AN EXPERIMENTAL ENQUIRY ON THE DISINFECTION OF FLOORS FOR PLAGUE.

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THIS investigation has been carried out under the orders of the Government of Bengal on the lines suggested by the Plague Commission in Chapter VI., paragraph 83, of their report, namely, by determining what strengths of the different disinfectants are required to be used in order to kill all the species of micro-organisms in the floor which are not considerably more resistant than the plague bacillus itself. Under these circumstances, any plague bacilli would be pretty certain to be destroyed, and this indirect method of ascertaining the strengths required for the purpose is necessitated by the impossibility of isolating plague bacilli from infected floors preventing direct experiment.

As not only the best but also the cheapest efficient disinfectant is required, some guide to the most likely ones to experiment with can be obtained by the simple method of ascertaining the strength required to kill the plague bacillus in test-tubes, as their effectiveness under these conditions is likely to afford some approximate guide to their relative power under more complicated conditions, and also allows of the elimination of those whose cost must evidently be prohibitive. The summary of previous observations given in paragraph 69 of the Plague Commission's report is of great value here, and they have been supplemented and extended in the experiments now under report. The strengths of various disinfectants required to kill the plague bacillus in from 10 to 15 minutes are summarized in the following table, which embodies the results of previous observers as well as my own.

Table of minimum strengths which kill plague bacilli in 10 *to* 15 *minutes in test-tubes.*

Disinfectant	Previous workers' results	The writer's results
Perchloride of mercury	1 in 5000 to 1 in 10,000	1 in 10,000
Perchloride of mercury dissolved in weak HCl	——	1 in 20,000
Permanganate of potash ...	1 in 10,000	1 in 10,000
Phenol	1 in 400 to 1 in 1000	1 in 750
Sulphuric acid	1 in 2000	1 in 1000
Hydrochloric acid	1 in 500	1 in 1500
Nitric acid	1 in 333	1 in 1200
Lysol	1 in 400	——
Formalin	——	1 in 750
Chloride of lime	1 in 100	——

NOTE.—In all my experiments the time-limit was 15 minutes, which accounts for the somewhat higher figures under some heads, as other workers have sometimes adopted a limit of only 10 minutes.

Taking the figures of the above table as a guide to the relative strengths of the disinfectants dealt with, and working out the cost of effective solutions of each, it was found that formalin and lysol were prohibitively expensive. Further, chlorinated lime is unsuitable, both on account of the cost of adequate strengths and its instability and varying composition. On the other hand, perchloride of mercury, phenol, permanganate of potash and the mineral acids are all sufficiently cheap to be worthy of careful examination.

The method suggested by the Plague Commission has been closely followed: only plates were found to be more efficient than agar slopes for the purpose of isolating the different organisms which survived the disinfection, in order to examine further their resisting powers; and consequently they were always used. As in most of the disinfections in towns, and nearly all those in villages, mud or rammed earth floors have to be dealt with, which present much greater difficulties than do paved floors, it was determined to test what strength of the different disinfectants were necessary for the effective treatment of mud floors, as such strengths would be still more reliable on impermeable floors, and hence would meet all requirements. A room with a mud floor, which had been leeped[1] with cowdung some weeks before, and had recently

[1] The process of leeping consists in mixing cowdung with water until it forms a paste, the latter being applied to the floor. At times mud, or a little chopped up straw or dried grass, is added to the paste. When dried the floor presents a fairly smooth surface, which however does not last long because of the formation of cracks and the breaking away of dried flakes, consequently the process of leeping has to be repeated every week or two. The paste is also applied after the manner of plaster to walls and ceilings. We are indebted to Captain H. Gidney, I.M.S., for this information.—G.H.F.N.

been inhabited by a sweeper, was selected for the experiments, and it proved to have a sufficiently varied bacterial flora to be admirably adapted for the work. Areas about two square feet in extent were nearly surrounded by low mud walls, and flooded with half a litre of the solutions, which were then allowed to run off. As soon as the surface had dried, which usually took between one and two hours, plugs of sterilised cotton-wool were rubbed over it and pieces with the adherent earth sown in broth. After incubation, plates were made, and the different species of colonies isolated in pure cultures. The resisting power of these to the same disinfectant previously used was then tested by exposing a loop-full of agar culture to different strengths of the disinfectant in test-tubes for 15 minutes, and then inoculating from them into broth, and noting in which the organisms grew. By comparing these results with those obtained in a similar manner with cultures of the plague bacillus, the resisting powers could be compared. If any organisms which had little or no greater resisting power than the plague bacillus itself were obtained from the floor after its disinfection, it was obvious that the strength used was not a safe or effective one. If, on the other hand, all the organisms isolated had much greater power of resistance than the plague bacillus, all weak ones having been destroyed, then the disinfectant might be relied on to kill the plague bacillus under similar circumstances.

EXPERIMENTS.

I. *Mineral acids.*—These have a powerful action on the plague bacillus, while they are also cheap. On the other hand, their action will be weakened by being partly neutralized by the alkalies present in the earth and cowdung of Indian floors, as is shown by the great effervescence which occurs on applying them. They will also have a deleterious effect on certain kinds of stone and cement with accompanying neutralization. They have been tested in strengths of 1 in 200, or from five to ten times that found necessary in test-tube experiments, with the following results:—

Sulphuric acid 1 *in* 200—500 were poured over two square feet of floor. Three different micro-organisms were subsequently obtained from the floor and severally exposed to different strengths of the acid for 15 minutes, and then inoculated into broth. One of them was killed by a 1 in 800 solution, but survived after exposure to a 1 in

1,000 one, being thus only slightly more resistant than the plague bacillus, while another survived in a 1 in 1,200 solution, but was killed by a 1 in 1,000 strength, being thus as feebly resistant as the plague bacillus itself. Sulphuric acid in a 1 in 800 solution, therefore, is not a reliable disinfectant of mud floors.

Hydrochloric acid 1 *in* 200 was tested in a similar manner to the above. Four different organisms subsequently cultivated from the floor were further tested in different strengths of this acid. One was highly resistant, being only killed by a 1 in 200 solution, surviving exposure to one of 1 in 300 strength. The other three were killed by a 1 in 1,000 solution, only surviving a 1 in 1,200 one, thus being very little more resistant than the plague bacillus. Hydrochloric acid in a strength of 1 in 200 is, therefore, not a reliable disinfectant of mud floors.

Nitric acid 1 *in* 200 was also tested as above, and two organisms isolated from the floor after its application were further tested as to their resisting powers in various strengths of this acid. Both were killed in 15 minutes by a 1 in 1,000 strength, only surviving a 1 in 1,200 solution, being thus scarcely more resistant than the plague bacillus. A 1 in 200 solution of nitric acid cannot, therefore, be relied on to kill the plague bacillus in a mud floor.

From the above experiments it appears that the mineral acids in strengths from five to ten times as great as those required to destroy the vitality of the plague bacillus in test-tube experiments are yet inoperative against organisms nearly or quite as weak as the plague bacillus when they are applied to mud floors. This is doubtless due to the rapidity with which they are neutralized when spread over mud floors. Stronger solutions would be too destructive for use as disinfectants on a large scale, so it is evident that these chemicals are not suitable for use by themselves as disinfectants against plague.

II. *Permanganate of potash* has a very powerful action on the plague bacillus in a test-tube, but is liable to be decomposed by the organic matter contained in a mud floor of an inhabited room. It has been tested in strengths of 1 in 1,000 and 1 in 500, or in quantities from ten to twenty times that which is effective against the plague bacillus in test-tube experiments, with the following results:—Two organisms were isolated from the mud floor after the use of a 1 in 1,000 solution. One of them was highly resistant, but the other, a Staphylococcus, was killed by a 1 in 6,000 solution of permanganate, and only resisted exposure for 15 minutes to a 1 in 10,000 solution,

being thus scarcely more resistant than the plague bacillus. A 1 in 1,000 solution of permanganate, therefore, cannot be relied on to destroy the plague bacillus in a mud floor. A 1 in 500 solution was therefore tested in the same way as before. A variety of organisms were isolated from the floor after its use, some of which were highly resistant sporogenic bacilli. Three other organisms were tested with the following results:—A short non-sporogenic bacillus was killed by a 1 in 1,200 solution, but survived 15 minutes in a 1 in 1,400 one. A small Coccus survived a 1 in 1,000 solution. Both germs were considerably more resistant than the plague bacillus. A third, a Staphylococcus, was destroyed by one of 1 in 2,000 strength, but survived a 1 in 5,000 one, being somewhat more resistant than the plague bacillus. As the number of colonies obtained after the disinfection from the floor was also very considerable, this experiment was not altogether satisfactory evidence of the efficiency of the solution used, so the same strength was tried again, a different portion of the floor being used as in the other experiments. Again, a number of colonies appeared in the plates, including a sporogenic bacillus and two species of Cocci, and on testing them, each resisted the action of a 1 in 500 solution of the permanganate for 15 minutes, being thus much more resistant than the plague organism, the result in this case being satisfactory. As the above two experiments did not give quite parallel results, the same strength solution was used once more, and yet again a number of organisms survived the process. On testing their resisting powers, no less than three different species of micro-organisms were found to be killed in 15 minutes by a 1 in 10,000 solution of permanganate of potash, being thus as feebly resistant as the plague bacillus itself. This failure, together with the large number of organisms which survived in each previous trial, shows that a 1 in 500 solution of this salt is not to be relied on as a disinfectant against plague in the case of mud floors. As this strength is twenty times as great as will kill the plague bacillus in test-tubes in 15 minutes, it is evident that the organic matter in the floor must have largely neutralized the permanganate solution; and as the amount of organic matter in different mud floors must vary greatly, it is evident that no reliance can be placed on this salt in solutions which would not be so strong as to be too costly, while the deleterious effect of organic matter on the solution would introduce an undesirable element of uncertainty which makes this chemical an unsuitable one for the purpose for which it is required.

III. *Phenol.*—This very cheap disinfectant was found to destroy the vitality of *Bacillus pestis* in test-tubes in 15 minutes in a strength of 1 in 750, while it survived exposure for the same time in a 1 in 1,000 solution. It was, therefore, tested in strengths of 1 in 100 and 1 in 50 in the same manner as before. After flooding with a 1 in 100 solution, a variety of organisms were isolated from the floor, some of which were found to have very little more resisting power than the plague bacillus, so that this strength was manifestly too weak to be effective against plague. On the other hand, a solution of 1 in 50 gave much more satisfactory results, as the following data show: in the first only three different organisms were obtained from the floor after disinfection, a Coccus and two sporogenic bacilli, all of which resisted exposure for 15 minutes to a 1 in 50 solution of phenol. In the second experiment five different organisms were recovered from the floor after disinfection. One was a highly resistant sporogenic bacillus frequently seen in this course of observations. The other four were further tested, and all of them survived 15 minutes in a 1 in 50 solution of phenol.

In both the last experiments phenol in a strength of 1 in 50 destroyed all the organisms in the floor which were not much more highly resistant than the plague bacillus, so that this strength proved to be an efficient disinfectant of mud floors, and *à priori* of impermeable ones.

IV. *Perchloride of mercury.*—This is the disinfectant which has been most extensively used in operations against plague in India; and as it has also been provisionally recommended by the Plague Commission, dissolved in dilute hydrochloric acid and in a strength of 1 in 1,000, it has been carefully tested. In the first place a 1 in 1,000 solution of the salt in water without the addition of any acid was used, and after the disinfection, three organisms were isolated from the mud floor. One of these was a spore-forming bacillus, which was only destroyed within 15 minutes by a 1 in 1,000 solution, while it survived a 1 in 1,200 one, being thus very highly resistant; the second was destroyed by a 1 in 2,000, but resisted a 1 in 4,000 one, being highly resistant; while the third only survived a 1 in 10,000 solution, being scarcely more resistant than the plague bacillus itself. A solution of corrosive sublimate of a strength of 1 part in 1,000 of water cannot, then, be relied on to kill the plague bacillus on the surface of a mud floor, no doubt on account of the precipitation of the salt under these conditions in an inert combination.

Acid perchloride of mercury.—Next, a trial was made of one part of the perchloride and two parts of strong hydrochloric acid in 1,000 of water, this being the solution which has been so extensively used in Calcutta during the last two or more years. This combination, I have found, kills the plague bacillus in test-tubes, when used in a strength of one part perchloride in 20,000 of water, the acid being in double the proportion of the salt as just mentioned. The 1 in 1,000 solution might reasonably have been expected to prove an efficient disinfectant, even of mud floors, against the plague bacillus, yet the four following experiments prove that it is not so. In the first trial three different organisms were isolated from the floor after disinfection, two of which were highly resistant sporogenic bacilli, which were only destroyed by solutions of the strength of 1 in 1,000 and 1 in 2,000, respectively, of perchloride in acid solution. The third, a Staphylococcus, was killed by a 1 in 4,000 solution, only resisting a 1 in 6,000 one, and thus being not very markedly more resistant than the plague bacillus. In the second experiment, in addition to two highly resistant sporogenic bacilli, two different Micrococci were isolated from the floor after disinfection. On testing them both were found to be destroyed by a 1 in 6,000 solution in 15 minutes, only resisting the action of a solution of 1 in 10,000, being thus very little more resistant than the plague bacillus. Thus in the first experiment the margin of safety was a small one, while in the second the disinfectant completely failed to destroy Micrococci of very little greater resisting power than the plague bacillus. The acid perchloride of mercury solution in a strength of 1 in 1,000 is therefore not a reliable disinfectant for use on mud floors, so that the failure of the extensive disinfection operations in Calcutta bustees last year can be easily understood.

As the failure of the above solution to disinfect mud floors appeared to be very possibly due to the precipitation of the mercury salt by the alkalies in the soil, owing to the amount of acid present being too small, it was resolved to try the same strength of perchloride, namely, 1 in 1,000, but with double the usual amount of hydrochloric acid, namely, four parts per 1,000, or 1 in 250, which in itself is an active agent in destroying the plague bacillus in test-tube experiments. Two experiments were carried out with this solution with the following results:— In the first trial three different organisms were recovered from the floor after the disinfection, one of which was a highly resistant sporogenic bacillus, frequently met with in these experiments. Another was a Coccus, which survived a 1 in 1,000 solution, being thus highly

resistant. The third was a Coccus, which was destroyed by a 1 in 6,000 solution, only resisting a 1 in 10,000 solution; being thus but little more resistant than the plague organism. In the second experiment, five different organisms survived the disinfecting process, and no less than two of these were subsequently found to be destroyed in 15 minutes by a 1 in 10,000 solution, being thus no more resistant than the plague bacillus itself, so that the latter, if it had been present, would in all probability have also survived the process, and the strength used was practically useless.

This result is a surprising one, for it shows that organisms may survive quite on the surface of a mud floor which has been flooded with a disinfectant mercurial solution in an acid medium 20 times stronger than that required to destroy the same organism in 15 minutes in a test-tube. That the organisms isolated from the floor were really present in it before the application of the disinfectant is certain, for the precaution was taken in this and in other experiments of covering up the patch with a large bell-jar immediately after the disinfectant had been poured on, and it was only removed at the time the sample of earth was taken within an hour or two, when the patch was dry, the room having been shut up and not visited in the meantime. Further, the organisms tested had also been isolated from undisinfected parts of the same floor, and the same species were repeatedly met with in different experiments. It is clear, then, that a 1 in 1,000 solution of perchloride of mercury, even with double the usual strength of hydrochloric acid, is not an efficient or reliable disinfectant for use in the case of mud floors, so that the strength so extensively used in various parts of India during the last few years is not sufficiently strong for village and bustee disinfections, although, as will be seen immediately, it is effective in the case of impermeable floors.

Experiments were next carried out with double the usual strength of acid perchloride, namely, one part of the mercury salt and two parts of hydrochloric acid in 500 of water instead of in 1,000, with the following results:—In the first trial no colonies were obtained from agar surface cultures inoculated from the broth tubes in which the disinfected earth had been sown, and on making plates only one very highly resisting sporogenic bacillus, which resisted exposure for 15 minutes to a 1 in 1,000 solution, was obtained. This was a highly satisfactory result. In a second experiment the same resistant sporogenic bacillus was obtained, together with a Coccus, which was considerably more resistant than the plague bacillus, also a good result. The very

small number of colonies isolated after the use of this 1 in 500 acid perchloride solution was as marked a feature of its use as the resisting power of those which survived, it equalling the 1 in 50 phenol solution in the latter respect, while it was superior to it in the former one. In the case of the particular floor used for these experiments, then, the 1 in 500 acid perchloride of mercury solution proved to be an efficient disinfectant for destroying organisms of as little resisting power as the plague bacillus, although in the ordinarily used strength of 1 in 1000 it proved to be unreliable.

Disinfection of impermeable floors.

In large towns a certain number of houses with paved floors may have to be disinfected, although Dr Hossack, who has had a large experience in the most crowded areas of Calcutta, informs me that plague cases comparatively rarely occur in such houses, if the floors are in good repair and clean, as in the best Bengali houses. On the other hand, in the filthy Marwari houses plague is very common. In view, however, of the great strength of the solutions found necessary for the efficient disinfection of mud floors, it seemed to be advisable to try if somewhat more dilute ones might not be sufficient for impermeable floors. Phenol and corrosive sublimate solution were used for this purpose.

Four experiments were carried out with a 1 in 1,000 solution of perchloride of mercury in weak hydrochloric acid as recommended by the Plague Commission, with the following results:—In the first two experiments the amount of solution used was only just enough to flood the floor area used, which was composed of flag stones. Of four plates made from four broth tubes sown with material from the disinfected surface as soon as it was dry, one proved to be quite sterile, and three only yielded a very few colonies. Staphylococci were isolated and further tested, but in each case they were found to require a strength of 1 in 2,000 acid perchloride solution to kill them in 15 minutes, so that they were at least ten times as resistant as the plague bacillus. In the remaining two experiments a slightly larger amount of the same solution was used, and the floors were found to have been completely sterilised. As the floor areas used formed a part of the small animal house, it was anything but sterile before disinfection. These results were highly satisfactory, and a 1 in 1,000 acid perchloride of mercury

solution may be relied on for the disinfection of impermeable floors against plague.

Four experiments were also carried out in a similar way on other portions of the same floor with a 1 in 100 solution of phenol, this being twice as dilute as was found necessary for the disinfection of mud floors. In three instances the number of colonies was small after the disinfection of the paved floor, while in the fourth it was somewhat larger. In each case (in addition to a highly resisting sporogenic bacillus in two instances) Staphylococci were separated, but all of them proved to be highly resistant forms, as they all survived exposure for 15 minutes to a 1 in 100 solution of phenol, being, therefore, much more resistant than the plague bacillus. It appears, then, that a 1 in 100 solution of phenol is an efficient disinfectant of impermeable floors against the plague bacillus, although it is not quite so powerful against the more highly resistant forms of micro-organisms as a 1 in 1,000 acid perchloride of mercury solution is. As with both chemicals some Cocci survived, it would not be advisable to try weaker solutions than these.

Summary and Conclusions.

The above experiments show that neither the mineral acids nor permanganate of potash are reliable disinfectants of mud floors against the plague bacillus, even when used in solutions which are many times as strong as are required to destroy the plague bacillus in test-tubes in 15 minutes. This is doubtless owing to the former being neutralized by the alkalies contained in the earth and in the cowdung used for leeping, and to the latter being precipitated and rendered inert by combining with organic matter, which is plentifully present in the leeped mud floors of rooms inhabited by the poorer classes in the large towns and villages of India, which are so often affected by plague.

Of the disinfectants which are sufficiently cheap to be employed on a large scale, there remain phenol and perchloride of mercury in acid solution, the former of which has given satisfactory results on mud floors in a strength of 1 in 50, while the latter is effective in a strength of one part of the mercury salt and two parts of strong hydrochloric acid in 500 of water. If we compare these strengths with those respectively required to destroy plague bacilli in 15 minutes in test-tubes, we find that 1 in 750 of phenol and 1 in 20,000 of acid perchloride of mercury solutions are effective under the latter cir-

cumstances. Thus it appears that in order to be sure of killing micro-organisms of as little resisting power as the plague bacillus on the surface of mud floors, 15 times as strong a solution of phenol and 40 times as strong a one of acid perchloride of mercury as are effective against the plague bacillus in test-tubes in 15 minutes must be used. Thus in proportion to its action on the plague bacillus in test-tubes, phenol is nearly three times as effective on a mud floor as is acid perchloride of mercury. This fact clearly indicates that a portion of the mercury salt is rendered inert by combining with certain constituents of the mud floor, probably albuminous substances, and this may explain the at first sight somewhat surprising failure of a 1 in 1,000 mercurial solution efficiently to disinfect the mud floor. However, when the strength of this solution was doubled, the acid being also doubled, satisfactory results were obtained with the mud floor tested, and the micro-organisms recovered after its use proved to be much more highly resistant than the plague bacillus, while they were fewer than even after the use of 1 in 50 phenol. Nevertheless, the neutralizing action of mud floors on the mercury solution will doubtless vary considerably in different places, and it is easy to conceive that in some instances this variable and unrecognisable factor may be considerably greater than in the case of the particular floor used in these experiments, and that even a 1 in 500 solution might be ineffectual under such circumstances. On the other hand, phenol appears to be free from this objection; and as it gave as good results in a strength of 1 in 50 as the acid perchloride did in one of 1 in 500, it must be considered the safer disinfectant in this strength (1 in 50) in the case of mud floors. In the case of impermeable floors either a 1 in 100 solution of phenol or a 1 in 1,000 one of acid perchloride of mercury is efficient, the latter being somewhat the more powerful of the two, which advantage is probably more than counterbalanced in practice by the disadvantage of using two different disinfectants, if the conclusion that phenol is the better in the case of mud floors is accepted. Both 1 in 50 phenol and 1 in 500 acid perchloride of mercury cost about one penny a gallon, the former being very slightly the cheaper of the two in such price-lists as I have been able to consult.

The variable effects of mud floors in partially neutralizing the disinfectant power of perchloride of mercury solutions may possibly account for the different estimations of the value of disinfection with this agent in different places and provinces, and also for the failure of

the very extensive operations of the year 1900 to prevent the recurrence of plague in the same houses and bustees during the recrudescence of the disease in Calcutta early in 1901. It must also be remembered that the above-noted strengths of disinfectants only act on the superficial layer of floors, etc., and have no influence on the presence of rats, by which, it is now pretty generally admitted, plague is in some indirect way spread—an important factor which must not be lost sight of in estimating the probable advantages of measures of disinfection. Still it is doubtless a good thing to destroy any plague bacilli lurking in a room which has been inhabited by a plague patient, and the practical outcome of the present enquiry is that the perchloride of mercury in hydrochloric acid must be used in at least double the strength that was provisionally recommended by the Plague Commission, and which has up to now, I believe, been very generally relied on in Calcutta. It is still better to substitute a 1 in 50 solution of phenol in every case when mud or earth floors or courtyards form any part of the area to be dealt with, as will be the case in the great majority of instances in which disinfection for plague is required.