with a broth culture of the plague bacillus and exposed on panes of sterile glass in the following situations:—(1) near ceiling, (2) on window sill, (3) on floor.—*Examination of the lower piece in each case showed that in all three it had been sterilized.*

(*b*) A culture of the plague bacillus growing on agar in a Petri capsule had portions of its surface protected by (1) a sterile leaf and (2) a small sterile feather.—*Attempts to sub-culture from under the feather and leaf proved sterile in each case.*

The following miscellaneous tests conclude the record of the experiments performed:—

1. A Petri capsule containing a nine days' growth on agar of hay bacillus was exposed upon the floor.—*The growth was completely sterilized.*

2. A Petri capsule containing a growth on agar of "Sarcina Lutea" was exposed upon the floor.—*Growth completely sterilized.*

3. A large handful of dry hay placed upon the floor. An infusion made from some of the hay taken from the interior of the bundle after the interval allowed for disinfection showed characteristic cultures of the hay bacillus.

As the primary object of the above experiments was to test the potency of the proprietary disinfectant known as compressed sulphur dioxide as a germicide in the presence of cultures of the plague bacillus, the following conclusions relate mainly to its efficiency in this respect. It is perhaps well again to draw attention to the fact that the size and structure of the experimental room gave exceptional advantages (especially for a native house) to the disinfectant, and enabled it to act with its maximum of efficiency.

Conclusions on compressed sulphur dioxide when tested as a germicide in relation to cultures of the bacillus of plague exposed in a room of the dimension and structure described in the preceding reports:—

1. That in such a room the contents of a cylinder of compressed sulphur dioxide destroys the vitality of cultures of the bacillus of plague when such cultures are freely exposed to its action, as also when they are protected by thin coverings of earth, wool, leaves, &c.

2. That the gas acts as an efficient aërial disinfectant in such a room; and also penetrates certain thicknesses of different substances likely to retain infectious material contained in the room and produces thereafter its characteristic effects.

3. That a small quantity of broth containing living plague bacilli placed in an open shallow vessel and exposed to the disinfectant in such a room is efficiently sterilized.

4. That the artificial addition of moisture to the atmosphere increases the potency of the disinfectant.

ERNEST L. MARSH, M.B., D.P.H. (Oxon.)
WILFRED WATKINS-PITCHFORD, E.R.C.S. (Eng.)

30th June 1898.

Report on the efficacy of Nitric Fumes as a disinfectant with special reference to the Bacillus of Bubonic Plague.

Upon the addition of metallic copper, zinc, or iron, or such organic substances as starch or saw-dust to nitric acid with or without the aid of heat, large quantities of nitric oxide gas are evolved. This gas is invisible, but combining immediately with the oxygen of the air produces the reddish-brown fumes of nitrogen tetroxide. Nitrogen tetroxide possesses disinfectant properties in virtue of its oxidising power.

The production of these fumes by copper, although not the most economical method, has been adopted in the present instance, because it is the most usual. The proportions thought to be requisite for disinfection are to be found in standard works on the subject. Thus in the text-book of Notter and Firth (*Theory and Practice of Hygiene, page* 691) directions to disinfect a room of a capacity of 1,000 cubic feet with nitrogen tetroxide require of copper shavings one ounce; of nitric acid, three ounces; of water, three ounces.

In the present experiments the room selected had dimensions $9\frac{8}{12}' \times 7\frac{9}{12}' \times 7\frac{2}{12}'$, for length, breadth and height respectively, so that a cubic capacity of 536 feet was available for the tests. This capacity on the basis of the above chemical data required of copper, 234 grains; of nitric acid and water, each 13 fluid drachms.

The various tests having been arranged in the prepared room, the copper, in the form of filings, was added to a measured quantity of acid and water contained in a glass jar standing in the middle of the floor. This done, the room was sealed and kept closed for nineteen hours.

Upon first entering the room after the interval allowed for disinfection, no trace of disinfectant odour was appreciable. Insect life was still represented by a large spider discovered alive near the ceiling.

It is worthy of note that a small quantity of copper remained unacted upon in the jar.

During the experiments the temperature of the room varied two degrees, *viz.*, between 83° and 85°F.

Following the plan of former reports on gaseous disinfectants the first experiments to be described are these which test the diffusibility and power of penetration possessed by the disinfectant. For this purpose use was made of starch paper moistened with a solution of iodide of potassium which in the presence of nitric fumes turns a deep blue colour owing to the liberation of iodine. The following experiments describe how starch paper so prepared was affected when exposed in different ways in the experimental room.

Experiments with iodised starch paper to show the powers of diffusion and penetration of nitric fumes.

Experiment I.—Shallow watch glasses containing prepared starch paper were exposed as follows:—

a. One at each corner of the room on the floor	...	*Feeble reaction only.*
b. One in a sconce at midheight	...	*Fairly strong reaction.*
c. One at ceiling level	...	*Distinct reaction less than b, but more than a.*

Experiment II.—Test-tubes (size 15 cm. × 1·5 cm.) with open mouths and containing prepared starch paper were exposed as follows:—

a. Two at the floor level	...	*No reaction.*
b. Two at midheight	...	*Indication of a feeble reaction.*
c. Two at the ceiling level	...	*The one showed no reaction, the other a very feeble reaction.*

Experiment III.—Test-tubes of larger diameter than those used in Experiment II containing prepared starch paper and having their mouths closed by different materials were exposed as follows:—

1. Two tubes (size 15 cm. × 2·5 cm.) suspended at midheight and having their mouths closed by—

a. One layer of dry Swedish filter paper	...	*Distinct reaction on upper edge of paper only.*
b. Two layers	...	*Very feeble reaction on upper edge of paper only.*

2. Two tubes (15 cm. × 1·75 cm.) standing upon the floor and having their mouths closed by—

a. One layer of dry loin-cloth material	...	*No reaction.*
b. Two layers	...	*Do.*

3. Two tubes (14 cm. × 2 cm.) suspended at midheight and having their mouths closed by plugs of absorbent wool. Thus—

a. Plug of 1·5 cm. thickness	...	*Feeble reaction.*
b. „ 2·5 „	...	*No reaction.*

Experiment IV.—A wide-mouthed shallow jar (depth about one inch) containing prepared starch paper and its mouth covered with a layer of dry loin cloth material was placed upon the floor.

Distinct reaction.

Experiment V.—A sheet of prepared starch paper protected by a layer of dry earth $\frac{1}{6}$th of an inch deep was exposed on the floor.

No reaction.

Experiment VI.—A large beaker (two-litre capacity) containing prepared starch paper at the bottom and at the upper part a plug of hay four inches in depth was stood upon the floor.

Very feeble reaction.

Consideration of these results proves that nitric fumes evolved from chemicals in the proportions employed, possess but feeble powers of penetration and comparatively slight powers of diffusion in a room of the capacity tested. It will be observed that at the floor level the action of the fumes was generally weak, that it was slightly more marked at the ceiling level, and most marked at midheight. The circumstance of certain of the test articles being suspended at midheight and under the ceiling in more particular relation to the fumes of the disinfectant as they became evolved would explain the more marked chemical reaction noted in some of the tests exposed in these situations.

The remaining experiments deal with the action of nitric fumes upon cultivations of the plague bacillus.

Experiments with cultures of the bacillus of plague exposed directly to the action of the disinfectant.

Experiment VII.—Petri capsule agar growths of the plague bacillus were exposed as follows:—

a. One at the floor level	...	*Vitality of plague bacillus destroyed, but culture contaminated during period of disinfection by other organisms which survived the attempted disinfection.*
b. One at midheight	...	*Plague bacilli not killed.*
c. One at ceiling level	...	*Plague bacilli not killed.*

The test in *a* was in the immediate vicinity of the evolving fumes, but in *b* and *c* this was not the case.

Experiment VIII.—Test-tube agar growths of the bacillus of plague were exposed with open mouths in the following situations and positions:—

a. One on the floor with mouth upwards		*Bacilli not killed.*
„ „ downwards.		
b. One at midheight with mouth upwards.		*Bacilli not killed.*
„ „ downwards.		
c. One at ceiling level with mouth upwards.		*Bacilli not killed.*
„ „ downwards.		

Experiment IX.—Broth cultures of the bacillus of plague were exposed in beakers in quantities of varying depths on a shelf at midheight as follows:—

a. Depth of broth culture	·3 cm.	(⅛ inch)	*Bacilli not killed.*
b. „	·625 „	(¼ „)	
c. „	1·25 „	(½ „)	
d. „	2·5 „	(1 „)	
e. „	5·0 „	(2 inches)	

Experiments with cultures of the bacillus of plague protected in various ways during exposure to the action of the disinfectant.

Experiment X.—A Petri capsule agar growth of the bacillus of plague was protected by a layer of dry loin-cloth material and hung face down at middle height.—*Bacilli not killed.*

Experiment XI.—A Petri capsule agar growth of the bacillus of plague was protected in part by a sterile feather, and in part by a sterile leaf:—*The parts of the growth protected by the leaf and feather, and even the unprotected parts all retained their vitality.*

The conclusion of these experiments suggests the following remarks:—

1. That nitric fumes as evolved in these experiments possess limited powers of diffusion and penetration. A thin layer of loose earth appeared to offer a positive impediment to their action.
2. That, except in one instance only, the disinfectant was not sufficiently powerful to destroy the vitality of cultures of the plague bacillus exposed in the experimental room.
3. That, practically considered, the amount of nitric oxide evolved by copper and nitric acid in the proportions employed cannot be trusted to effect the proper disinfection of a room.

ERNEST L. MARSH, M.B., D.P.H. (Oxon.).
WILFRED WATKINS-PITCHFORD., M.B. (Lon.), F.R.C.S. (Eng.)

Plague Research Laboratory,
Bombay, 30th June 1898.

No. 316 OF 1898.

FROM

To W. M. HAFFKINE, ESQUIRE, C.I.E.;

THE SECRETARY TO THE SURGEON-GENERAL WITH THE GOVERNMENT OF BOMBAY.

Plague Research Laboratory, Mazagon,
Bombay, 30th June 1898.

SIR,

I have the honour to forward two reports on the investigation forming in the Plague Research Laboratory, by Drs. E. L. Marsh and W. W. Pitchford, serving under me, on the disinfecting power of—

(*a*) compressed sulphur dioxide,[1]—the present investigation forming a conclusion to the two others, which had been carried out in this laboratory by Surgeon-Major W. B. Bannerman, I. M. S.,[2] and Dr. E. L. Marsh,[3] and have been reported already; and of

(*b*) vaporised carbolic acid.[4]

2. It will be seen from these reports that both, sulphur and carbolic fumes, have a considerable penetrating and disinfecting power, and should render service within the limits in which they may be applied. Both appear to reach and affect plague bacilli not only exposed on open surfaces, but also protected by one or several thin layers of cotton material, cotton-wool, filter paper, &c.

3. The effect of sulphur dioxide appears to be superior to that of carbolic fumes. Thus, while a layer of a quarter of an inch of dry or moist earth is not sufficient to protect the plague bacilli against the action of sulphur fumes,—carbolic fumes fail to penetrate even a layer of one-sixth of an inch of earth taken dry,—that is, in a condition which appears to be favourable to the penetra-

(1) *Vide* p. 8, (2) p. 1, (3) p. 2, (4) p. 4.

* The Report on Nitric Fumes, p. 11, was forwarded on a date subsequent to the date of this letter.—W. M. H.

tion of this gas. As regards bactericidal power, also, the sulphur fumes, falling upon the surface of a sporiferous culture of *Bacillus subtilis*, sterilize this within 24 hours, while carbolic fumes leave it alive.

4. The manipulation of the cannisters containing compressed sulphur dioxide is also easier than that of the boiling carbolic acid in Scott's disinfector, as will be seen from the remarks made in the reports.

5. Unfortunately, the use of these cannisters makes one dependent on the existence of a special supply of them, whereas a hot kettle and carbolic acid may be got easily anywhere.

6. And, what is still more important, wearing and clothing apparel, and metallic surfaces, as well as certain objects of furniture, will be deteriorated more by sulphurous than by carbolic fumes.

7. Humidity of the atmosphere is favourable to the action of the sulphurous fumes, while it seems to impede the penetrating power of carbolic fumes. In selecting the one or the other for use, the condition of the atmosphere in the place, or the time of the year, should, therefore, be taken into consideration.

8. When appreciating the results of the above experiments from a more general point of view, the following circumstances are to be taken into account.

In respect of their bactericidal action, gaseous disinfectants, including sulphur and carbolic fumes, are all decidedly inferior to fluid ones. Whichever effect is observed under the action of fumes, a much more certain and prompt one is always secured by a solution of perchloride of mercury, or of carbolic acid, or of many other substances. Thus,

(*a*) When, in immediate contact with a contaminated surface, sulphur dioxide produces an effect in eight, or twelve, or twenty-four hours, the liquid disinfectants achieve the same effect in one, or two or five minutes.

(*b*) Gaseous disinfectants take a considerable time to get mixed with the air in small interspaces and to penetrate through a layer of earth, or clothing, or bedding, while a liquid disinfectant, poured over the same object, is generally absorbed and reaches the contagion immediately.

(*c*) A gaseous disinfectant is applicable only to rooms where all openings can be closed, so to say, hermetically, and requires all the labour necessary for closing them; while the application of a fluid disinfectant, when done by a pump or other appropriate instrument, is a matter of a few minutes, can be done in rooms of any description or in any condition, and requires no preliminary preparation whatever.

(*d*) As mentioned, after these few minutes of action all infected surfaces reached by the liquid disinfectant can be pronounced as safe with a much greater degree of certainty, than after 24 hours' action of disinfecting fumes; whereas, therefore, in the latter case, the inhabitants have to be evicted, provided with shelter, and kept out of their dwellings for 24 hours, in the former they may be permitted to remain there even during the few minutes of operation, if they so wish.

9. Thus, the advantages of a gaseous disinfectant are to be sought for not in the direction of a greater or more prompt effect upon the objects actually reached by the disinfectant, but only in the direction of its possibly reaching the contagion in places and under conditions where a liquid disinfectant may fail to do so. Taking into account the power with which a liquid can be thrown, by means of a pump, against walls, ceilings and floors, and objects of furniture, it is evident that it is only deep and hidden crevices that would escape contact with the fluid; and it is with regard to such crevices that one might expect from disinfectant fumes the greatest amount of benefit.

10. Unfortunately, as it appears from the adjoined reports and from those sent in previously, the sulphur and the carbolic fumes mix freely with the air in open spaces, but, when the circulation of air is limited or entirely arrested, *their diffusion into interstices, and even into places of considerable capacity, is absolutely insufficient to secure disinfecting action.* This is demonstrated by

their not affecting plague microbes grown in the distant half of test-tubes of ordinary size, left with their openings unplugged, and placed in horizontal, or vertical position, with the mouths upwards or downwards, on the floor, or midheight of walls, or on the upper parts of these, close to the ceiling.

I consider this result as depriving gaseous disinfectants of almost all advantages which can be expected from them, in comparison with fluid ones.

11. However, the following may be said in favour of these disinfectants:—

(*a*) Many of them possess a powerful deodorising effect.

(*b*) Whenever the conditions happen to permit of the inhabitants of a room being, without inconvenience, evicted for 12 or 24 hours, and the room itself is such as can have all its openings effectively closed, it may be useful to fill it with disinfecting fumes with the object of killing the insect-life in the air, on the walls and in the shallow interstices, as insects in infected rooms are likely to be carriers of contagion.

12. But the general tendency to seek protection, during epidemics, in bon-fires, fumigation, gaseous disinfectants, &c., is based upon a notion that infection exists in, and may be carried and communicated by, "vicious air". This notion is deprived of any experimental foundation whatever; on no occasion was there reason, in the least sufficient, to believe that the air was the actual vehicle of contagion in any of the diseases which have been put into the category of the so-called "air-born diseases"; and all investigations made up to the present go to show that air is free from pathogenic microbes.

I have the honour to be,
Sir,
Your most obedient Servant,
W. M. HAFFKINE.

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