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L & M.

~~Legislative~~ (189) Department.

ORDINARY MS.

G.O., No. 1273 P.

DATED 8-10-28

[Despatch Abstract.]

Mr Hankin's report on the disinfection of floors.

Recording letter from the Secretary to Govt., N.W.P. and Oudh forwarding a copy of ——— with a mixture of mud and cowdung.

BACK PAPERS.

G.O.	No.	Department.	Date.	G.O.	No.	Department.	Date.

FURTHER PAPERS.

G.O.	No.	Department.	Date.	G.O.	No.	Department.	Date.

Local & Munl. (189 8) Department.

CURRENT No. 19848

From The Secretary to Govt. N.W. Provinces and Oudh.

Order No: 1273 P. dtd 8-10-98.

Recorded
8 10 98

No. 790 / XVI

Dated 22-9-98

Received 29-9-98

Registered 30-9-98.

Enclosures / book

Spare copies.

Note The information on pages 31-37 of the pamphlet is the same as that printed on page 421 et seq of Watson, Vol II & is nearly 1 year 3 months old
8/10/98

SUBJECT.

Forwarding a copy of the report by Mr. Hankin about the disinfection of floors with a mixture of mud and cowdung.

8/10/98

BACK PAPERS.

G.O.	No.	Department.	Date.
	944P 29-8-98.		

FURTHER PAPERS.

G.O.	No.	Department.	Date.

No. 79 OF 1898.
XVI-4

LOCAL & MUNICIPAL DEPT.
CURRENT No. 944P

ALL COMMUNICATIONS TO GOVERNMENT SHOULD GIVE THE No., DATE AND SUBJECT OF ANY PREVIOUS CORRESPONDENCE, AND SHOULD NOTE THE DEPARTMENT QUOTED.

From

THE SECRETARY TO GOVERNMENT,
N.-W. PROVINCES AND OUDH,

To

The Secretary to the Government of Madras
Local and Municipal Department

Dated Nainital the 22nd September 1898.

SIR,

Sanitation DEPARTMENT.

In Compliance with the request contained in your telegram dated the 24th August 1898, I am directed to forward a copy of the report by Mr. Hankin on pages 30 et seq of which will be found the information required by the Government of Madras.

I have the honour to be
Sir,
Your most obedient servant,

H. McAuley
Under Secretary to Government
N/W P and Oudh

P. No. 4136—17-2-98. 1,000—P.D.

Investigations on Plague by Mr. E. H. Hankin, Chemical Analyser and Bacteriologist, North-West Provinces and Oudh and Central Provinces, 1897.

B 1441

Investigations on Plague by Mr. E. H. Hankin, Chemical Analyser and Bacteriologist, North-West Provinces and Oudh and Central Provinces, 1897.

s 1441

No. 455.

From

E. H. HANKIN,
Chemical Examiner and Bacteriologist,
N.-W. P. and Oudh and Central Provinces ;

To

SURGEON-MAJOR-GENERAL H. COOK,
Surgeon-General with the Government of Bombay.

Dated Agra, 23rd July 1897.

SIR,

I have the honour to enclose a copy of my report on the vitality of the bubonic microbe in grain, and a report to you on certain questions connected with the vitality of the bubonic microbe. These, with the reports previously sent to you, complete my account of my work on the plague microbe carried out while I was in Bombay. I have sent an abstract of my work to the President of the Bubonic Plague Research Committee. Lack of time has made it impossible for me to combine all my results into one report, as I am now carrying on my duties as Chemical Examiner and Bacteriologist.

I have the honour to be,
Sir,
Your most obedient servant,
E. H. HANKIN.

From

E. H. HANKIN,
Chemical Examiner and Bacteriologist,
N.-W. P. and Oudh and Central Provinces;

To

SURGEON-MAJOR-GENERAL H. COOK,
Surgeon-General with the Government of Bombay,
Bombay.

Dated Agra, 23rd July 1897.

SIR,

I have the honour to forward the following report on certain questions connected with the vitality of the bubonic microbe.

2. A question of great importance in connection with the etiology of bubonic plague is whether the microbe has a habitat outside the bodies of men or animals suffering from the disease. M. Yersin appears to be able to find it with facility in the earth of infected houses. He has met with it at some distance from the surface at a distance to which it is not likely to have been carried in recent excreta of patients, thus suggesting that the microbe can live and thrive in the soil of infected houses. If this is the case, it becomes necessary not only to remove the patient from an infected house and to disinfect his excreta, but also to dig up and disinfect the soil of the locality for a considerable depth before we can be sure that the infection has been removed.

M. Yersin states that this soil microbe is completely devoid of virulence for rats and mice, and so the question arises how he can be sure that the microbe he found was that of plague. Researches that I have carried on for several months to test his assertion have shown me that by existing methods the isolation of the bubonic microbe, when mixed with the microbes commonly present in dirt, is almost impossible.

Professor Calmette, to whom I appealed for help, very kindly communicated to me a method which, I presume, is that of M. Yersin, for the isolation of the plague microbe from soil. A small quantity of the suspected soil is injected under the skin of the mouse. As soon as the mouse is very ill and apparently about to die, it should be killed, and cultures made from its heart-blood. This method has given me as little success as others. I have injected both rats and mice with varying quantities of suspected substances. On their death, I have inoculated their organs on to many kinds of food media. In most cases ten or twelve culture tubes were inoculated from each mouse. But with one probable exception I have failed to isolate undoubted plague microbes.

I have however discovered in all kinds of substances microbes having many superficial resemblances to those of plague. For instance, a microbe isolated from a mouse dead after inoculation with some ground-up weevils resembled the plague microbe in several respects. When spread over the surface of agaragar, its culture resembled that of plague in giving an appearance like ground glass when looked at from behind by obliquely transmitted light. When grown in bouillon containing pieces of fat, this microbe produced a growth identical in appearance with that of plague so long as the culture was not touched. The stalactites of growth hanging down from the fat drops were at once seen to be adherent to the latter on gently shaking the tube, thus differentiating the growth from that of plague. Microscopic appearances of the cultures of this microbe resembled those of young plague cultures. As they grew older, a few of the short bacilli appeared to be swollen up and two or three times as long as the normal form. But in no case were true involution forms met with in any way resembling those of plague. The growth of this microbe on agar was far more scanty than that of plague. Of five mice inoculated with large quantities of cultures of this microbe, one survived and the others died after intervals of 8 to 24 days. Another microbe that I noted at the time as suspicious was isolated from a

mouse that died after inoculation with an extract of some rice stored in a godown in which rats had been dying. It produced involution forms, but slightly different from those of the plague microbe. It differed from the plague microbe in that its growth on agaragar was more copious, resembling somewhat that of Bacillus Coli Communis, and further in that the growth after a few days acquired a brown colour. There was no great probability that the rice had become infected from the rats, as it had but recently been placed in the godown and was stored in a well closed bin supported on legs. Many different species of microbes were commonly met with in cultures made from mice that had succumbed to inoculation with dirt and other substances. Occasionally in such mixed cultures, colonies that were in contact with others after the lapse of several days appeared to enlarge and on microscopic examination presented appearances of involution forms similar to those of plague. But in no case was it possible to obtain such involution-form-producing microbes in pure culture and to further examine their properties.

Among other reasons for the difficulty in isolating the plague microbe when it is in the presence of others, is the fact that as, it occurs in tissues, it often shows an unreadiness to develope on agaragar. If such tissues are inoculated on to agaragar, growth may only occur at the commencement of the track of the needle. If the inoculating needle has been moved in a zigzag movement, the growth may be only at the turns of the zigzag. Apparently growth only results where a comparatively large quantity of the inoculated material is present. If foreign microbes are present in the tissues, this unreadiness to grow when inoculated on to agaragar appears to be greatly increased. The members of the Austrian Plague Commission told me that they had had a similar experience. In mice dead after plague inoculation it frequently happens that cultures made from the seat of inoculation remain sterile, while cultures made from the spleen and heart-blood produce typical plague growth. It is possible that this result has to do with the chance entry of foreign microbes at the wound made when inoculating the mouse. In a series of experiments I infected sheep's wool and cotton wool (unsterilised) with the bubonic plague microbe, and at different intervals injected small quantities of extracts of such wool into mice. Some times the mice survived, even when inoculated from the wool at a time when the analogy of my experiments on grain suggested that the microbe had not died out. In other cases the mice died, and all cultures made from their organs remained sterile. Owing to this irregularity, I was unable to obtain in my experiments on the duration of the infection in clothing, wool, &c., any clear and regular results similar to those I had obtained from grain. One practical point that I came across was that the microbe grows more readily in agaragar clarified by the addition of a small quantity of chopped-up meat than in agaragar clarified by the ordinary method with white of egg.

It is needless to describe all the work that I did in attempting to find the plague microbe in soil, &c., from infected houses. The following few examples will suffice :—

(*1*) *House in Cross Lane, Currey Road.*—Professor Muller found this house while carrying out his plague inspections. He kindly telephoned for me, and I arrived about an hour after he had left. On opening the door of the single room, about ten feet square, that was inhabited by the family, he found three persons, the mother and two daughters, lying on the floor suffering from plague. The mother and one daughter were at once taken away to hospital. The other daughter, a girl of about eight years old, died while they were removing her, and the body was left in the corner of the room. The father, Dattu Koli, a mill-hand, had died on the preceding day. Another child had died four days earlier. Thus the whole family had been carried off. On entering the ill ventilated room I found it in a state of great disorder. Clothes, food and filth of all sorts covered the floor. In one corner was the cooking place and numerous brass vessels. Another corner was occupied by the nanee or drain, in which were some human dejecta. Another corner contained an altar with a brass image of Ganesha, the god of wisdom. The body of the little girl was in the other corner. The room was pervaded by an overpowering smell that produced nausea. I spent about an hour collecting specimens in the room, and also helped the Sanitary Inspector to burn the clothes and

property. I arrived at my laboratory within an hour of leaving the place, and immediately proceeded to inject extracts of the specimens I had collected into mice. The specimens of earth of the mud and cowdung-floor were all taken from the surface and at places in which traces of dejecta were visible.

The following is a list of the injections made into mice :—

Number of Mouse.	Substance injected.	Result.
1	Earth from floor	Death after 24 hours.
2	„ „	„ 96 „
3	Another specimen of earth	„ 24 „
4	„ „	„ 24 „
5	Another specimen of earth	Survived.
6	„ „	„
7	Another specimen of earth	Death after 48 hours.
8	„ „	Survived.
9	Another specimen of earth	Death after 48 hours.
10	„ „	„ 8 days.
11	Stain of dried saliva found on a stool ...	Survived.
12	„ „ „ ...	Death after 18 days.
13	Water in brass lota on floor	„ 72 hours.
14	„ „	„ 5 days.
15	„ in large degchie	Survived.
16	„ „	„
17	Dejecta from corner of drain	Death after 24 hours.
18	„ „	„ „
19	Millet found between grindstones ...	„ „
20	„ „ ...	Survived.
21	Stain on clothing (? vomit)	Death after 25 days.
22	„ „	Survived.
23	Drain outside house	Death in 48 hours.
24	„ „	„ after 5 days.
25	Dust from bamboo lamp-frame over shrine ...	Survived.
26	„ „ „ ...	„
27	Stain of saliva mixed with betel on edge of drain	Death in 24 hours.
28	Stain of saliva mixed with betel on edge of drain	Survived.
29	Dust from a shelf	„
30	„ „	Death in 24 hours.
31	Canvas sacking used as bedding ...	„ „
32	„ „ ...	„ after 12 days.
33	Salt found in an earthen pot	Survived.
34	„ „	„
35	Partly eaten pudding made of rice on floor by corpse	„
36	Partly eaten pudding made of rice on floor by corpse	„
37	Washings of three cigarettes found on the floor	„
38	Washings of three cigarettes found on the floor	„

Of the twenty mice that died mentioned in the above table, 17 were subjected to further examination by inoculation into a second series of mice. In each case the spleen was ground up in bouillon, and two or three drops inoculated into mice. Of the 17 mice of this second series, only 4 died. From each of these four mice other mice were inoculated, some with quantities of the spleen, others with only traces. Altogether in this experiment 94 mice were inoculated. Numerous agaragar cultures were inoculated from the spleens and other organs of every mouse that died.

The plague microbe was not detected in a single case, although there is every probability that the room was badly infected. The microscopic appearances in mice Nos. 10 and 31 were somewhat suspicious of plague, but no plague microbes could be isolated. With regard to mouse No. 24, that had

been inoculated from dirt in the drain outside the house, passages were made from its spleen through three mice. From the last of these mice a microbe was isolated that gave involution forms somewhat similar to those of plague, but it grew on agaragar more vigorously than plague, and did not respond to Pfeiffer's serum test.

(2) *Dairy in Mazagon.*—Many plague cases had occurred in and near a large dairy in Mazagon. The cow-sheds were in a large courtyard partly surrounded by small houses. The following specimens were injected into mice:

(1) Water of a well used for the cows.
(2) Water found on manhole of drain near infected houses at back of cow-shed.
(3) Ditto ditto ditto.
(4) Drain water outside an infected house.
(5) Liquid running from a latrine towards a gully.
(6) Water from drain of cattle-shed at point of entering gully.
(7) Drain from disinfected house in which cases had occurred.
(8) Water from trough used for cattle.
(9) Milk.
(10) Do.
(11) Liquid in front drain of latrine near cow-shed.
(12) Cotton seeds cooked, used as food for buffaloes.
(13) Grain used as food for buffaloes.
(14) Ditto.
(15) Dung of buffalo that was in bad health.
(16) Earth of whitewashed latrine.
(17) Water of Nawab's tank in the neighbourhood.

In none of the above substances was the plague microbe detected, though a somewhat suspicious microbe was found in No. 7.

(3) During the height of the plague, vultures living at the Towers of Silence were often noticed to be ill. One of these animals was found unable to walk and was sent to me in a basket. Its saliva and dejecta were found to contain microbes resembling those of plague in microscopic examination. Two mice injected with traces of its dejecta passed on the day of its arrival died, but in neither case could the plague microbe be isolated. The dejecta passed three days later were not virulent to mice. The vulture recovered in a few days.

(4) *Chillum from the Parel Hospital.*—Two native soldiers employed as orderly cooks in the Parel Hospital were attacked with the pneumonic form of plague. A woman of the same caste as the soldiers (Brahmin) employed in the same cook-house and a Brahmin who was in the habit of visiting her at the kitchen, were attacked with plague a few days before the two soldiers were affected. In the case of the woman the cervical glands were enlarged. Other servants employed in the cook-house of different castes escaped. The above persons being of the same caste were in the habit of smoking tobacco from the same chillum together. The female plague patient recovered. Surgeon-Captain Thomson informed me that, while she was ill, she smoked with the two soldiers from the same chillum. The chillum was sent to me together with a piece of dirty rag wound round its orifice as a mouthpiece. From this the following mice were inoculated:—

Mouse (1) with a quantity of dirt from inside the chillum near the mouth end.
" (2) ditto ditto ditto.
" (3) a trace of ditto ditto.
" (4) with several drops of extract of the rag.
" (5) ditto ditto ditto.
" (6) with a trace of the above.

Only the last-mentioned mouse died. Microscopically a few bacilli resembling those of plague were visible in its spleen, but all cultures made from its spleen and other organs remained sterile, and a second mouse inoculated from it remained healthy. Leaving it as an open question whether the two soldiers had obtained their pneumonic plague from infection from the chillum, in view of the fact that the woman had her cervical glands affected, it is highly probable that the chillum had become infected. Nevertheless, though examined as soon as it came into my possession, no plague microbes could be detected.

(5) *Suspected materials from Sewri.*—At the time of my visit to this place to collect the following specimens, plague had been going on for some weeks. Most of the following specimens were collected in a field at the back of the village that was used as a latrine and that was in an indescribably filthy condition. The salt water pond found at the far side of this field appeared to be the margin of a disused salt-pan. Within two hours of collection the following substances or extracts made from them were injected into mice:—

Number of Mouse.	Substance injected.	Result.
1	One-third of four ants found near dejecta in field	Death in 24 hours.
2	Ditto	Survived.
3	Ditto	"
4	Water from salt pond near dejecta ..	Death in 24 hours.
5	Ditto ...	Died in 2 hours.
6	Ditto ...	" 2 "
7	Human dejecta on sea side of village ...	" 4 days.
8	Cloth of rezai on which woman had died on preceding day	Survived.
9	Earth by cactus hedge saturated with urine &c.	Died in 24 hours.
10	Waste water by stand-pipe at south end of village...	" 13 days.
11	Fly caught on human dejecta	" 4 "
12	Ants from nest in infected house ...	" 24 hours.
13	Ditto	Survived.

Mice Nos. 5 and 6 probably died owing to the excessive quantities of salt in the water with which they were injected. An extensive œdema was found at the seat of inoculation in each case. Some of the liquid that exuded from the œdema was inoculated into two mice. Both survived.

A microbe was isolated from the heart-blood of No. 4, probably identical with that of plague. Its growth on agaragar resembled that of plague in general appearance, and in that, when touched with an inoculating needle, it was found to be sticky, and on withdrawing the needle it drew out into threads. It differed from the plague microbe however in that involution forms were formed on agaragar within 24 hours, and in the fact that its growth in bouillon resulted in an uniform turbidity. The involution forms made by this microbe were however so strikingly similar to those of plague, that I thought it well to subject the matter to further investigation, and in the first place to see whether the plague microbe could not be modified by cultivation in media containing an excess of salt. The following experiment was carried out:—

A series of agaragar tubes were prepared containing from one to ten per cent. of salt. These were inoculated with a culture of a plague microbe that had been isolated from a rat some time before. When examined on the following day, it was found that the tubes containing one and two per cent. of salt respectively showed a fairly good growth. In the tube containing two per cent. a commencement of the formation of involution forms could already be seen. In the tube containing three per cent. very large and exaggerated involution forms were visible. Scarcely any of the microbes present showed forms normal to ordinary agaragar cultures at that stage. Irregular rod-shaped and pear-shaped forms preponderated. In the tube containing four per cent. of salt and in the tubes containing higher percentages of salt, no development had occurred after 24 hours. Forty-eight hours after inoculation, however, involution forms were met with in the tube containing 4 per cent. of salt. The growth to the naked eye appeared to be very slight. Among the involution forms present, irregularly spherical and spindle shaped forms preponderated. In some cases irregular bacillar forms appeared to be growing out of spheres. From the tube containing two per cent. of salt, another series of tubes were inoculated. The tube of this series containing two per cent. of salt developed excellent involution forms in 24 hours. The plague microbe thus grown for two generations on salt was

inoculated into several tubes of bouillon. In each case uniform turbidity of the bouillon resulted. The bouillon tubes were further inoculated on to agaragar, and pure cultures of the plague microbe resulted.

On these grounds it appeared to me to be probable that the microbe isolated from mouse No. 4 was that of plague modified by its sojourn in salt water. A further proof was obtained by injecting a third of a culture into each of two rabbits, one of which had previously received 3 c. c. of Haffkine's plague vaccine. The protected animal survived, and the other died in four days. Involution forms resembling those of plague were found in mixed cultures obtained from mouse No. 7, but as I did not succeed in isolating this microbe, I cannot definitely say that it was that of plague, although it appeared to me to be such at the time.

The above are examples of a long series of researches that have led me to doubt the statement that the plague microbe can be found by existing methods in the soil of infected localities.

A satisfactory method of isolating the plague microbe, similar to that discovered by Dunham for cholera, is much to be desired. My observation that the plague microbe is very resistant to reducing agents suggested to me that possibly such a method might be found by the use of such substances. My sudden removal from Bombay prevented me from working out this point, but the following experiment carried out under my direction by Dr. Srinivasa Rau is of interest.

A solution was prepared containing ferrous sulphate 10 per cent., tartaric acid 10 per cent., citric acid 1 per cent. The solution was made faintly alkaline with ammonia. I had previously found that the bubonic plague microbe was not destroyed by immersion for 24 hours in water containing a proportion of the above mixture corresponding to a half per cent. of ferrous sulphate. From one to eight drops of the above mixture were added to a series of bouillon tubes which were then inoculated with unsterilised tap water to which the plague microbe had been added. On the following day each bouillon tube was inoculated on to agaragar. The cultures thus obtained from the bouillon tubes containing from 1 to 6 drops of the solution contained many kinds of microbes, while those obtained from the tubes containing 7 and 8 drops only contained colonies of one other microbe besides that of plague according to Dr. Rau. Though it does not seem to me to be probable on general grounds that this method could separate the plague microbe from those with which it may often be associated, the point here raised appears to me to be worth working out.

The above described experiments with agaragar containing different quantities of salt has led to the discovery of a rapid method of identifying the microbe of plague that has been worked out in my laboratory by Surgeon-Captains Leumann, James, and Thomson, who have tested plague microbes from many different sources in this manner. In order to prove that this method is reliable it is necessary to show that other microbes, when grown on salt agaragar, do not produce similar involution forms. This I have done with a large number of microbes from dirty water, &c. In many cases the growth on salt agaragar appeared to differ morphologically from the normal growth, but in no case was any appearance similar to that of plague involution forms met with.

According to M. Yersin (Annales de l'Institut Pasteur, volume VIII., page 662), flies can suffer from plague. I have worked out the relations of certain insects to this disease, and strongly doubt whether they are a frequent mode of transmission of the malady. A bug caught on a patient in Mandvi was ground up and injected into a rat. The animal died five days later apparently not of plague. Four rats were inoculated with ten bugs each from some that were brought to me from the infectious diseases hospital. Two of the rats survived. Of the two that died, one was found to have died from plague. I obtained some ants from a house in which numbers of rats were dying. The servant employed to collect these rats died a little later of plague. The ants had been caught coming out of a hole in which a dead rat was found. A mouse bitten on the ear by one of these ants (a large black species) died of plague, as did another mouse into which eight other ants were injected. Ten days later five mice were inoculated with a fresh lot of ants obtained from this house, only one of which died, apparently not of plague. The avidity with which ants attack rats dead of plague led me to investigate the matter further. I kept ants in captivity and fed them with infected materials. At intervals they were examined, and though at first I thought that they retained the infection for several days, further experiments have led me to the conclusion that this is not the case. A large number of ants were also examined from localities in which human plague cases were occurring, but in which no rats had been at about that time dying of plague. In none of these cases were any grounds obtained for believing that the plague microbe was present. Thus although ants and other insects that attack rats dead of plague may be concerned in spreading the infected material about the house, it does not appear to be likely that the insects themselves form a repository for the infection. I have also examined numerous weevils and beetles found in suspected grain from infected houses, &c., without in any case detecting the microbe of plague.

The search for the microbe outside the bodies of men and animals suffering from the disease having thus led mainly to negative results, an attempt was made to obtain some idea of its probable behaviour in the outside world by testing the duration of its life in various substances that might possibly act as its nidus.

As it is impossible adequately with existing methods to test for the plague microbe in such a substance as cowdung, owing to the presence of innumerable foreign microbes, many of which can grow more rapidly than that of plague, it was found necessary to add the plague microbe to the substances to be tested after they had been sterilised. If the plague microbe dies out in a given time in such substances in the absence of other microbes, it is probable that it would die out more quickly were other microbes present, though it must be remembered that this is only an inference. To prevent the loss of volatile substances during the process of sterilising, the substances tested were, as a rule, sterilised under steam pressure in hermetically sealed tubes.

The observations recorded in my report on disinfectants that the microbe of plague is exceptionally sensitive to the action of certain organic acids might lead to the inference that it is unlikely to be able to exist for long in all substances having an acid reaction. For instance, in the report referred to I have described experiments carried out by Dr. Srinivasa Rau under my direction that showed that as soon as milk has been kept long enough to acquire a well marked acid reaction, it has the power of destroying the bubonic microbe. But that such a generalisation is not permissible is shown by the following facts.

While carrying out my experiments on the action of acids in destroying the bubonic microbe, the question occurred to me whether this action was due to any specific action of the acids tried, or whether on the other hand it was merely due to the absence of a neutral or alkaline reaction from the media in which the microbes were suspended. To decide between these alternatives, it would be necessary to find an acid that exerted no antiseptic action on the microbe of plague. The fact that solutions of tartaric acid readily permit the growth of various fungi led me to test the action of this substance. The results obtained were as follows :—

Percentage of acid present.	Result on inoculating into gelatine.
0 control	†
·2	†
·4	†
·8	†
1	†
2	†
3	†

The method of experimenting was the same as that employed in my experiments on antiseptics.

Thus the bubonic plague microbe is not destroyed by exposure for a short time to the action of 3 per cent. tartaric acid.

In some experiments carried out with Dr. Srinivasa Rau it was found that the microbe is in certain cases unharmed by a prolonged exposure to urine having a clearly marked acid reaction. The following experiments were carried out.

(1) Fresh urine of acid reaction was placed in a glass tube hermetically sealed and sterilised. Another specimen of urine that had been allowed to putrefy and that had acquired an alkaline reaction was similarly treated. On cooling the two tubes were inoculated with plague, and at the intervals stated the latter were inoculated further to gelatine. The results were as follows :—

Interval.	Fresh Urine.	Putrefied Urine.
At once, control	†	†
After ½ hour	†	†
After 1 hour	†	†
After 1½ hours	†	–
After 2 hours	†	impure.
After 2½ hours	†	–
After 24 hours	–	–

Thus according to this experiment the plague microbe can remain alive for at least 2½ hours in acid urine, but dies out within 24 hours. In putrefied urine of alkaline reaction it seemed to die out more quickly.

In another experiment mice were employed. Fresh acid urine and stale alkaline urine were sterilised as before. Both were inoculated with plague. In each case a fine knife was dipped into the mixture, and a mouse was then pricked with it near the root of the tail. Both of these mice were found dead of plague three days later. Pieces of sterilised dirty rags were wetted with the infected urine and placed in Petri's capsules until the following day. They were then taken out of the dessicator and kept in the room. On the following day, that is to say two days after the commencement of the experiment, pieces of the rags were extracted with bouillon and injected into two mice, both of which remained in good health. Two days later another pair of mice were similarly treated with the same result. The day after the commencement of the experiment, the bubonic microbe was found by inoculation on to agaragar to be still alive in the rest of the infected urine that had been left in the tube. The putrefied infected urine unfortunately by this time had become impure.

In another experiment some acid fresh urine was infected with plague and inoculated at intervals to gelatine, and thence to agaragar to control the purity of the resulting growth. The following are the results :—

Interval,	Results in gelatine.
At once	†
After ½ hour	†
After 1 hour	†
After 1½ hours	†
After 2 hours	†
After 2½ hours	†
After 3 hours	†
After 24 hours	†

The following are the results of two experiments carried out with alkaline and acid putrid urine respectively. The two specimens of urine had been sterilised in hermetically sealed tubes as in the earlier experiments. It was found that the reaction was still acid and alkaline in the two cases respectively after sterilisation :—

Interval.	Acid putrid urine.	Alkaline putrid urine.
At once, control	†	†
After ½ hour	†	†
After 1 hour	†	†
After 1½ hours	†	†
After 2 hours	†	†
After 2½ hours	†	†
After 3 hours	†	†

The purity of only the first and last tubes in each series was controlled by further inoculation on to agaragar. In each case tubes were inoculated after 24 hours, but both of them turned out to be impure.

To summarise the above results, the plague microbe was found to remain alive for 24 hours two out of three times in acid sterilised urine. The experiments were not sufficiently extended for it to be possible to arrive at any conclusion as to whether the microbe could exist for a longer or shorter time in urine that had undergone the ammoniacal putrefaction.

In acid human fæces, on the other hand, it appears to die out, as shown by the following experiment carried out by Dr. Rau. The material was sterilised in a sealed tube and infected with plague. From this mixture inoculations were made to gelatine, and thence on to agaragar with the following results :—

Interval.	Growth in gelatine.	Growth on agaragar.
At once, control	†	†
After ½ hour	†	
After 1 hour	†	
After 1½ hours	†	
After 2 hours	†	†
After 2½ hours	–	–
After 3 hours	–	
After 24 hours	–	–

I have obtained similar results in cowdung having an acid reaction. More commonly, however, cowdung has an alkaline reaction, and in this substance the microbe appears to be able to remain alive a longer time as shown by the following experiments. In view of the fact that cowdung is so often used mixed with mud for making the floors of houses in India, these results are not without interest from the practical standpoint.

Some normal alkaline cowdung was mixed with twice its weight of water and boiled. It was then centrifugalised and the supernatant liquid was put into a tube hermetically sealed and sterilised in the autoclave. The reaction of the liquid after this treatment was still alkaline. It was infected with plague, and then inoculated at the intervals stated below into gelatine. Forty-eight hours later the gelatine was in certain of the tubes further inoculated into agaragar to control its purity, with the following results :—

Interval.	Growth in gelatine.	Growth in agar.
At once, control	†	†
After ½ hour	†	
After 1 hour	†	
After 1½ hours	†	
After 2 hours	†	
After 3 hours	†	†
After 24 hours	†	†
After 48 hours	†	†
After 72 hours	†	impure.

The above experiment was carried out by Dr. Srinivasa Rau.

In the following experiment solid cowdung was sterilised in a sealed tube and infected with plague. The following are the results :—

Interval.	Growth in gelatine.	Growth on agar.
At once, control	†	†
After ½ hour	†	
After 1 hour	†	
After 2 hours	†	
After 3 hours	†	
After 24 hours	†	†
After 48 hours	†	†
After 72 hours	†	†
After 96 hours	–	–

2. My experiments have been carried out with numerous specimens of the following articles :—

Linseed.	Castor seed.
Yellow rapeseed.	Poppyseed.
Brown „	Wheat (new hard red).
Tilseed or Gingelly seed	„ (another lighter variety).
Groundnuts.	Flour.

3. In order to test whether the microbe was still present in a living condition in a specimen of previously infected grain, my method has been to make an extract of this grain and to inject it into a mouse. The great susceptibility of mice to bubonic plague and to infectious diseases in general, while advantageous in one sense, has been disadvantageous in another, in that the mice might have died owing to the presence of other microbes than the bubonic in the grain. In some cases the *post-mortem* appearances have suggested that this has occurred ; but as it is impossible to give an absolute proof that the bubonic microbe had nothing to do with the death of the animal in these cases, I have in general regarded the survival of the mouse as the only adequate proof that the microbe in question was no longer living in the previously infected grain.

4. My first experiment was carried out with the bubonic plague microbe derived from cultures on agaragar. Two cultures, each about a week old, were employed. Of these, one had been derived from a patient in Bombay, the other had been recently isolated from a rat found dead of the plague. The growth was scraped off the surface of the jelly and well shaken up in 15 cubic centimetres of bouillon. The mixture was then placed in a burette. A sample of the grain to be tested, weighing about a kilogram, was placed in a previously sterilised stoppered foot-glass. One cubic centimetre of the bouillon emulsion of the microbe was then poured into the foot-glass, care being taken that it should fall into the centre of the grain. The stopper was replaced, and the foot-glass was immediately violently shaken for about a quarter of an hour. At the end of this time all traces of moisture from the bouillon that had been added had completely vanished. On shaking, a cloud of dry dust could be seen in the air in the foot-glass. In order to avoid inhaling this presumably infected dust, the next part of the experiment was carried out in the open air. The grain was poured from the foot-glass into the linen bag in which I had received the specimen. From the bag a bulk of about 40 cubic centimetres was poured into a measuring glass that had been previously sterilised. This small bulk of grain was then wetted with sterile bouillon and stirred up with a glass rod. About a half to a quarter of this bouillon was sucked up into a pipette and injected into a mouse. The white mice employed had generally been in my possession for some time. Each lived in a separate cage. The cage before being occupied had in all cases been sterilised in an autoclave. The pipettes employed for the injections were always disinfected by boiling in water previous to use. The skin of the mouse at the point of inoculation was previously to the injection rubbed with a mixture of carbolic acid, water and alcohol.

5. The extracts made in this manner, as soon as possible after the infection of the grain, were injected into mice with the following results :—

Mouse, treated with extract of Linseed,			died within 24 hours.
„	„	„	Yellow rapeseed, died within 72 hours.
„	„	„	Brown „ „ 24 „
„	„	„	Tilseed „ 48 „
„	„	„	Groundnuts „ 24 „
„	„	„	Castor seed „ 24 „
„	„	„	Poppyseed „ 24 „
„	„	„	Wheat (new hard red), died within 48 hours.
„	„	„	„ (of another kind) survived.
„	„	„	Flour, died within 48 hours.

6. Two days after the infection of the grain, specimens were again taken extracted in exactly the same manner as before and injected into another series of mice with the following results :—

Mouse, treated with extract of Linseed,			died within 7 days.
„	„	„	Yellow rapeseed, died within 24 hours.
„	„	„	Brown „ „ 4 days.
„	„	„	Tilseed, survived.
„	„	„	Groundnuts, died within 11 days.
„	„	„	Castor seed „ 48 hours.
„	„	„	Poppyseed „ 48 „
„	„	„	Wheat (first variety), survived.
„	„	„	„ (second variety) „
„	„	„	Flour, died within 48 hours.

7. At this time I found out that an extract of castor seeds to which no bubonic microbes had been added was equally fatal as the infected seeds to mice, and consequently was obliged to omit this article from my experiments. The reason of this is that the castor seeds contain a poison known as ricin, which is intensely active on subcutaneous injection. On stirring up the seeds with bouillon as described caused a fatal dose to pass into solution. The mouse injected with such an extract would certainly die whether or not the bubonic microbe was present. If I made the extract of the seeds so rapidly that a fatal dose of the poison would not be dissolved, I could have no certainty that sufficient time had elapsed to allow the microbes to pass into suspension in the bouillon.

8. Four days after the commencement of the experiment, extracts of the grain were again made as before and injected into mice as follows :—

Mouse, treated with extract of Linseed,			died after 13 days.
„	„	„	Yellow rapeseed, survived.
„	„	„	Brown „ „
„	„	„	Tilseed, survived.
„	„	„	Groundnuts „
„	„	„	Poppyseed „
„	„	„	Wheat (first variety), survived.
„	„	„	„ (second variety) „
„	„	„	Flour, survived.

9. Eleven days after the commencement of the experiments extracts were again injected. A change in procedure was made in this case in that the grain, instead of being merely stirred in a measuring glass with a glass rod in order to produce the suspension in the bouillon, were slightly ground up in a mortar until the mixture of bouillon and débris was so thick that I could with difficulty suck it up into the pipette. The results obtained were as follows :—

Mouse, treated with extract of Linseed,			survived.
„	„	„	Yellow rapeseed, survived.
„	„	„	Brown „ died within 24 hours.
„	„	„	Tilseed, died within 7 days.
„	„	„	Groundnuts, died within 24 hours.
„	„	„	Poppyseed survived.
„	„	„	Wheat (first variety), survived.
„	„	„	„ (second variety) „
„	„	„	Flour, survived.

10. Thirteen days after the commencement of the experiment extracts were again injected into mice with the following results. In this, as in later experiments, the grain was extracted by grinding it in a mortar with the bouillon.

Mouse, treated with extract of Linseed,			survived.
„	„	„	Yellow rapeseed, died after 24 hours.
„	„	„	Brown „ survived.
„	„	„	Tilseed, survived.
„	„	„	Groundnuts, survived.
„	„	„	Poppyseed „
„	„	„	Wheat (first variety), survived.
„	„	„	„ (second variety) „
„	„	„	Flour, survived.

11. The mouse treated with the yellow rapeseed extract showed no appear. ances microscopically or otherwise of having succumbed to bubonic infection. There was nothing noteworthy at the seat of inoculation in the back, but there

was an extensive blood-stained œdema on the underside of the neck, but appearances suggesting that it had died from some accidental infection.

12. The above experiment indicates that the bubonic microbe derived from pure cultures perishes within thirteen days after being added to the above-mentioned specimens of grain and seeds. The question now arises whether the microbe would show a greater or lesser degree of resistance when derived from the organs of animals dead of the disease. A reply to this question is given in the following experiment.

13. The spleen of a rat and the spleen, liver and œdema from a mouse—both animals having died after plague inoculation—were ground up in a sterile mortar with powdered glass and mixed with 10 cubic centimetres of bouillon. One cubic centimetre of the mixture was added to half a kilogram of each of the specimens of grain immediately and violently shaken as in the previous experiment. Extracts were at once injected into mice with the following results :—

Mouse, injected with Linseed, died within 48 hours.
" " Yellow rapeseed, survived.
" " Brown " died in 5 days.
" " Tilseed, died in 6 days.
" " Groundnuts, died in 6 days.
" " Poppyseed, survived.
" " Wheat (second variety), died in 4 days.
" " Flour, died in 4 days.

14. The fact that two mice in this series survived and that the others only succumbed after an unusual delay may be due to the fact that the grains were extracted very rapidly with the bouillon and not ground up in it as in the preceding and following experiments. Consequently it is probable that fewer microbes than usual became suspended in the bouillon extracts.

15. Two days later, extracts were again made and injected into mice as follows :

Mouse, treated with extract of Linseed, died within 24 hours.
" " " Yellow rapeseed, died within 24 hours.
" " " Brown " " "
" " " Tilseed, survived.
" " " Groundnuts, died in 24 hours.
" " " Poppyseed " 3 days.
" " " Wheat, survived.
" " " Flour "

16. Four days after the commencement of the experiment extracts were again made and injected as follows :—

Mouse, treated with extract of Linseed, died in 5 days.
" " " Yellow rapeseed, died in 3 days.
" " " Brown " " 2 "
" " " Tilseed, survived.
" " " Groundnuts "
" " " Poppyseed "
" " " Wheat "
" " " Flour "

17. The above mice that died showed no appearances under the microscope or culturally suspicious of bubonic plague. Their spleens were injected into three other mice which have remained in good health.

18. Six days from the commencement of the experiment extracts were again injected into mice with the following results :—

Mouse, treated with extract of Linseed, survived.
" " " Yellow rapeseed, survived.
" " " Brown " "
" " " Tilseed, survived.
" " " Groundnuts "
" " " Poppyseed "
" " " Wheat "
" " " Flour "

19. Thus, in this experiment grain infected with the organs of animals dead of the plague lost its infectious power completely within six days.

20. The remaining point of the enquiry is the resisting power of the microbes as it exists in secretions of the human patient. For this purpose I chose the sputum in which the microbe has been shown by Surgeon-Captain Childe, Professor of Pathology in the Grant Medical College, to exist in large numbers and in a very virulent condition.

21. Sputum recently obtained from a patient was mixed with an equal volume of bouillon. One cubic centimetre of the mixture was immediately added as before to each of the specimens of grain. Owing to an accident, the linseed could not be used in this experiment.

22. Extracts made immediately were injected into mice with the following results :—

Mouse, treated with extract of Yellow rapeseed, died within 24 hours.
" " " Brown " " 24 "
" " " Tilseed " 24 "
" " " Groundnuts " 24 "
" " " Poppyseed " 24 "
" " " Wheat " 48 "
" " " Flour " 24 "

23. Two days later extracts were again made and injected. The results were as follows :—

Mouse, treated with extract of Yellow rapeseed, died within 24 hours.
" " " Brown rapeseed " 24 "
" " " Tilseed " 24 "
" " " Groundnuts " 28 "
" " " Poppyseed " 24 "
" " " Wheat " 48 "
" " " Flour " 24 "

24. Four days after the commencement of the experiment extracts were again injected as follows :—

Mouse, treated with extract of Yellow rapeseed, survived.
" " " Brown " died within 24 hours.
" " " Tilseed, survived.
" " " Groundnuts, survived.
" " " Poppyseed "
" " " Wheat, died within 48 hours.
" " " Flour "

25. Six days after the commencement of the experiment extracts were made and injected with the following results :—

Mouse, treated with extract of Yellow rapeseed, survived.
" " " Brown " "
" " " Tilseed, survived.
" " " Groundnuts, survived.
" " " Poppyseed "
" " " Wheat "
" " " Flour, died in 3 days.

26. No reason could be obtained from *post-mortem* appearances or cultures for thinking that the last-mentioned mouse had died of bubonic plague. Further, the flour had shown no special capacity for nourishing the bubonic microbe in my other experiments, so that it would appear to be probable that the bubonic microbe had died out in this case as in the other. Nevertheless, I am continuing my experiments with flour, and hope to make a separate report to you on the subject in a few days.

27. Earlier experiments carried out with another kind of grain (bajri) has shown me that the bubonic microbe in this case dies out within 2 days.

28. In these experiments I had kept the grain damp, so that eventually it became covered with mildew. In the experiments now described, by keeping the grain in a dry condition in bags, I imitated more closely the conditions that exist in commerce. Under such conditions the microbe of bubonic plague appears to die out within from 4 to 6 days. In a few instances, my experiments

have suggested that it may survive for periods extending up to 13 days. But it must be remembered that my experiments had to be carried out with the aid of mice owing to the impossibility of obtaining a sufficient supply of healthy rats in Bombay at the present time. It is more probable that the death of the mice in these exceptional instances was due to the accidental presence of other microbes than that unusually resistant forms of the bubonic microbe were present.

I have the honour to be,

Sir,

Your obedient servant,

E. H. HANKIN.

From

E. H. HANKIN,

Chemical Examiner and Bacteriologist,

N.-W. P. & Oudh;

To

SURGEON-MAJOR-GENERAL H. COOK,

Surgeon-General with the Government of Bombay.

Hurdwar, 28th June 1897.

SIR,

I have the honour to submit the following report on the action of disinfectants on the microbe of bubonic plague.

2. Generally speaking, two chief methods of testing the action of disinfectants are in use. In the first the microbe is mixed with a solution of the antiseptic of known strength, and after a definite interval a small quantity of the mixture is injected into an animal susceptible to the microbe employed. If the microbe is still alive, it reproduces and causes the death of the animal. If the microbe has been killed by the disinfectant the animal will remain in good health. Although this method has been found useful in the case of certain spore-forming microbes, it is not of general applicability, in that it is possible that the microbe might become attenuated by the action of the antiseptic, and thus incapable of killing the animal employed. In the other method the mixture of the microbe with the disinfectant is inoculated into sterilised food media. If the medium remains sterile, it is concluded that the microbe has been killed by the disinfectant. Except in one or two experiments I have used this method. In carrying it out, it was first necessary to obtain a series of solutions of the antiseptic of known and increasing strengths. Especially in the case of the weaker disinfectants it was always possible that foreign and resistant microbes might be present, and hence before commencing the experiment it was necessary to sterilise these solutions. In the case of non-volatile disinfectants this could be easily done by placing the solutions in test tubes plugged with cotton wool and sterilising them under steam pressure in the autoclave. In the case of volatile disinfectants, on the other hand, a more complicated procedure was necessary. A series of test tubes each containing exactly 5 cubic centimetres of water was prepared and sterilised. A strong solution of the antiseptic of known strength was then prepared, hermetically sealed up in a tube and sterilised in the autoclave. It was then opened under aseptic precautions, and measured quantities were taken out by means of a sterilised pipette and placed in the test tubes of water. It was then necessary to add the bubonic microbe to these solutions. My previous work on the fate of the bubonic microbe in grains and other articles of export had shown me that this microbe is almost equally resistant whether obtained from solid or liquid cultures or from the organs of animals dead of the disease. If there is any difference it is the microbe existing in agaragar cultures that is the more resistant. Consequently, in all my experiments I have employed emulsions of well grown agaragar cultures. Five cubic centimetres of sterilised water were poured into the test tube containing the culture under suitable precautions. By means

of a glass bristle the film of growth was well rubbed up in the liquid until a fairly homogeneous emulsion was produced. Usually four drops of this emulsion were added to each of the tubes containing the disinfectant solutions. After from five to ten minutes a measured quantity of the mixture of microbes and disinfectant was removed by means of a capillary pipette and placed in a tube of gelatine. In my earlier experiments I used bouillon for this purpose. In the case of other microbes bouillon would give perfectly reliable results. I am indebted to the members of the German Plague Commission, however, for information as to a source of error that would occur with the bubonic microbe if bouillon is used. The bubonic microbe will only grow in cultures in the presence of free oxygen. If inoculated into traces into bouillon after being subjected to the action of antiseptics, there is the possibility of its falling to the bottom of the tube where the supply of oxygen is insufficient and hence of its refusing to grow. To avoid this risk the German Commission worked with gelatine plate cultures which after inoculation were kept in an ice box. I found it more convenient to employ test tubes containing small quantities of nutrient gelatine. After inoculation these tubes were kept at the room temperature lying in an oblique position so that the gelatine was in a thin layer. As different specimens of gelatine do not always show the same power of supporting the life of the bubonic microbe, I usually took the precaution of inoculating each mixture of the microbe and disinfectant into two tubes containing nutrient gelatine of different strengths. If the control tube in one set of tubes did not grow well, that of the other series was almost certain to be normal and the results of that series were recorded. The control tube, it may be explained, was a tube containing sterilised water to which no antiseptic had been added. It was inoculated with the emulsion of the microbe at the same time as the other tubes, and similarly the microbes it contained were further inoculated into gelatine.

In the case of growth resulting in the gelatine tubes, a most important part of the experiment was to obtain a proof that this growth was that of the bubonic microbe and not of some other microbe. The growth in gelatine, as in bouillon, is in some cases somewhat characteristic. After 24 to 48 hours, if the tube has been inoculated with only a trace of the microbe, a few light floculi may be seen sticking to the side of the tube. If the tube is moved so that the liquid it contains is disturbed, it is seen that these floculi are readily detached and broken up in the liquid. If the tube is slightly shaken the floculi may be completely broken up and suspended in the liquid, which may then appear perfectly transparent as if no growth was present. So delicate are these floculi and so easily broken up, that simply roughly picking up the tube may, owing to the consequent shaking, make the growth nearly or completely invisible. Hence it is necessary to keep the tubes for at least a week in order to be certain that no growth is present. At the end of this time the growth will be so copious that there is no risk of overlooking it, but its appearance cannot be regarded as sufficiently characteristic. For this reason, in every case I have inoculated the gelatine on to agaragar in order to learn whether or not a pure culture of the bubonic microbe was present.

A somewhat unexpected source of error here presented itself. When inoculated on to agaragar the bubonic microbe produces a culture that is distinguishable after it has grown for three or four days from that of most other microbes by the practised eye. The parts of the culture inoculated with a small quantity of material contain numerous minute not readily confluent colonies. Near the bottom of the tube where a larger quantity of material was deposited by the inoculating needle, the agaragar is covered with a continuous growth whose appearance has been described by Kitasato as resembling ground glass. In certain cases, and especially if the tube has been inoculated from the organs of animals dead of plague, the growth may be local. For instance, if the inoculating needle has been moved in a zigzag track over the surface of the agar, growth may only ensue at and near the turns of the zigzag, or the growth may be limited to the commencement of the track. Apparently the microbe in such cases only grows where it has been deposited in a quantity or in a layer on the surface of the agaragar. When the microbe of plague is inoculated from 20 per cent. gelatine on to agaragar, it grows readily over the whole surface of the track of the needle. The growth has the typical "ground glass" appearance. It happened,

however, in my experiments that certain other microbes, that occasionally occur as impurities, when inoculated from 20 per cent. gelatine on to agaragar, instead of acquiring their ordinary cultural characters, form a growth possessing a ground glass appearance extremely difficult to distinguish from that of the bubonic microbe. Hence in experiments on the action of disinfectants on this microbe, the appearance of the agar culture cannot be relied on to prove that a pure culture was present. It is necessary to examine the culture microscopically after sufficient time has elapsed to permit the development of involution forms. This may involve a delay of a week or more before a conclusion can be arrived at as to the purity of the culture. To obviate this necessity, I have in certain cases made use of a test for the microbe that I discovered some time ago. If the microbe is grown in agaragar containing three or four per cent. salt, the involution forms develope extremely rapidly. In favourable cases they may be found in numbers 24 hours after the culture has been inoculated. A rather large quantity of material should be employed for inoculating the culture. It is not possible to give a definite figure as to the amount of salt necessary as it appears to vary in different cases. The involution forms at first formed appear as spheres, sausage, or lemon-shaped objects larger than are usually found in on ordinary agaragar cultures. Surgeon-Captain James, who has been working in my laboratory in Bombay, has tried this method on bubonic microbes of many different origins and has found them in every case to give this peculiar reaction. Surgeon-Captain Thomson has repeated this work and is at present investigating the technique most favourable for producing the effect.

In the following description of my experiments, I have in every case mentioned the occurrence of impurities where such were found. As I was working in a small draughty and overcrowded laboratory the avoidance of impurities was a matter of some difficulty.

3. *Action of the Phenols and their allies on the Bubonic Microbe.*

The following experiment illustrates the action of carbolic acid on the bubonic microbe. As above described tubes containing solutions of carbolic acid of different strengths were inoculated with measured quantities of the microbe, and after five to ten minutes the mixture of microbes and disinfectant were inoculated further into gelatine. In this experiment each disinfectant solution was inoculated into four tubes. Of these, two contained 2 per cent. gelatine, and the other two gelatine of twenty per cent. One set of tubes was kept at air temperature (about 32 Cent.). The other set was kept in an ice box whose temperature was about ten degrees lower. The results obtained were as follows :—

Per cent. of disinfectant.	Kept at air temperature.		Kept in ice box.	
	After 5 minutes inoculated into 2 per cent. gelatine.	After 20 minutes inoculated into 20 per cent. gelatine.	After 5 minutes inoculated into 2 per cent. gelatine.	After 20 minutes inoculated into 20 per cent. gelatine
0 control	†	†	†	†
·5	†	†	†	†
1	–	†	–	–
2	–	–	–	–
3	–	impure.	–	–
4	–	–	–	–

In the above table the sign "†" means that a culture of plague was produced in the gelatine tube inoculated from the mixture of microbes and carbolic acid of the strength indicated in the left hand column. The sign "–" means that the gelatine tube remained sterile.

From the above experiment it appears that a half per cent. solution of carbolic acid even after 20 minutes action is incapable of destroying the plague microbe. Disinfection is not certainly produced by 1 per cent. in the same time. Other experiments gave similar results. Kitasato found that 1 per cent. of carbolic acid was necessary to destroy the plague microbe; $\frac{3}{4}$ per cent. was incapable of destroying it in an hour's time. According to Abel it is not destroyed even in six hours by a one per cent. solution. Consequently the plague

microbe cannot be regarded as particularly sensitive to the action of carbolic acid.

Carbolic acid is not much used in practice on a large scale. Its place is usually taken by a substance known in India commercially as "phenyle." This is a distillation product containing various phenols and cresols. Its action on the bubonic microbe was tested with the following results :—

Percentage of disinfectant.	After 5 to 10 minutes inoculated into 20 per cent. gelatine.	After 5 to 10 minutes inoculated into 1 per cent. gelatine.
0 control.	†	†
·1	–	–
·25	–	–
·5	–	–
·75	–	–
1·0	–	–

Consequently phenyle appears to be more active than carbolic acid on the microbe of plague.

Izal gave the following results :—

Percentage of disinfectant.	After 5 minutes.	After 10 minutes.	After 20 minutes.	After 1 hour.
0 control.	†	†	†	†
·1			impure.	–
·2	–	–	–	impure.
·4	impure.	–	–	–
·6	–	–	–	–
·8	–	impure.	–	
1·0	–	–	impure.	
2·0	–	–	–	
3·0	–	–	–	

Thus izal in a strength of ·1 per cent. or one in a thousand appears to kill the plague microbe in an hour. In a strength of one in five hundred it appears to kill it in 5 minutes. Similar results were obtained in another experiment.

The objection may be raised to both of the last named disinfectants that they are insoluble in water merely forming in it a milky emulsion. Although this emulsion appears to be quite capable of destroying the microbe under the conditions of the above experiments it would be rash to conclude that it would be capable of killing it to the same extent under the conditions that exist in nature. For instance, if the microbe is contained in partially dried human excreta, or if it has soaked into a cowdung floor, a soluble disinfectant might penetrate the surrounding organic matter and destroy it. On the other hand, it is difficult to imagine how the same penetrative power could be possessed by the globules of an emulsion. Further experiments are necessary before a definite opinion can be expressed on this point.

Such an objection cannot be raised against lysol, another disinfectant of the same class. This consists of cresols &c. mixed with a quantity of soap. Owing to the presence of the latter substance the active substances it contains are soluble in water in all proportions. An experiment gave the following results :—

Percentage of disinfectant.	After 5 minutes inoculated into gelatine.	After 15 minutes inoculated into gelatine.
0 control.	†	†
·25	–	–
·5	–	–
1·0	–	–
2·0	–	–
3·0	–	–

Similar results were obtained in another experiment.

It is well known that naphthaline is not a disinfectant; nevertheless it has been used widely by the general public in Bombay. As there was a possibility that the impure commercial napthaline had some antiseptic action, I have put the matter to a test, together with a patent preparation known as "napthosublimate". The following results were obtained:—

Percentage of disinfectant.	Pure naphthaline.	Impure naphthaline.	Naphthosublimate.
0 control.	†	†	†
·01	†	†	†
·02	†	†	†
·04	†	†	†
·06	†	†	†
·1	†	†	†
·15	†	†	†

Naphthaline is insoluble in cold water. In the above experiments I made an emulsion of the substance by suspending a measured quantity in boiling water. While still hot I added the requisite quantities to the series of test tubes of sterilised water. Hence the napthaline was in each case more finely divided, and more likely to come into contact with the microbes than would be the case in practice. Nevertheless no antiseptic action could be detected.

4. Action of corrosive sublimate and copper sulphate on the Plague Microbe.

The above metallic salts were tested with the following results:—

Percentage of disinfectant.	Copper sulphate.	Corrosive sublimate.
0 control.	†	†
·02	†	–
·04	†	–
·06	impure.	–
·08	†	–
·1	–	–
·15	–	–

Abel, whose method of work seems to have been somewhat different from mine, finds that ·02 per cent. of sublimate disinfects agaragar cultures within an hour, and that ·1 per cent. disinfects within 10 minutes.

Copper sulphate, under the name of "Nela tutiya" can be obtained in almost any village, and as shown by the above experiment might be used as a disinfectant in cases of necessity. A five per cent. solution of copper sulphate was used by the French Government in 1892 in combating cholera. The objection may, however, be raised against both the above disinfectants that they are precipitated by albuminous and other organic substances, and that they hence lack the penetrative power required in a disinfectant for solid dejecta &c.

The fact that solutions of sublimate are somewhat apt to decompose on keeping is not of serious import with regard to the use of this substance on a large scale, as under these conditions the solution is usually freshly made up. A practical difficulty however exists in the fact that sublimate is not always readily soluble. Both in Bombay and elsewhere it has been noticed that the "sublimate" used in disinfecting houses does not completely dissolve in the water employed. I have recently had an opportunity of investigating the matter, and I find that a part of the so-called sublimate refuses to dissolve even in boiling water. Apparently a part of this sublimate is made of "ruskapoor". This is a bazár product consisting of a mixture of sublimate and calomel. The solution obtained in practice possesses some disinfecting power as I have found by experiment, but it is probably not so efficient as would be a similar solution made of pure sublimate.

5. Action of alkalies on the microbe of Bubonic Plague.

As shown by the following experiments this microbe is remarkably resistant to the action of alkalies.

The percentages of ammonia expressed in the following table represent the amount of a saturated watery solution of ammonia that was added to the sterilised water employed. In the case of the caustic potash every precaution was taken that the specimen employed should be fresh and active:—

Percentage of disinfectant.	Ammonia in 20 per cent gelatine.	Ammonia in 2 per cent gelatine.	Caustic Potash in 20 per cent gelatine.	Caustic Potash in 2 per cent gelatine.
0 control.	†	†	†	†
·25	†	†	†	†
·5	†	†	†	†
1·0	†	impure.	†	†
2·0	†	impure.	–	–
3 0	†	†	–	–

In the following experiment with quicklime, the greatest precautions were taken to obtain this substance in its most active condition. Perfectly fresh katni lime was obtained and mixed with water that had been recently boiled to avoid the risk of any of the lime being neutralised by carbonic acid that might otherwise have been present. The following results were obtained:—

Percentage of disinfectant.	Inoculated into 20 per cent. gelatine.	Inoculated into 2 per cent. gelatine.
0 control.	†	†
·25	–	†
·5	–	–
·75	–	–
1·0	–	–
2·0	–	–
3·0	†	†

I took great pains to prove that the cultures obtained from the 3 per cent. lime solution, or rather suspension, were pure, and I have no doubt of the fact. A source of error in experiments with alkalies may here be mentioned. Although the microbe of plague is not readily killed by antiseptics, its growth in liquid cultures is readily inhibited by a too strong alkaline reaction. In the above experiment it is possible that the amount of lime solution added to the gelatine might alter the reaction and so make the food medium less well fitted for the development of the microbe. The two per cent. gelatine used in the above experiment was slightly acid (amphotere) in reaction and therefore less likely to be altered in this way.

In practice, however, lime is not likely to be used under such favourable conditions for the development of its disinfectant action. Especially in the hot and damp climate of many parts of India it readily absorbs water and carbonic acid from the atmosphere and thus becomes changed into carbonate of lime which is without action on bacteria. The following experiment more closely imitates the practical conditions of its employment.

A basket full of the fresh lime, as used in the above experiment, was allowed to stand in the verandah of my laboratory for several days. By this time the lime had changed into a loose powder. This was well stirred up so that the specimen taken should represent the average. The following results were obtained:—

Percentage of Lime.	After 10 minutes in 20 per cent. gelatine.	After 10 minutes in 2 per cent. gelatine.	After ½ hour in 2 per cent. gelatine.
·0 control	†	†	†
25	†	†	†
·5	†	†	†
·75	†	†	†
1·0	–	–	impure.
2·0	†	†	†

Water dissolves 0·13 per cent. of lime, so that the solutions of lime used in the above experiments were more than saturated. In view of the above it may be asked whether whitewashing of suspected houses is a valid precaution against the plague. With regard to the outside of the house, it is not likely that it is the source of infection, and so long as whitewashing is in native hands, this part of the work is likely to be considered as the most important because it is most easily visible to an inspecting officer. For instance, a few months ago a native official in a Native State, that was threatened with the plague, published an order that only the outside of the houses were to be whitewashed in order to avoid annoying the inhabitants. Rogers Pacha tells me that in combating cholera in Egypt the native employés were very apt to whitewash only the exterior of a house, hoping that the sanitary officer would infer that the inside had also been done. *A priori* it might be urged that whitewashing the inside of the house would be useful because it would involve a turning out of the inhabitants and a ventilation of their belongings while the work is proceeding. I have no wish to deny that this is likely to be the case, but it is probable that the end could be reached in a more effective way.

Whitewashing has another disadvantage in that lime combines with and neutralises many of the more commonly used disinfectants. For instance, being an alkali it combines with acids which, as will be shown below, are efficient disinfectants for the bubonic microbe. With carbolic acid and phenyle it forms carbolates which, according to Notter, are of but slight disinfectant power. With sublimate it forms an insoluble compound, probably destitute of antiseptic action. If sulphur is burnt in a freshly whitewashed house, the sulphurous acid produced is likely to combine with the lime, producing calcium sulphite, a substance of doubtful antiseptic power.

6. *Action of Acids on the Microbe of Bubonic Plague.*

The microbe of plague is extremely sensitive to the action of acids.

I have tested the action of certain organic acids on this microbe, and the results appear to have some bearing on the epidemiology of the disease. It may be mentioned that "aromatic vinegar" was much used in the Middle Ages in Europe for destroying the contagion of plague. As will be seen from the following experiments, its essential constituent, acetic acid, has a strong action on the plague microbe.

Formic acid was tested with the following results:—

Percentage of Disinfectant.	Growth in gelatine 20%.
·0 control	†
·1	†
·5	†
1·0	–
2·0	impure.
3·0	–

The following results were obtained with acetic acid in a series of experiments:—

Percentage of Acid.	With 10% gelatine.	With 20% gelatine.	With 2% gelatine.	With 20% gelatine.
0 control	†	†	†	†
·1	†	†	†	†
·2			†	†
·3			–	†
·4			–	†
·5	impure.	†	–	†
·7			–	–
1·0	–	–	–	–
2·0	–	–		
3·0	–	–		

The following experiments were carried out with lactic acid:—

Percentage of Acid.	With 10 per cent. gelatine.	With 2 per cent. gelatine.	With 20 per cent. gelatine.
0 control ...	†	†	†
·01 ...		†	†
·03 ...		†	†
·05 ...		†	†
·07 ...		–	†
·1 ...	–	–	†
·2 ...		–	†
·3 ...		impure.	–
·5 ...	–		
1·0 ...	–		
2·0 ...	–		
3·0 ...	–		

To summarise the above results the bubonic microbe is destroyed in five minutes by

Formic acid	of a strength	of 1 in 100.
Acetic acid	„ „	1 in 142.
Lactic acid	„ „	1 in 333.

Most articles of diet either possess an acid reaction or acquire it on decomposition owing to the development of the above acids or of others closely allied to them. In the case of fish and meat a strongly marked acid reaction is present. In view of the above results it appears scarcely likely that these substances should retain or convey the bubonic plague infection. If grain and other substances containing carbohydrates are kept in a damp place, fermentations set in which usually, but not always, result in the appearance of fatty acids. Hence it is difficult to understand how the bubonic infection could lurk in rotten grain as has been held by Dr. Waters and others. Shortly after it has been obtained, milk in this country acquires an acid reaction owing to the development of lactic acid. Its action on the bubonic microbe has been investigated by Dr. Srinivasa Rau in my laboratory. The following is an account of his experiments.

Three test tubes of milk were taken. One contained milk fresh from the dairy. It possessed a faint acid reaction. Another contained milk whose reaction had been made faintly alkaline by the addition of soda. The third contained milk that had been kept from the preceding day and whose reaction was strongly acid. These three specimens were sterilised in hermetically sealed tubes in the autoclave. On cooling they were inoculated with the bubonic microbe. At the intervals stated below the infected milk was inoculated on to agaragar with the following results:—

	Fresh Milk.	Sour Milk.	Alkaline Milk
At once ...	Growth	Growth	Growth.
After half an hour...	No plague but one foreign colony.	Sterile	Do.
After 1 hour ...	Sterile	Do.	Do.
After 1½ hours ...	Do.	No plague but foreign colonies.	Impure.

In another similar experiment milk having an amphotere reaction was employed, and the microbe was found to remain alive for 24 hours.

In the following experiment the conditions were varied in that the infected milk was inoculated into gelatine instead of agaragar:—

	Fresh (amphotere) Milk.	Alkaline Milk.	Sour Milk 24 hours old.
At once	+	+	+
After half an hour ...	+	+	+
After 1 hour ...	+	+	–
After 1½ hours ...	+	+	–
After 2 hours ...	+	+	–
After 2½ hours ...	+	+	–
After 3 hours ...	+	+	–

Each of the above gelatine cultures was inoculated into agaragar, and proved to be pure. Other experiments confirmed these results. Thus as soon as milk has been kept long enough to acquire a well marked acid reaction, it possesses the power of destroying the bubonic microbe.

Although the tissues of a living animal, so far as is known, possess an alkaline reaction, those of a dead animal possess an acid reaction which undergoes a great increase on the occurrence of *rigor mortis*. Such acidity is found in the tissues of animals dead of plague. In some experiments carried out in my laboratory by Dr. Rohak to test this point, it was found that before *rigor mortis*, the acidity in the body of a mouse from which the head skin and viscera had been removed, corresponded to 3·76 c. c. of decinormal soda solution per 10 grammes of mouse. The acidity of another mouse estimated after the development of rigor mortis required 5·7 c. c. for its neutralisation. The acidity of two mice dead of plague was found to correspond to 5·79 and 4·37 c. c. of soda solution respectively. It is possible that the presence of this acid in the bodies of persons dead of plague may aid in causing the disappearance of the microbe after burial. The point obviously is deserving of further investigation.

As may be expected the action of mineral acids on the bubonic microbe is yet stronger than that of organic acids. The following table shows the results obtained with nitric and hydrochloric acids :—

Percentage of Acid.	Nitric Acid.			Hydrochloric Acid.	
	A.	B.	C.	A.	B.
0 control	+	+	+	+	+
·01	+	+		+	+
·03				+	
·05	+	+	+	+	+
·07	+		+		
·1	+	–	impure	+	–
·2	+	–		impure	–
·3			–	–	–
·5	–	–	–	–	–
·7		–			
1·0		–	–	–	–

The following experiments were carried out with sulphuric acid and a mixture of four parts of sulphuric and one part of nitric acids :—

Percentage of Acid.	Sulphuric Acid.			Mixed Acids.	
	A.	B.	C.	A.	B.
0 control	+	+	+	+	+
·01	+	+	+	+	+
·02		+	+	+	+
·03	+	impure	+	+	+
·04		–	–	–	–
·05	–	impure	+	+	–
·07	–	impure	–	–	–
·1	–				

The above results may be summarised as follows. The bubonic microbe was found to be destroyed in the above experiments by

Nitric acid in a strength of 1 in 333.
Hydrochloric acid „ 1 in 500.
Sulphuric acid „ 1 in 1,429.

The great cheapness of sulphuric acid, the probability of its exerting a continued action, and its activity in destroying the bubonic microbe suggest that it might be introduced with advantage as a disinfectant. Provided the soil is not highly calcareous it might be used in a strength of 1 in 250 for disinfecting infected floors. It will be noticed that in the last experiment I tested the action of a mixture of four parts of sulphuric acid with one part of nitric acid on the bubonic microbe, and that its activity appeared to be equal to that of sulphuric acid alone. The waste acids from the Government Cordite Factory are of about this composition, and thus might find employment as disinfectants.

7. Action of reducing agents on the Bubonic Microbe.

Among reducing agents, ferrous sulphate has been frequently recommended for the treatment of sewage and filth. It will be interesting to examine its action on the bubonic microbe.

Ferrous sulphate in solution readily absorbs oxygen from the air. Consequently in the following experiment measured quantities of solid ferrous sulphate were weighed out and added to recently sterilised and still hot water. After the mixture the solutions were again heated. The following results were obtained :

Percentage of ferrous sulphate.	Growth in gelatine.
0 control	+
·1	+
·2	+
1·0	impure.
2·0	+

In the following experiment ferrous sulphate was obtained in alkaline solution by the following method. A solution was made containing 10 per cent. of ferrous sulphate, 10 per cent. of tartaric acid, and 1 per cent. of citric acid. The mixture was made faintly alkaline with ammonia. A dark green liquid resulted, but no precipitation occurred. The percentages stated in the following table are those of the iron present on the supposition that it existed as ferrous sulphate. The following are the results :—

Percentage of Disinfectant.	After 10 minutes.	After 24 hours.
0 control	+	+
·5	+	+
1·0	+	–
2·0	+	–
3·0	+	–
4·0	+	–
5·0	+	–
6·0	+	–
10·0	+	–

That is to say, a solution containing 10 per cent. of this strong reducing agent, *i. e.* the solution originally prepared, had no power of destroying the bubonic microbe during 10 minutes action. Even a half per cent. solution did not exert an antiseptic action after 24 hours. Control observations were made that showed that the above solutions did possess a strong reducing action after sterilising, that is to say, as used in my experiment.

The researches of Wilm and other observers show that the bubonic microbe is destroyed by a few hours' drying in a dessicator. It occurred to me that it would be interesting to learn whether it would die out so rapidly if dried in a current of hydrogen gas. To test this I blew a series of bulbs on a glass tube. The portions of the tube between each pair of bulbs were drawn out, so that afterwards each bulb could be easily fused off. Hydrogen from a Kipp's apparatus was passed through a wash-bottle containing silver nitrate, and another containing lead nitrate, to purify it. It then passed through a large wash-bottle containing strong sulphuric acid to remove its moisture. From this it passed through the row of bulbs. The apparatus was kept in action for 24 hours to expel all traces of oxygen. A bouillon culture of plague was then centrifugalised under aseptic precautions. The supernatant liquid was then poured off, leaving a sediment consisting of a dense mass of the microbes suspended in a few drops of bouillon. The current of hydrogen was then turned off, and the upper bulbs of the series slightly warmed to expel some of the hydrogen. The distal end of the row of bulbs was then placed in the mass of bacilli in the centrifuge tube. A few drops of the suspension was thereupon drawn up owing to the contraction of the hydrogen on the cooling of the bulbs. By tilting and turning the tube the whole of the inside of the glass was wetted with the suspension of the miscrobes. The tap from the hydrogen apparatus was then opened and in the current of dried hydrogen the film was rapidly evaporated to dryness. After the hydrogen had passed through the row of bulbs for the whole day, it was hermetically sealed at both ends in a blowpipe flame. One bulb was then fused off, ground up in a sterile mortar with a few drop of bouillon, and the resulting liquid injected into a mouse. This mouse died of plague. Other bulbs were fused off and similarly injected into mice on succeeding days. In this way the microbe was found to be still alive five days after the commencement of the experiment.

It is impossible to draw a definite conclusion from the above experiment, because owing to the interruption of my work consequent on my transference to Hurdwar, I have not been able to carry out analogous experiments in which the microbe should be evaporated to dryness under similar conditions in a current of air or oxygen. At the same time, in view of the asserted influence of ventilation in destroying the infection of plague, the above result is at least suggestive.

8. *The action of oxidising agents on the Bubonic Microbe.*

The above experiments have shown that this microbe is resistant to reducing agents. It will now be shown that it is extremely susceptible to the action of certain oxidising agents.

The following experiments were carried out with chloride of lime. The specimen employed was perfectly fresh and strongly smelling of chlorine. The results obtained are as follows :—

Percentage of Disinfectant.	After 5 minutes in 20 per cent. gelatine.	After 5 minutes in 1 per cent. gelatine.
0 control	†	†
·01	—	—
·05	—	—
·3	—	—
·5	—	—
·7	—	—
1·0	—	—

With regard to chloride of lime it may be noted that it loses its disinfectant power when kept in improperly closed vessels. When fresh it has the characteristic and disagreeable smell of chlorine. Owing to this simple fact not being generally known, a merchant of Bombay at the commencement of the epidemic was able to sell many barrels of chalk as chloride of lime. The liberation of hypochlorous acid and chlorine from the chloride of lime depends on the absorption of carbonic acid from the air or on the addition of acids. It must be borne in mind that this substance must not be mixed with lime or other alkalies.

Potassium permanganate is not generally recognised as a useful disinfectant. Although some microbes are so resistant to its action that they are only destroyed by it in a five per cent. solution (Koch), my experiments show that it is an extremely potent destroyer of the bubonic microbe. This observation is so contrary to what might have been expected that I thought it advisable to subject it to an extended test. The following table shows my first results :—

Percentage of Permanganate.	A	B	C	D	E	F	G	H	I	J
0 control	†	†	†	†	†	†	†	†	†	†
·005		impure.	—	†						
·01	—	—	—	†	—	—	—	impure.	—	—
·02	—	—	—	—	—	—	—	—	—	—
·03	—	—	—	—	—	—	—	—	—	—
·04	—	—	—	—	—	—	—	—	—	—
·05	—	—	—	—	—	—	—	—	—	—
·06	—	—	—	—	—	—	—	—	—	—
·07	—	—	—	—	—	—	—	—	—	—
·08	—	—	—	—	—	—	—	—	—	—
·1	—	—	—	†	—	—	—	—	—	—

Experiment "D" is not comparable with the others, in that the solutions of permanganate employed were those that had been employed two days before the experiment "C." The tubes containing ·005 and ·01 per cent. were at the time of reinoculation completely reduced. Before reinoculation of the tube containing ·1 per cent. sufficient gelatine was added to it to completely reduce the permanganate present. Consequently in these three tubes the microbe was not exposed to the action of permanganate, but only to its decomposition products. As shown by the above results it was thus proved that the microbe of plague is not harmed by these decomposition products in the strength used in my experiments. That the microbe was really dead in the above series of tubes was also proved by injecting a few drops of each solution into mice. This was done with the tubes that had not been reduced and with the last tube in the series, in which as already stated the permanganate had been reduced. All the mice remained in good health except the one inoculated from this last tube, which died of plague.

Thus the above experiments show that under the conditions of the experiments the bubonic microbe is destroyed within five minutes by permanganate in a strength of 1 in 10,000. Experiment "C" suggests that even diluter solutions would be efficient.

The chief objection to the use of permanganate is that it is so readily destroyed by all sorts of organic matter. Hence if used to disinfect microbes as they are found in nature there is a risk of its being reduced so rapidly by the filth mixed with the microbes that the latter would remain unaffected. I noticed that in certain of the tubes containing the diluter solutions of permanganate in the above experiments, on the following day the permanganate was completely reduced. In other words, that the permanganate had first destroyed the bubonic microbes and afterwards had been reduced by the organic matter inevitably present. It appeared to me to be worth while to put this matter to a further test.

Permanganate solutions of different strengths were poured into a series of well grown agaragar cultures. The thick film of culture was then at once stirred up with a sterilised needle and mixed with the permanganate solution. Sufficient of the solution was in each case added to the culture tube to cover the agaragar when the tube was held in an erect position. At intervals stated below the suspension of the microbes mixed with permanganate was inoculated

into gelatine. Although the amount of organic matter present was far more than enough to reduce the small quantity of permanganate employed, the following results were obtained :—

Percentage of Permanganate.	1 Minute.	3 Minutes.	4 Minutes.	5 Minutes.
·05 ...				
·1 ...	–	–	–	
·2 ...	–			–
·3 ...	–			–
·4 ...	–			–
·5 ...	–			–
·7 ...	–			–
·8 ...	–			–
·9 ...	–			–
1·0 ...	–			–

In inoculating the gelatine tubes two or three drops of the emulsion of the microbes and permanganate solution was removed with a capillary pipette and placed in the gelatine. Hence there is a probability that masses of the culture film were carried over into the gelatine tubes. Thus the above experiment indicates that the permanganate possesses more power of penetrating than might have been expected.

In another experiment a half per cent. solution of permanganate was poured on to a well grown agar culture. The film of culture was not disturbed, so that it would have to be penetrated by the permanganate solution for the disinfecting action to be exerted. At intervals stated below the culture was tilted so as to bring part of the growth out of contact with the permanganate solution and a visible quantity of the growth was removed by means of a glass bristle and inoculated into bouillon. The results were as follows :—

After 1 minute ... ... Growth.
„ 2 minutes ... ... Sterile.
„ 3 „ ... ... do.
„ 5 „ ... ... do.

The addition of sulphuric acid in the proportion of 294 parts of acid to 316 parts of permanganate increases the amount of oxygen that it liberates. It occurred to me that it would be of interest to see if in this way its action on the bubonic microbe could be increased. In order to find a difference in the action of permanganate under these two conditions it was obviously necessary to work with diluter solutions than those previously employed. Experiment showed that heating in the autoclave often resulted in the complete reduction of the permanganate, partly owing to the presence of organic matter in the tap water, and partly owing to the presence of dirt on the inside of the tubes. In the following experiment after making up the solutions they were merely heated to boiling point in a water-bath. In each case the solution containing ·001 per cent. of the permanganate was found to be reduced. These tubes were therefore used as control. The following are the results :—

Percentage of Permanganate.	Acidified Permanganate.	Neutral Permanganate.	Alkaline Permanganate.
·001 control ...	†	†	†
·002 ...	– *	– *	– *
·003 ...	– *	– *	– *
·004 ...	– *	–	– *
·005 ...	– *	–	–
·006 ...	– *	–	–
·007 ...	– *	–	–
·008 ...	– *	–	–
·01 ...	–	–	–
·05 ...	–	–	–

The tubes marked with an asterisk were found to be completely reduced on the following day. The alkaline series of permanganate solutions was made by the addition of one cubic centimetre of normal soda solutions to 50 c.c. of a ·01 solution of permanganate that was used in making up the diluter solutions employed in the experiment. The experiment was repeated with the additional precaution that the test tubes were first boiled in acidulated permanganate solution, then washed in a solution of sulphurous acid, and then again boiled in neutral permanganate solution. In this way organic matter capable of acting on the permanganate was removed from the inside of the tubes. The solutions employed were made up not in tap water but in distilled water containing three-quarters per cent. of common salt. The following are the results :—

Percentage of Permanganate.	Neutral Permanganate.	Acid Permanganate.
0 control... ...	†	†
·001	†	†
·002	–	–
·003	–	–
·004	–	–
·005	–	–
·006	–	–
·007	–	–
·008	–	–
·009	–	–
·01	–	–

Thus the above experiments did not show that acid when added to the permanganate increased its action on the bubonic microbe. On the other hand, it appeared that whether in acid neutral or alkaline solution if there was enough permanganate present to produce a clearly marked pink colour the microbe was destroyed within five minutes.

The evidence in my possession leaves practically no room for doubt that potassium permanganate when used in well water every three days can check a waterborne cholera outbreak. But there is reason for believing that the cholera microbe is far more resistant to the action of permanganate than is the microbe of bubonic plague. Hence it might be concluded that permanganate might be employed in checking the latter disease. But owing to the ease with which permanganate is destroyed by organic matter, and owing to the fact that articles most in need of disinfection are apt to contain large quantities of such matter, it is probable that such strong solutions of permanganate would be necessary, that the expense and inconvenience of the method would call for consideration. That this would be the case is indicated by the following facts.

The part of a room probably most liable to infection and most in need of disinfection is the floor. Among the poorer classes in this country, floors are generally covered with a mixture of cowdung and mud. Cowdung contains substances that rapidly destroy permanganate, as is shown by the following experiment.

Ten grammes of cowdung were boiled in a litre of water. A hundred cubic centimetres of the mixture was poured into each of a series of foot-glasses. Progressively increasing quantities of permanganate solution of a strength of one per cent. were mixed with the contents of the series of foot-glasses. It was found that the addition of 4 c.c. of the permanganate solution was necessary to produce a red colour lasting for a quarter of an hour. In the foot-glasses containing smaller quantities the colour rapidly vanished owing to the reduction of the permanganate. Now the quantity of permanganate contained in 4 c.c. of the solution was ·04 grammes. The quantity of cowdung contained in the liquid to which it was added was 1 gramme. Consequently ·04 grammes of permanganate are necessary to act on one gramme of cowdung. Therefore in disinfecting a cowdung floor, each gramme of cowdung must absorb a quantity

of liquid containing ·04 grammes of permanganate. Let us make an assumption probably in excess of the truth, to the effect that each gramme of cowdung absorbs one cubic centimetre of liquid. Then for disinfection to ensue, each cubic centimetre of the liquid must contain ·04 grammes of permanganate. If one cubic centimetre contains this quantity, then 100 cubic centimetres will contain 4 grammes, or in other words a four per cent. solution would have to be employed.

8. General Considerations.

The conditions of the above experiments appear to be more favourable to the disinfectant than would as a rule be the case in practice. If a disinfectant is unable to destroy the microbe in ten minutes, in the absence of foreign substances that might tend to neutralise or mask its action, it is not likely to be always able to destroy the microbe within a reasonable time under the conditions that obtain in practice. This reason while justifying the condemnation of a disinfectant from the results of such experiments, necessitates caution in using such experiments to recommend a disinfectant. In practice a disinfectant is not required to sterilise test tubes containing suspensions of microbes, but to treat infected floors, clothing, drains, &c. The floor may be made of cowdung, which generally, though not always, has an alkaline reaction, and this alkali may tend to neutralise acid disinfectants or to hinder the liberation of chlorine from chloride of lime. Clothing and drains are likely to contain mucous, albumenous and other substances which may precipitate sublimate and other metallic disinfectants. I am at present carrying out a series of experiments on the disinfection of cowdung floors, which I hope will form the subject of a separate report. An adequate disinfection of a cowdung floor appears from my researches to be a far more difficult matter than is commonly supposed.

I have the honour to be,
Sir,
Your most obedient servant,
E. H. HANKIN.

From

THE CHEMICAL EXAMINER AND BACTERIOLOGIST,
N.-W. P. and Oudh and Central Provinces;

To

SURGEON MAJOR-GENERAL H. COOK,
Surgeon-General with the Government of Bombay,
Bombay.

Dated Agra, 17th July 1897

SIR,

I have the honour to submit a further report on disinfection against the bubonic microbe, comprising an account of some experiments that I have recently carried out in infected villages.

2. In my earlier report on the action of disinfectants on the bubonic microbe, I stated that the conditions of my experiments appeared to be more favourable to the disinfectant than would as a rule be the case in practice. Hence it appeared to me to be advisable to carry out some experiments in which practical conditions would be more closely imitated.

Owing to the fact that the excreta of men and rats suffering from plague are likely to fall on to the floors of houses, and that such excreta are likely in in some but not in all cases to contain the plague microbe, and owing further to the probability that the infection of plague gains entrance to the human body in the majority of cases through the skin of the feet and legs, it is probably the floors of houses and latrines that are most in need of disinfection. The floors of houses of the lower classes in India are usually covered with a mixture of mud and cowdung. My experiments therefore have been in the first place

directed to examining the means of disinfecting such floors. The most satisfactory way of carrying out such a test would be to impregnate a cowdung floor with the microbe of plague, and then to test for its presence after the application of the disinfectant. Unfortunately, however, this is at present impossible, as no reliable test exists for the presence of the plague microbe when mixed with others. Hence I have had to experiment with floors that had not been infected. By estimating the numbers of ordinary microbes present before and after the application of the disinfectant, some idea can be formed of the activity of the latter on a cowdung floor. No doubt some of the microbes present are more resistant than the plague microbe to the action of disinfectants, others on the other hand are likely to be as sensitive, and hence it would probably not be safe to employ a disinfectant against plague that had no action in decreasing the numbers of harmless microbes present on an ordinary floor.

The method I have employed was to remove as much of the material of the floor as could be taken up on the end of a flattened needle. This was put into a test-tube containing melted agaragar, mixed up well with the food medium, and the mixture was then allowed to solidify. On the following day the number of colonies that had developed was counted. It is needless to say that this method does not give results of any absolute value. If for instance the material of the floor was dry, it is likely that most of the microbes present in the specimen removed for examination would adhere to the particles of sand and dirt, and hence each particle would produce only a single colony. If on the other hand the particles were damp, it is likely that the microbes adhering to them would more readily become suspended and distributed in the agaragar, and thus produce a large number of colonies. Though the method has no absolute value, it has some relative value if repeated sufficiently often. If a specimen taken before disinfection produced a great number of colonies, and a specimen taken after disinfection produced no colonies after 24 hours, we may infer that some disinfectant action has been exerted. It would not be safe to conclude that the specimen taken after disinfection was absolutely sterile, for microbes might be, and as a matter of fact were, generally present that could not produce colonies after 24 hours, but that did so after a longer interval.

The following results were obtained in some preliminary observations:—

	Number of colonies developed after 24 hours.
Earthen floor of my laboratory in Hurdwar (a grass hut), before "lepoing"	800
Do. do. ...	584
Floor of Dhobie's house (mixed mud and cowdung) ...	19,000
Do. ½ inch from the surface	1,400
Do. sleeping rooms	28,000
Mud floor of Ghascut's room	13,400
Do. ¼ inch from surface	640
Floor of bearer's room recently "lepoed"	10,500
Do. ½ inch from the surface	6,700
Fresh cowdung	7,500
Do.	11,.00
Earth out of doors exposed to sun...	7,000
Do. do.	4,000

Experiments to test the action of different disinfectants were carried out on the mud floor of the grass hut that served me for a laboratory in the village in which I was working in the following manner. The floor had been covered with the ordinary mixture of mud and cowdung some days before. Areas about a foot square were divided off from each other by ridges of earth. Two specimens of the mud mixture of each area were examined bacteriologically, as described above, before pouring on the disinfectant. The disinfectant to be tested was then poured on to the surface, so that the whole of it was wetted, but no attempt was made to rub it in. After this at different intervals other specimens were taken and tested. In every case two specimens were taken from different parts of the area. The figures in the following table give the average of the two results. For the sake of clearness, I have not given the actual number of colony-producing microbes found, but I have given the relative numbers that developed for every hundred colonies found in the control observations. For instance, for every hundred colonies present before treatment with sublimate, half an hour afterwards only 60 were present, and 24 hours later the relative number had risen to 130, as shown by the first line in the table:—

Disinfectant.	Before treatment.	After ½ hour.	After 1 hour.	After 2 hours.	After 24 hours.
A. Sublimate 1 in 1,000	100	60	55	40	130
B. Sublimate 1 in 1,000, plus hydrochloric acid 2 in 1,000	100	...	43	...	1
C. Chloride of lime 1 per cent. ...	100	...	112	63	315
D. Potassium permanganate 1 per cent ...	100	98	55	76	86
E. Sulphuric acid 1 per cent... ...	100	...	79	57	35
F. Permanganate and sulphuric acid each 1 per cent.	100	17	12	11	7
G. The same mixture diluted ten times ...	100	31	49	131	112
H. Sulphuric acid 1 in 250	100	87	63	45	112
I. Sulphuric acid 1 in 500	100	97	140	142	300
J. Sulphuric acid 1 in 500, plus permanganate 1 in 1,000	100	68	72	63	161
K. Phenyle 1 per cent	100	...	248	166	138
L. Lysol 1 per cent	100	...	72	67	220

After the addition of the disinfectants the treated areas were protected from dust by means of covers placed over them.

It will be noted that the only substance tried that produced anything approaching a complete disinfection was corrosive sublimate in an acidulated solution. Chloride of lime, phenyle, and lysol appear to be without much action on the microbes of a cowdung floor, although as is known they are energetic disinfectants of microbes suspended in test tubes of bouillon. A comparison of experiments D, E, and F shows that a mixture of permanganate and sulphuric acid acts more strongly than either of these substances alone. The same deduction can be drawn from experiments I and J. As already explained, moistening a cowdung floor is likely to lead to an increase of the number of colonies that will appear in these experiments. The specimens taken before treatment were obtained from the floor while it was dry. Those taken after treatment were taken when it had for some time been moistened with the disinfectant solution. This is the most probable cause of the increase in the number of colonies developed after the addition of the solution of phenyle in experiment K.

The following observations were made in houses in course of disinfection. The first case investigated was in a large two-storied pucca built house. This house had been evacuated about two weeks before my visit, and the inhabitants had cleaned it up and had most of the rooms whitewashed. On the first floor I found a small room that the coolies had not thought noticeable enough to whitewash. One or two persons appeared to have used it as a latrine. This is referred to below as room A. The relatively small numbers of microbes or rather colonies met with before disinfection is probably due to the room being pucca built and thoroughly dried. This room was treated with a neutral solution of sublimate of a strength of 1 in 1,000. Room B was a badly smelling place on the ground floor. It had not been cleaned previously. It was treated with sulphuric acid of a strength of 1 in 250 with about half the quantity of permanganate. The specimens were taken about a quarter of an hour and again 24 hours after disinfection. The following are the results :—

Locality.	Numbers of colonies in specimens taken. Before.	¼ hour after.	24 hours after.
(1) Dirt from floor of Room A ...	2,080	5	11
(2) Ditto ...	960	6	19
(3) Ditto ...	1,280	256	1,440
(4) Ditto ...	640	...	30
(5) Dirt from floor of Room B ...	1,100	44	117
(6) Ditto ...	2,000	24	35
(7) Ditto ...	...	171	29
(8) Ditto ...	...	25	440
(9) Dirt from wall of Room B ...	400	1	34
(10) Ditto ...	...	88	1,280
(11) Cowdung floor of cook-room ...	1,200	...	544
(12) Ditto ...	...	...	640
(13) Ditto ...	...	...	12,000
(14) Ditto ...	...	...	768

The cook-room had been previously whitewashed. On the day of my visit it was treated with neutral sublimate solution.

Other observations were carried out in another village. No local cases of plague had occurred there, but some rats had been dying of plague in the house of one of the leading inhabitants. This was a large pucca built ill-ventilated and dirty house. A large quantity of grain was stored in one of the rooms on the ground floor. The owner told me that the doors of the rooms had to be kept shut owing to the depredations of monkeys. The upper stories of the house seemed in great part to be given up to the use of these animals. Acidulated sublimate solution was used to disinfect this house, and I noticed that the disagreable smell of dirt was still present in each room after disinfection had been carried out. The following results were obtained :—

Locality.	Numbers of colonies developed in tubes inoculated with specimens taken. Before disinfection.	After ½ hour.
(1) Brick courtyard	9,600	208
(2) Ditto	28,000	1,100
(3) Outer room, south side, kutcha floor ...	1,800	1,100
(4) Ditto	2,500	78
(5) Kutcha stable floor outside granary ...	25,000	120
(6) Ditto ...	35,000	43
(7) Pucca living room	1,900	128
(8) Ditto	3,560	192
(9) Cook-room	...	352

In drawing conclusions from the above results, it must be borne in mind that the action of acidulated sublimated solution, judging from an earlier experiment, is only likely to reach its maximum after two hours at least, and that the specimens in the above experiment were taken only fifteen minutes after the application of the disinfectant.

Recent work has shown that sulphur fumigation cannot be relied on to destroy microbes. Experiments carried out by the German Health Office have shown that though gaseous disinfectants may destroy the microbe in experiments on a small scale, they will not do so in large rooms as the gases do not show sufficient penetrative power. I carried out the two following experiments to further test the matter.

In the first experiment a small godown in the bungalow in which I was staying was treated. The room was closed up as well as was practicable. The men employed in the village to fumigate with sulphur were called in and told to treat the room with sulphur as was customary. They first brought in a portable stove containing a bright coal fire. On this they put a degchie of water to produce steam. Another similar fire was then brought in. On this they threw a handful of flowers of sulphur, which owing to the strength of the fire was almost immediately consumed. Then one of them rushed into the room and threw a small quantity of methylated spirit on to the fire containing the sulphur. Owing to the presence of two brisk fires in the room, the greater part of the oxygen present was likely to combine with the coal rather than with the sulphur. I was told that as a rule in fumigation with sulphur such bright coal fires were not employed, but only enough fire to cause the sulphur to burn. Under the conditions described it was natural that no disinfection should take place. Specimens of dirt taken before fumigation produced from 1,800 to 3,200 colonies. Specimens taken from the same positions after fumigation produced from 2,400 to 5,000 colonies. Some agaragar plague cultures were placed in different parts of the room, and after the fumigation were inoculated on to fresh agaragar. In all cases a typical growth was produced.

The second experiment was carried out in another small room in the same bungalow. Before fumigation water was thrown over the walls and ceiling of the room, so that plenty of aqueous vapour should be present in the air. The sulphur was burnt in the following manner. About two seers of flowers of sulphur were placed in each of two earthenware pans. Enough methylated spirit was added to it to make the powdered sulphur stick together, so that it could be moulded into a thick column. The columns were then lighted and

the door closed. The sulphur burnt for a long time, and a yellowish fog gradually developed in the air in the room. The following results were obtained :—

	Before fumigation.	After fumigation.
Dirt from floor...	370,000	1,700
Ditto	14,400	6,000
Ditto	...	4,800
Ditto	...	400

The above results show that a very slight impression on the microbes present had ensued. As in the previous experiment, agaragar plague cultures were placed in different parts of the room before fumigation. After fumigation each culture was inoculated on to fresh agar and in each case it grew. In two of the tubes, however, the colonies were not very numerous, suggesting the possibility that some slight effect had been produced on the microbes present. It may be objected to this part of the experiment that the bubonic microbes were present in test tubes closed in each case by a plug of cotton wool, and that thus they were to some extent protected from the action of the gas. But in practice the sulphurous acid gas would have to penetrate far more serious obstacles than loose cotton wool if it is to be of any use at all. Another condition that might not always exist in practice was in favour of the sulphur, namely, that the bubonic microbes exposed to its action were moist. Sulphur when burnt produces sulphur dioxide. This is only disinfectant when it has combined with water forming sulphurous acid. Hence microbes in a damp condition are more likely to be affected than if they are dry.

There remains another way of treating an infected floor which, where practicable, is likely to give better results than can be obtained with a chemical disinfectant, namely, by exposing it to the action of fire. The following experiment was carried out to test this possibility.

An area of earth, about ten feet square, under the shade of a tree was covered with a mixture of mud and cowdung. The smooth surface thus produced was allowed to dry during four days. Then a small portion of the surface was again covered with fresh cowdung and mud. The whole was then covered with a layer of dry grass about two inches thick. In addition bamboos about an inch thick were laid side by side at about half an inch distance from each other over about half the area. The grass was then set alight, and after the fire had gone out the surface was tested with the following results :

	Before burning.	After burning.
(1) Surface afterwards covered with a thick layer of grass	1,600	1
(2) Do. do. ...	1,080	...
(3) Surface covered with a thin layer of grass ...	...	400
(4) Half an inch below the part of the surface covered with a thick layer of grass ...	3,200	22
(5) Do. do. ...	2,000	100
(6) Two inches below above surface... ...	...	900
(7) Freshly made surface afterwards covered with grass and bamboos	7,300	...
(8) Do. do. ...	2,400	...
(9) Half an inch below this surface	...	...
(10) Do. do. ...	...	2
(11) Two inches below above surface... ...	...	84

Thus in the above experiment complete disinfection appears to have been produced by the burning of a layer of grass two inches thick. Where the heat was greater, owing to the combustion of the bamboos, the disinfectant action appears to have reached to some distance below the surface. Although probably the quickest and most certain method of freeing an earthen floor from infection, the method of firing must be, for obvious reasons, limited in its applicability. It was tried with apparent success in an infected chawl in Bombay inhabited by the families of policemen. Grass as in my experiment was burnt all over the floors and so far as I am aware no more cases of plague occurred. Though the method can thus be carried out with the help of intelligent supervision, it would probably lead to accidents if employed in a town on a large scale. In most cases however it can be used in public latrines, which are frequently made of

corrugated iron or other incombustible material. Both on the general grounds that human excreta are apt to contain the microbe of the disease and on the *à posteriori* evidence of the Hong Kong epidemic, it is probable that public latrines may become sources of infection, and consequently are greatly in need of efficient disinfection. Owing to the existence of caste and other prejudices, the disinfection of public latrines is very often shirked. Further, if ordinary disinfectants are used, it is not easy for an inspecting officer to know whether the work has been properly carried out. Hence I suggest that public latrines in infected areas should be frequently treated with burning grass or other fuel. The sweeper could put a bundle of grass into each compartment and light it. Afterwards the ashes could be washed out with a dilute solution of sulphuric acid (1 to 250 of water).

The probable efficiency of disinfectants is not the only factor to be taken into consideration. Their cost must also be noted. Taking commercial prices for large quantities, the cost of various possible disinfectants is as follows :—

	Number of gallons of the solution obtainable for one rupee.
(1) Sublimate 1 in 1,000	15
(2) Sublimate 1 in 1,000 *plus* hydrochloric acid 2 in 1,000	14
(3) Sulphuric acid 1 in 250	1,920
(4) Sulphuric acid 1 in 250 *plus* 1 in 500 of permanganate of potassium	128
(5) Phenyle 1 in 100	144

The above prices do not include cost of transit, which is higher for the acids than for the solid disinfectants.

Another question that must be taken into consideration in choosing a disinfectant is whether its action is likely to be lasting, whether in other words its employment is likely to make the medium unsuited for a considerable time for the life of the bubonic microbe. This is possibly more important in the case of bubonic plague than with other diseases. Not only in this disease is a disinfected area liable to be reinfected by human patients, but in addition in many cases this may occur through the agency of infected rats. Hence a disinfectant that merely destroys the microbes present at the time, and that has no lasting action, is not sufficient for the purpose. As shown by previous experiments, acidulated sublimate solution is the only one of the disinfectants tried that showed a clearly good action on a cowdung floor. Unfortunately, however, solutions of mercuric chloride, even with the addition of volatile hydrochloric acid, are by no means stable, especially when mixed with the material of a cowdung floor. Dilute sulphuric acid, on the other hand, is not volatile, and, if used in sufficient quantities, is likely for some time at least to make the floor an unsuitable medium for the bubonic microbe. Although, as shown in previous experiments, there are many microbes in a cowdung floor not readily affected by it, in the strength recommended, the special sensitiveness of the bubonic microbe to acids in general, and to sulphuric acid in particular, makes it likely that the latter substance will be efficient when used on such a floor. The acid will slowly diffuse to the deeper layers and combine with calcium salts and various alkaline substances that may happen to be present. But the upper layers, which are in most need of disinfection, are most likely to be affected, and the acid will be less quickly neutralised if the floor has been treated with acid sublimate solution on the preceding day. In a locality in which the soil is calcareous, sulphuric acid is not likely to be a convenient disinfectant for cowdung floors.

A further consideration bearing on the choice of an antiseptic depends on a view that is generally held by those having experience of plague, namely, that the virus is sooner or later destroyed by ventilation. As I have elsewhere shown, the plague microbe is somewhat resistant to the action of drying, and hence it is difficult to believe that the asserted benefit of ventilation is only due to dessication tending to destroy the microbe. Its action is more likely to be due to the oxidation processes that are produced by the evaporation of water in the presence of air and light. Many authorities have brought forward

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