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[Despatch Abstract.]

Plague Bacillus and others. Communicating to Collector the report on the germicidal action of certain disinfectants on the ——

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Government {1898} of Madras.

LOCAL AND MUNICIPAL.

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G.O., No. 740 P., 2nd August 1898.

AUGUST.

Plague Bacillus.

Communicating to Collectors and others, the report on the germicidal action of certain disinfectants on the —.

Deptt 26 1/8/98

GOVERNMENT OF MADRAS.

LOCAL AND MUNICIPAL DEPARTMENT.

READ—the following paper:—

Report on the germicidal action of certain disinfectants on the bacilli of plague, by Wilfred Watkins-Pitchford, dated Petit Laboratory, Bombay, 22nd April 1898.

I have the honour to submit the following observations upon the germicidal action, with reference to the bacillus of plague, of the following substances:—

Carbolic Acid.	Common Salt.
"Phenyle," and "Lawes' Disinfecting Fluid."	Formic Acid.
"Izal."	Acetic "
"Lysol."	Lactic "
Pure Naphthaline.	Tartaric "
Commercial Naphthaline.	Nitric "
"Naphtho-sublimate."	Hydrochloric Acid.
Corrosive Sublimate (Perchloride of Mercury).	Sulphuric "
Sulphate of Copper (Blue Stone).	Sulphate of Iron (Green Vitriol).
Ammonia water.	Chloride of Lime.
Caustic Potash.	Permanganate of Potash.
Quick Lime.	Bromine water.
Borax (Sodium Biborate).	Hot water.

On pages 16—30 of Mr. Hankin's report upon his investigations into plague for the year 1897 the effect of most of these substances upon the bacillus is discussed. My results are to a large extent corroboratory of those of Mr. Hankin.

It should be remembered that the action of these various substances upon the *bacillus pestis* has been observed in watery media only, a watery solution or suspension of the drug being brought to act upon a watery emulsion of the bacillus. How substances in a solid form (powder) or gaseous form (disinfectant fumes) act upon the bacillus, and how the bacillus is influenced by disinfectants when it itself is contained in a solid medium such as earth, form subjects for enquiry which are now being pursued.

In the observations recorded in this report the methods adopted by Mr. Hankin have to a large extent been imitated, these methods having been varied only when it appeared probable that greater accuracy would be obtained by the change.

It has been considered advisable to use tap water for the making of all solutions to be tested, for the reason that, when applied practically in the disinfection of dwellings, clothing, &c., the solvent of the chemical would be tap water rather than distilled water. Ferrous sulphate alone has, for special reasons, been tested with distilled water. Sterile test-tubes containing sterile solutions, or suspensions, in varying strengths of the chemical are inoculated by means of a capillary pipette with a few drops of a watery emulsion of an agaragar culture of the bacillus. Upon repeating some of the experiments recorded in this report with emulsions of plague cultures of an age different to that of the cultures used in the first experiments, a marked variation in the vitality of the bacillus was observed. This variation appears

to be, in the main, that a young culture (and therefore the more virulent pathogenically) is less resistant to a germicide than an old culture. A probable explanation of the greater resisting power of an old bacillus is that its surface has become denser and less permeable to the poisonous fluid. The discovery of this variation in the resisting power of the *bacillus pestis*, whatever may be its complete explanation, has led one to quote after each summary of the action of a drug the age and pedigree of the growth used in testing it.

After allowing the chemical to act for one, five, ten, &c., minutes (the exact period being noted in each case) some of the mixture of chemical solution and bacilli is drawn into a sterile pipette and transferred to nutrient gelatine. A gelatine of 15% strength is used for all the tests; this strength of gelatine is liquid at "room temperature" which throughout all the experiments varied from 80°F. to 86°F. A separate pipette is used for the transference of each strength of inoculated chemical to its gelatine tube.

After the lapse of a week the gelatine tubes so treated, and which have been kept in a dark cupboard at room temperature, are examined; if no growth is evident, the bacillus has been destroyed by its exposure to the chemical. If growth has occurred, it becomes necessary to ascertain whether this is of the plague bacillus or of some other one accidentally introduced during the experiment. For this purpose a drop of the gelatine with growth is first examined directly by the microscope; should the appearance of the bacilli not be absolutely typical, a further specimen of the gelatine growth is inoculated upon agaragar. The growth upon the latter medium is examined both macroscopically and microscopically at the end of two to four days.

It may not be altogether out of place to note here the microscopic appearances of the unstained bacillus as grown in different media. A pure culture, not more than two to three weeks old, grown in 15 % gelatine presents remarkable microscopic characters. If a drop of the culture be examined without dilution with water, the bacterial growth appears as faintly-defined slightly granular masses which can be resolved by the eye into what appear as chains of minute cocci embedded in a homogeneous ground substance; one or two detached bacilli of the usual appearance may be seen floating free, chains of bacilli are conspicuous by their absence.

A drop of broth culture (unstained and undiluted with water) exhibits somewhat similar appearances; but in this case the zoogloea like masses can more readily be resolved into groups of bacilli; short chains of from two to six typically shaped bacilli are also seen as well as many free organisms.

A fragment of an agar culture rubbed up in a drop of water and examined directly shows a few clusters of bacilli (especially in old cultures), but the organisms for the most part will be seen to float freely and unconnected with one another. These individual bacilli will be seen, in the case of a young culture, to have a shape resembling an hour-glass with the isthmus of the two bulbs very broad and short. Occupying almost the whole of each bulb of the hour-glass will be seen a dark spot of a flattened lenticular shape the plane surface of which looks towards that of its fellow in the opposite pole. The appearance of two dark lenticular spots with their flat surfaces opposed and close to one another is the first thing which strikes the eye of one examining the normal unstained bacillus. When floating in water two bacilli are frequently seen adhering side to side and their four polar spots being then about equidistant from one another give, at first sight, a tetrad-like appearance. In older cultures the compressed hour-glass form is accompanied by others resembling in shape a life-preserver, a large-headed drum-stick or a sphere. The shank portion of the elongated forms may be either straight or bent. The heads of the "life-preserver" and "drum-stick" show for some time a polar spot of small size and the same can be seen at either side of a recently formed sphere.

These polar spots appear to be due to the greater collection and perhaps condensation of the protoplasm in these regions. As the spots are readily visible in the living and unstained bacillus, the phenomenon cannot be one due to staining, as some have thought. The detection of these spots I consider to be a most valuable aid to the rapid identification of the germ.

The macroscopic appearances of an agar culture of the bacillus are usually supposed to be sufficiently characteristic to greatly assist in identifying the organism. It is found, however, that a "ground-glass haze" indistinguishable from that due to plague growth may result from the growth of other organisms. Especially is this the case when the inoculation of the agar has been obtained by allowing a few drops of the infected fluid to run over its surface. A "ground-glass haze" growth closely simulating plague is produced, especially by an aërobic organism which, morphologically, resembles the tetanus bacillus.

An irregularity in the action of progressively stronger solutions of the chemical under examination, will be noticed on more than one occasion in the following tables; that is to say, a certain percentage has failed to destroy the vitality of the bacillus, although both a weaker and a stronger solution succeeded in doing so. That this irregularity is due to some mischance in the experiment seems probable, but the occurrence of an irregularity in Mr. Hankin's experiment with sulphuric acid coinciding almost exactly with one in my table for that drug, is suggestive of some other explanation of this paradox. An impairment of the vitality of the bacillus, short of its actual death, is frequently observed: for instance the ·3% lactic acid tube afforded a very scanty growth of the germ, the ·2% tube gave a vigorous growth, the ·5% tube was quite sterile. The same phenomenon of impaired reproductive power is noticed in the observations upon the action of heat.

Carbolic Acid.—Crystals of pure carbolic acid are dissolved in water contained in test-tubes and the solutions sterilised in the autoclave. Before inoculation with the bacillus the solutions are allowed to cool to room temperature.

In the following tables a + sign indicates the survival of the bacillus, a — sign indicates that its vitality has been destroyed.

A test-tube containing sterile water only, referred to as 0 %, or the "control tube," has in each experiment been inoculated with some of the same emulsion as that used for the tubes containing the chemical. After the transference of some of the contents of the control tube to nutrient gelatine, the appearance of a growth of bacillus pestis in the latter is taken as evidence of the vitality of the emulsion.

Time of exposure of emulsion to action of drug.

	% age.	1 minute.	5 minutes.	15 minutes.	20 minutes.	1 hour.	6 hours.
Carbolic Acid	0	+	+	+	+	±	±
	·5					±	±
	1		—	—	—	—	
	2	—	—				
	3	—					
	4						

2 % solution destroyed vitality in 5 minutes.
4 % " " in 1 minute.

Emulsion of agar growth 6 days old, from agar growth 12 days old from body.

"*Phenyle*".—This preparation makes a remarkably milk-like and slightly alkaline emulsion when diluted with water. Dilute solutions (such as a ·75 %) are comparatively innocuous if drunk; hence a caution in the use of this disinfectant, from its milk-like appearance in water, appears hardly necessary. That "phenyle water" is comparatively harmless to animal life, one has proved by experiment. It is believed, and has been asserted by many natives, that the use of phenyle water as a disinfectant is dangerous both because it induces illness in men by contaminating their food, and also by causing the death of the rats in the drains and elsewhere; some have even asserted that rats washed with phenyle water and obliged to drink it develop plague in consequence. I have now in my laboratory two wild rats which for sixty-five days and forty days, respectively, have not been allowed any drink but phenyle water (2 per cent.) and both of which have for thirty days eaten no food that had not been soaked in the same fluid. One of these rats has, on two occasions, had a drachm of phenyle water injected into its stomach, and has been obliged to swim

about in a pail of the disinfectant. Both of these animals appear in the best of health, and now take their phenyle food and water without hesitation. The justifiability of such experiments as these must be obvious to those who are cognisant of the extreme ignorance and great prejudices of many of the people most interested in the suppression of plague.

—	% age.	1 minute.	5 minutes.
"Phenyle"	0	+	±
	·1		±
	·25		—
	·5	—	—
	·75	—	—
	1	—	—

·25 % in water destroyed vitality in 5 minutes.
·5 % " " in 1 minute.

The emulsion of the bacillus had a similar origin to that used with carbolic acid.

"*Lawes' Disinfecting Fluid*".—An examination of this article showed that it closely resembles and is probably identical with "Phenyle". It proved fatal to the bacillus in exactly the same proportion.

"*Izal*"—

% age.	1 minute.	5 minutes.	10 minutes.	1 hour.
0	+	+	+	±
·05				±
·1		—	—	—
·4		—	—	—
·8	—	—		
1	—			

·1 % destroyed vitality in 5 minutes.
·8 % " " in 1 minute.

The same emulsion used as that for carbolic acid.

"*Lysol*"—

% age.	1 minute.	5 minutes.	10 minutes.	1 hour.
0	+	+	+	+
·05		—	—	—
·1		—	—	—
·25		—	—	
·5	—	—		
·1	—	—		

·05 % destroyed vitality in 5 minutes.
·5 % " " in 1 minute.

Same emulsion of bacillus as used for carbolic acid.

Pure Naphthaline—

Commercial Naphthaline.—A suspension of either of these insoluble substances in water neither destroys the vitality nor retards the growth of the *bacillus pestis*.

% age.	Pure naphthaline.	Commercial naphthaline.
	Exposed for 15 minutes.	Exposed for 15 minutes.
0	±	±
·5	±	±
1	±	±
2	±	±
4	±	±

"*Naphtho-sublimate*".—The specimen of this proprietary article tested afforded similarly negative results. The emulsion used for these three substances was of an agar growth 15 days old from an 18-day agar growth directly from the body.

Corrosive Sublimate—

—	% age.	1 minute's exposure.
First test	0	+
	·005	—
	·02	—
	·05	—
	·08	—
	·1	—
	·15	—

Emulsion of an agar growth 5 days old, from a 2-day old culture from a bubo.

—	% age.	1 minute.	5 minutes.
Second test ..	0	+	+
	·005	+	—
	·02	—	—
	·05	—	—
	·08	—	—
	·1	—	—
	·15	—	—

·005% destroyed vitality in 5 minutes.
·02 % „ „ in 1 minute.

Emulsion of agar growth 17 days old, from a broth culture 49 days old, 3 days from the body.

Sulphate of Copper—

—	% age.	1 minute.	5 minutes.
First test	0	+	+
	·005	+	+
	·02	+	+
	·05	+	+
	·08	+	+
	·1	+	+
	·15	+	+
	·25	+	+
	·5	—	—
	1	—	—
	2	—	—
	5	—	—

·5% destroyed vitality in 5 minutes.

Emulsion of growth 5 days old, from an agar culture 2 days from bubo.

—	% age.	5 minutes.	5 minutes.
Second test ..	0	+	+
	·005	+	+
	·02	+	+
	·05	+	+
	·08	+	+
	·1	+	—
	·15	+	—
	·25	—	—
	·5	—	—
	1	—	—
	2	—	—
	5	—	—

·15% destroyed vitality in 5 minutes.
·25% „ „ in 1 minute.

Emulsion of growth 19 days old, from a broth culture 7 weeks old, 3 days from the body.

Ammonia Water.—Test solutions of ammonia were prepared by the method adopted for all substances, the efficacy of which would be reduced by heat sterilisation. To test-tubes containing known quantities of sterile water, the appropriate quantity of a 5% solution was added by means of a sterile pipette. The 5% solution was obtained by the addition of one cc. of strong liquid ammonia (·880) to nineteen cc. of sterile water.

From the accompanying table it will be seen that this powerful volatile alkali has, even in a proportion (5%) which smells strongly of the gas, no germicidal action upon the B. pestis :—

% age.	5 minutes.
0	+
·25	+
·5	+
1	+
2	+
3	+
5	+

Emulsion of agar growth 3 days old, from an agar growth 2 days, from bubo.

Caustic Potash—

% age.	1 minute.	5 minutes.
0	+	+
·25		+
·5		—
1		—
2	+	—
3	+	+
5	+	+

Emulsion of agar culture 5 days old, from agar culture 2 days, from bubo.

The irregularity of the germicidal effect of the chemical as shown in this table has already been commented upon.

Quick Lime.—Perfectly fresh and hard quick lime, sterilised in a hydrogen flame, was added in measured proportion to water previously freed from carbonic acid gas and sterilised by heat. The ·1 % tube was the only one which did not contain solid slaked lime in suspension.

% age.	5 minutes.
0	+
·1	—
·25	—
·5	—
·75	—
1	—
2	—
3	—

·1% solution destroyed vitality in 5 minutes.

Emulsion of agar growth 3 days old from agar growth 8 days from bubo.

Borax—Sodium bi-borate—and *Common Salt*—Sodium chloride.—The reputed preservative and disinfectant action of these two substances induced one to test their germicidal power. The result was entirely negative : a 10% solution of either substance failed to destroy the vitality of the plague bacillus even after fifteen minutes' exposure.

Organic Acids :—

Formic Acid—

°/o age.	5 minutes.
0	+
·25	+
·5	—
1	—
2	—
4	—

·5 °/o destroyed vitality in 5 minutes.

Tartaric Acid—

°/o age.	5 minutes.
0	+
1	—
2	+
4	—
5	—

4°/o destroyed vitality in 5 minutes, although germicidal action appears irregular.

Acetic Acid.—Glacial acetic acid was used, and solutions prepared in a similar manner to that used for ammonia.

°/o age.	1 minute.	5 minutes.
0	+	+
·1		+
·25		+
·5		+ feeble growth.
·75		—
1	—	—
2	+	+
4	—	—

Germicidal action appears irregular.

Lactic Acid—

°/o age.	1 minute.	5 minutes.
0	+	+
·01		+
·03		+
·05		+
·07		+
·1		+
·2		+
·3		+ feeble growth.
·5		—
1	—	—
2	—	—
4	—	—

·5°/o destroyed vitality in 5 minutes.
1°/o ,, ,, in 1 minute.

The emulsion used for testing formic, acetic, tartaric and lactic acids was prepared from an agar growth 16 days old, from a broth culture 7 weeks old, from an agar culture 2 days, from the body.

Mineral Acids.—Solutions of hydrochloric acid were obtained sterile like those of ammonia; solutions of nitric and sulphuric acids were first prepared and then sterilised in the autoclave.

°/o age.	Hydrochloric Acid.		Nitric Acid.		Sulphuric Acid.	
	1 minute.	5 minutes.	1 minute.	5 minutes.	1 minute.	5 minutes.
0	+	+	+	+	+	+
·03		—		+		+
·05		+		+		+
·07		—		+		+
·1		—		—		—
·2		—		—		—
·3		—	—	—	—	—
·5	—	—	—	—	—	—
·7	—	—	—	—	—	—
1	—	—	—	—	—	—
	·07 °/o destroyed in 5 minutes. ·5 °/o ,, in 1 minute.		·2 °/o destroyed in 5 minutes. ·5 °/o ,, in 1 minute.		·2 °/o destroyed in 5 minutes. ·5 °/o ,, in 1 minute.	
	Emulsion of agar growth 16 days old, from 2 days' agar growth from bubo.				Agar growth 16 days old, from 7 weeks' broth culture, 3 days from body.	

Sulphate of Iron—Green Vitriol.—This substance owing to its power of absorbing oxygen from and thus reducing organic material has long held the reputation of a valuable germicide. The results of both Mr. Hankin's experiments and my own show that this salt even in perfectly unreduced solutions has but little practical utility as a plague germicide. Difficulty was experienced in obtaining sterile solutions of ferrous sulphate unoxidised. The following method proved successful.

A crystal of the salt weighing exactly four grammes held in sterile forceps was dipped into absolute alcohol and then inflamed; when the alcohol had burnt out, the crystal was dropped into a flask containing 100 cc. of cold, distilled, sterile water. From the 4 °/o solution which thus resulted, measured quantities were removed by means of a sterile graduated pipette and added to measured quantities of sterile distilled water in test-tubes.

°/o age.	1 minute.	5 minutes.
0	+	+
·1		+
·2		+
1		+
2		+
4	—	—

4 °/o destroyed vitality in 1 minute.

Emulsion of agar growth 16 days old, from 7 weeks' broth culture from agar growth 3 days from the body.

Oxidising Agents.—Chloride of lime, potassium permanganate, bromine water.

Chloride of Lime.—A solution of this substance when pure and freshly prepared consists of a mixture of the chloride with the hypochlorite of calcium; the latter salt when acted upon by an acid in the presence of moisture is a powerful oxidiser.

°/o age.	1 minute.	5 minutes.
0		+
·005		+
·05		—
·1		—
·25	—	
·5	—	
1	—	

·05 °/o destroyed vitality in 5 minutes.
·25 °/o ,, ,, in 1 minute.

Emulsion of agar growth 16 days old, from 6 weeks' broth culture, from 4 days' agar growth, from bubo.

Permanganate of Potash.—Mr. Hankin states this to be "an extremely potent destroyer of the bubonic microbe"; this certainly seems to be the case, and for its rapidity and certainty of action in dilute solutions one seems justified in placing it on the same footing as perchloride of mercury. The reasons why permanganate cannot be relied upon to disinfect dwelling-houses, drains, &c., have been stated in Mr. Hankin's report.

Weak solutions of permanganate were found to be reduced by exposure to the action of heat in the autoclave. Some crystals were therefore weighed, and sterilised by exposure to dry heat at 120° C.; by adding these to cold sterile water a solution of known strength was obtained, and from this the solutions of varying strengths were prepared as described under sulphate of iron.

%age.	1 minute.	5 minutes.
0	+	+
·0025		+
·005		+
·01		+
·02	—	—
·04	—	—
·06	—	—
·08	—	—
·1	—	—

·02 % destroyed vitality in 5 minutes.
·04 % ,, ,, in 1 minute.

Emulsion of agar growth 16 days old, from 6 weeks' broth culture, from 4 days' agar growth, from bubo.

Bromine water is being sold in Bombay as a disinfectant under the title of "Professor Gajjar's Disinfecting Fluid." A specimen of this fluid gave the following results:—

%age.	1 minute.	5 minutes.
0	+	+
·05		+
·1		+
·4		+
·8	—	—
1	—	—
2	—	—

·8 % destroyed vitality in 1 minute.

Emulsion of agar growth 20 days old, from 7 weeks' broth culture, from agar growth 3 days, from body.

Hot water.—Twenty-four test-tubes, each containing 1 cc. of watery emulsion of an agar growth (26 days old, from 6 weeks' broth culture, from agar culture, 3 days from the body) were placed in a beaker of cold water. In a twenty-fifth test-tube containing 1 cc. of water was placed a thermometer. The water in the beaker was gradually heated. At 114° F. a test-tube was removed, and some of its contents inoculated on to sterile nutrient agar; similarly at every increase of one degree of temperature a tube was removed and tested by inoculation. The following results were noted:—

The bacillus suspended in water and heated to 120° F. appears to have its power of growth slightly increased; from 120° F. to 126° F., the vitality appears to be progressively reduced, and after 126° F. it is not possible to obtain a cultivation from it.

Water heated to 127° F. or above appears therefore to destroy the vitality of the bacillus; this temperature can be borne by the hand for a few seconds only.

If the microbe of plague can exist in the surface layers of the earth floor of an infected room (a proposition open to dispute) it seems probable that it could be destroyed by pouring *boiling* water all over the surface.

In summarising these few observations, only those substances will be considered which in a solution of 1 part in 400 or more of water destroyed the bacillus of plague in 5 minutes. The efficient strength of each is quoted.

Perchloride of mercury	1 in 5,000

(in one experiment this proportion was successfully reduced to 1 in 20,000).

Permanganate of potash	1 in 5,000
Chloride of lime	1 in 2,000
"Lysol"	1 in 2,000
Hydrochloric acid	1 in 1,428
Quick lime	1 in 1,000
Sulphate of copper	1 in 1,000
Izal	1 in 1,000
Nitric acid	1 in 500
Sulphuric acid	1 in 500
Phenyle or Lawes' fluid	1 in 400

It will be seen that perchloride of mercury, permanganate of potash, chloride of lime and "lysol" proved to be by far the most potent substances examined. That most classical of germicides—carbolic acid—cannot be included, its efficient strength being but 1 in 50.

The various circumstances under which these different substances act to their best advantage has been described in other reports.

Perchloride of mercury is a thoroughly reliable germicide. As already known, its power is enhanced by the addition of a certain quantity of hydrochloric acid. The drawback to its indiscriminate use is its very poisonous nature.

Permanganate of potash though non-poisonous is speedily rendered impotent in the presence of oxidisable organic material.

Chloride of lime possesses the merits of cheapness and great efficiency. It is not dangerous, in any marked degree, to animal life.

"Lysol" is a potent germicide, but its cost is against its use on a large scale.

Quick lime is a harmless disinfectant, and is readily obtainable in most parts of the country.

Sulphate of copper exerts its germicidal properties in a somewhat irregular and uncertain manner.

Phenyle is fairly cheap. It is harmless to animals.

Very hot or boiling water is an excellent surface disinfectant for plague; its practical application would probably be inconvenient.

ORDER—No. 740 P., dated 2nd August 1898.

Communicated to all Collectors, President, Municipal Commission, Madras, Surgeon-General and Sanitary Commissioner, Madras.

(True Extract.)

H. TREMENHEERE,
Secretary to Government.

To all Collectors (A, F, G, H, J, K).
,, Surgeon-General.
,, Sanitary Commissioner.
,, President, Municipal Commission, Madras.
Editors' Table.

Exd. C. Parthasarathy.

Government (1898) of Madras.

LOCAL AND MUNICIPAL.

S. A

Proof Submitted for approval.

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After allowing the chemical to act for one, five, ten, &c., minutes (the exact period being noted in each case) some of the mixture of chemical solution and bacilli is drawn into a sterile pipette and transferred to nutrient gelatine. A gelatine of 15% strength is used for all the tests; this strength of gelatine is liquid at "room temperature" which throughout all the experiments varied from 80°F. to 86°F. A separate pipette is used for the transference of each strength of inoculated chemical to its gelatine tube.

After the lapse of a week the gelatine tubes so treated, and which have been kept in a dark cupboard at room temperature, are examined; if no growth is evident, the bacillus has been destroyed by its exposure to the chemical. If growth has occurred, it becomes necessary to ascertain whether this is of the plague bacillus or of some other one accidentally introduced during the experiment. For this purpose a drop of the gelatine with growth is first examined directly by the microscope; should the appearance of the bacilli not be absolutely typical, a further specimen of the gelatine growth is inoculated upon agaragar. The growth upon the latter medium is examined both macroscopically and microscopically at the end of two to four days.

It may not be altogether out of place to note here the microscopic appearances of the unstained bacillus as grown in different media. A pure culture, not more than two to three weeks old, grown in 15 % gelatine presents remarkable microscopic characters. If a drop of the culture be examined without dilution with water, the bacterial growth appears as faintly-defined slightly granular masses which can be resolved by the eye into what appear as chains of minute cocci embedded in a homogeneous ground substance; one or two detached bacilli of the usual appearance may be seen floating free, chains of bacilli are conspicuous by their absence.

A drop of broth culture (unstained and undiluted with water) exhibits somewhat similar appearances; but in this case the zoogloea like masses can more readily be resolved into groups of bacilli; short chains of from two to six typically shaped bacilli are also seen as well as many free organisms.

A fragment of an agar culture rubbed up in a drop of water and examined directly shows a few clusters of bacilli (especially in old cultures), but the organisms for the most part will be seen to float freely and unconnected with one another. These individual bacilli will be seen, in the case of a young culture, to have a shape resembling an hour-glass with the isthmus of the two bulbs very broad and short. Occupying almost the whole of each bulb of the hour-glass will be seen a dark spot of a flattened lenticular shape the plane surface of which looks towards that of its fellow in the opposite pole. The appearance of two dark lenticular spots with their flat surfaces opposed and close to one another is the first thing which strikes the eye of one examining the normal unstained bacillus. When floating in water two bacilli are frequently seen adhering side to side and their four polar spots being then about equidistant from one another give, at first sight, a tetrad-like appearance. In older cultures the compressed hour-glass form is accompanied by others resembling in shape a life-preserver, a large-headed drum-stick or a sphere. The shank portion of the elongated forms may be either straight or bent. The heads of the "life-preserver" and "drum-stick" show for some time a polar spot of small size and the same can be seen at either side of a recently formed sphere.

These polar spots appear to be due to the greater collection and perhaps condensation of the protoplasm in these regions. As the spots are readily visible in the living and unstained bacillus, the phenomenon cannot be one due to staining, as some have thought. The detection of these spots I consider to be a most valuable aid to the rapid identification of the germ.

The macroscopic appearances of an agar culture of the bacillus are usually supposed to be sufficiently characteristic to greatly assist in identifying the organism. It is found, however, that a "ground-glass haze" indistinguishable from that due to plague growth may result from the growth of other organisms. Especially is this the case when the inoculation of the agar has been obtained by allowing a few drops of the infected fluid to run over its surface. A "ground-glass haze" growth closely simulating plague is produced, especially by an aërobic organism which, morphologically, resembles the tetanus bacillus.

An irregularity in the action of progressively stronger solutions of the chemical under examination, will be noticed on more than one occasion in the following tables; that is to say, a certain percentage has failed to destroy the vitality of the bacillus, although both a weaker and a stronger solution succeeded in doing so. That this irregularity is due to some mischance in the experiment seems probable, but the occurrence of an irregularity in Mr. Hankin's experiment with sulphuric acid coinciding almost exactly with one in my table for that drug, is suggestive of some other explanation of this paradox. An impairment of the vitality of the bacillus, short of its actual death, is frequently observed: for instance the ·3% lactic acid tube afforded a very scanty growth of the germ, the ·2% tube gave a vigorous growth, the ·5% tube was quite sterile. The same phenomenon of impaired reproductive power is noticed in the observations upon the action of heat.

Carbolic Acid.—Crystals of pure carbolic acid are dissolved in water contained in test-tubes and the solutions sterilised in the autoclave. Before inoculation with the bacillus the solutions are allowed to cool to room temperature.

In the following tables a + sign indicates the survival of the bacillus, a — sign indicates that its vitality has been destroyed.

A test-tube containing sterile water only, referred to as 0 %, or the "control tube," has in each experiment been inoculated with some of the same emulsion as that used for the tubes containing the chemical. After the transference of some of the contents of the control tube to nutrient gelatine, the appearance of a growth of bacillus pestis in the latter is taken as evidence of the vitality of the emulsion.

Time of exposure of emulsion to action of drug.

	% age.	1 minute.	5 minutes.	15 minutes.	20 minutes.	1 hour.	6 hours.
Carbolic Acid	0	+	+	+	+	+	+
	·5				—	—	
	1		—	—			
	2		—				
	3		—				
	4	—	—				

2 % solution destroyed vitality in 5 minutes.
4 % " " in 1 minute.

Emulsion of agar growth 6 days old, from agar growth 12 days old from body.

"*Phenyle*".—This preparation makes a remarkably milk-like and slightly alkaline emulsion when diluted with water. Dilute solutions (such as a ·75 %) are comparatively innocuous if drunk; hence a caution in the use of this disinfectant, from its milk-like appearance in water, appears hardly necessary. That "phenyle water" is comparatively harmless to animal life, one has proved by experiment. It is believed, and has been asserted by many natives, that the use of phenyle water as a disinfectant is dangerous both because it induces illness in men by contaminating their food, and also by causing the death of the rats in the drains and elsewhere; some have even asserted that rats washed with phenyle water and obliged to drink it develop plague in consequence. I have now in my laboratory two wild rats which for sixty-five days and forty days, respectively, have not been allowed any drink but phenyle water (2 per cent.) and both of which have for thirty days eaten no food that had not been soaked in the same fluid. One of these rats has, on two occasions, had a drachm of phenyle water injected into its stomach, and has been obliged to swim

about in a pail of the disinfectant. Both of these animals appear in the best of health, and now take their phenyle food and water without hesitation. The justifiability of such experiments as these must be obvious to those who are cognisant of the extreme ignorance and great prejudices of many of the people most interested in the suppression of plague.

—	% age.	1 minute.	5 minutes.
"Phenyle"	0	+	+
	·1		+
	·25		—
	·5	—	—
	·75	—	—
	1	—	—

·25 % in water destroyed vitality in 5 minutes.
·5 % " " in 1 minute.

The emulsion of the bacillus had a similar origin to that used with carbolic acid.

"*Lawes' Disinfecting Fluid*".—An examination of this article showed that it closely resembles and is probably identical with "Phenyle". It proved fatal to the bacillus in exactly the same proportion.

"*Izal*"—

% age.	1 minute.	5 minutes.	10 minutes.	1 hour.
0	+	+	+	+
·05				+
·1		—	—	—
·4		—	—	—
·8	—	—		
1	—			

·1 % destroyed vitality in 5 minutes.
·8 % " " in 1 minute.

The same emulsion used as that for carbolic acid.

"*Lysol*"—

% age.	1 minute.	5 minutes.	10 minutes.	1 hour.
0	+	+	+	+
·05		—	—	—
·1		—	—	—
·25		—	—	
·5	—	—		
1	—	—		

·05 % destroyed vitality in 5 minutes.
·5 % " " in 1 minute.

Same emulsion of bacillus as used for carbolic acid.

Pure Naphthaline—

Commercial Naphthaline.—A suspension of either of these insoluble substances in water neither destroys the vitality nor retards the growth of the Bacillus pestis.

% age.	Pure naphthaline.	Commercial naphthaline.
	Exposed for 15 minutes.	Exposed for 15 minutes.
0	+	+
·5	+	+
1	+	+
2	+	+
4	+	+

"*Naphtho sublimate*".—The specimen of this proprietary article tested afforded similarly negative results. The emulsion used for these three substances was of an agar growth 15 days old from an 18-day agar growth directly from the body.

Corrosive Sublimate—

—	% age.	1 minute's exposure
First test	0	+
	·005	—
	·02	—
	·05	—
	·08	—
	·1	—
	·15	—

Emulsion of an agar growth 5 days old, from a 2-day old culture from a bubo.

—	% age.	1 minute.	5 minutes.
Second test ..	0	+	+
	·005	+	—
	·02	—	—
	·05	—	—
	·08	—	—
	·1	—	—
	·15	—	—

·005% destroyed vitality in 5 minutes.
·12 % " " in 1 minute.

Emulsion of agar growth 17 days old, from a broth culture 49 days old, 3 days from the body.

Sulphate of Copper—

—	% age.	1 minute.	5 minutes.
First test	0	+	+
	·005		+
	·02		+
	·05		+
	·08		+
	·1	+	+
	·15	+	+
	·25	+	+
	·5	+	—
	1	—	—
	2	—	—
	5	—	—

·5% destroyed vitality in 5 minutes.

Emulsion of growth 5 days old, from an agar culture 2 days from bubo.

—	% age.	5 minutes.	5 minutes.
Second test ..	0	+	+
	·005		+
	·02		+
	·05		+
	·08		+
	·1	+	—
	·15	+	—
	·25	—	—
	·5	—	—
	1	—	—
	2	—	—
	5	—	—

·15% destroyed vitality in 5 minutes.
·25% " " in 1 minute.

Emulsion of growth 19 days old, from a broth culture 7 weeks old, 3 days from the body.

Ammonia Water.—Test solutions of ammonia were prepared by the method adopted for all substances, the efficacy of which would be reduced by heat sterilisation. To test-tubes containing known quantities of sterile water, the appropriate quantity of a 5% solution was added by means of a sterile pipette. The 5% solution was obtained by the addition of one cc. of strong liquid ammonia (·880) to nineteen cc. of sterile water.

From the accompanying table it will be seen that this powerful volatile alkali has, even in a proportion (⅕%) which smells strongly of the gas, no germicidal action upon the B. pestis :—

% age.	5 minutes.
0	+
·25	+
·5	+
1	+
2	+
3	+
5	+

Emulsion of agar growth 3 days old, from an agar growth 2 days, from bubo.

Caustic Potash—

% age.	1 minute.	5 minutes.
0	+	+
·25		+
·5		—
1		—
2	+	—
3	+	+
5	+	+

Emulsion of agar culture 5 days old, from agar culture 2 days, from bubo.

The irregularity of the germicidal effect of the chemical as shown in this table has already been commented upon.

Quick Lime.—Perfectly fresh and hard quick lime, sterilised in a hydrogen flame, was added in measured proportion to water previously freed from carbonic acid gas and sterilised by heat. The ·1 % tube was the only one which did not contain solid slaked lime in suspension.

% age.	5 minutes.
0	+
·1	—
·25	—
·5	—
·75	—
1	—
2	—
3	—

·1 solution destroyed vitality in 5 minutes.

Emulsion of agar growth 3 days old from agar growth 8 days from bubo.

Borax—Sodium bi-borate—and *Common Salt*—Sodium chloride.—The reputed preservative and disinfectant action of these two substances induced one to test their germicidal power. The result was entirely negative: a 10% solution of either substance failed to destroy the vitality of the plague bacillus even after fifteen minutes' exposure.

Organic Acids :—

Formic Acid—

°/₀ age.	5 minutes.
0	+
·25	+
·5	—
1	—
2	—
4	—

·5 °/₀ destroyed vitality in 5 minutes.

Tartaric Acid—

°/₀ age.	5 minutes.
0	+
1	—
2	+
4	—
5	—

4°/₀ destroyed vitality in 5 minutes, although germicidal action appears irregular.

Acetic Acid.—Glacial acetic acid was used, and solutions prepared in a similar manner to that used for ammonia.

°/₀ age.	1 minute.	5 minutes.	
0	+	+	
·1		+	
·25		+	
·5		+	feeble growth.
·75		—	
1	—	—	
2	+	+	
4	—	—	

Germicidal action appears irregular.

Lactic Acid—

°/₀ age.	1 minute.	5 minutes.	
0	+	+	
·01		+	
·03		+	
·05		+	
·07		+	
·1		+	
·2		+	
·3		+	feeble growth.
·5		—	
1	—	—	
2	—	—	
4	—	—	

·5°/₀ destroyed vitality in 5 minutes.
1°/₀ ,, ,, in 1 minute.

The emulsion used for testing formic, acetic, tartaric and lactic acids was prepared from an agar growth 16 days old, from a broth culture 7 weeks old, from an agar culture 2 days, from the body.

Mineral Acids.—Solutions of hydrochloric acid were obtained sterile like those of ammonia; solutions of nitric and sulphuric acids were first prepared and then sterilised in the autoclave.

°/₀age.	Hydrochloric Acid.		Nitric Acid.		Sulphuric Acid.	
	1 minute.	5 minutes.	1 minute.	5 minutes.	1 minute.	5 minutes.
0	+	+	+	+	+	+
·03		—		+		+
·05		+		+		—
·07		—		+		+
·1		—		—		—
·2		—		—		—
·3		—	—	—	—	—
·5	—	—	—	—	—	—
·7	—	—	—	—	—	—
1	—	—				
	·07 °/₀ destroyed in 5 minutes. ·5 °/₀ ,, in 1 minute.		·2 °/₀ destroyed in 5 minutes. ·5 °/₀ ,, in 1 minute.		·2 °/₀ destroyed in 5 minutes. ·5 °/₀ ,, in 1 minute.	
	Emulsion of agar growth 16 days old, from 2 days' agar growth from bubo.				Agar growth 16 days old, from 7 weeks' broth culture, 3 days from body.	

Sulphate of Iron—Green Vitriol.—This substance owing to its power of absorbing oxygen from and thus reducing organic material has long held the reputation of a valuable germicide. The results of both Mr. Hankin's experiments and my own show that this salt even in perfectly unreduced solutions has but little practical utility as a plague germicide. Difficulty was experienced in obtaining sterile solutions of ferrous sulphate unoxidised. The following method proved successful.

A crystal of the salt weighing exactly four grammes held in sterile forceps was dipped into absolute alcohol and then inflamed; when the alcohol had burnt out, the crystal was dropped into a flask containing 100 cc. of cold, distilled, sterile water. From the 4 °/₀ solution which thus resulted, measured quantities were removed by means of a sterile graduated pipette and added to measured quantities of sterile distilled water in test-tubes.

°/₀age.	1 minute.	5 minutes.
0	+	+
·1		+
·2		+
1		+
2		+
4	—	—

4 °/₀ destroyed vitality in 1 minute.

Emulsion of agar growth 16 days old, from 7 weeks' broth culture from agar growth 3 days from the body.

Oxidising Agents.—Chloride of lime, potassium permanganate, bromine water.

Chloride of Lime.—A solution of this substance when pure and freshly prepared consists of a mixture of the chloride with the hypochlorite of calcium; the latter salt when acted upon by an acid in the presence of moisture is a powerful oxidiser.

°/₀age.	1 minute.	5 minutes.
0		+
·005		+
·05		—
·1		—
·25	—	
·5	—	
1	—	

·05 °/₀ destroyed vitality in 5 minutes.
·25 °/₀ ,, ,, in 1 minute.

Emulsion of agar growth 16 days old, from 6 weeks' broth culture, from 4 days' agar growth, from bubo.

Permanganate of Potash.—Mr. Hankin states this to be "an extremely potent destroyer of the bubonic microbe"; this certainly seems to be the case, and for its rapidity and certainty of action in dilute solutions one seems justified in placing it on the same footing as perchloride of mercury. The reasons why permanganate cannot be relied upon to disinfect dwelling-houses, drains, &c., have been stated in Mr. Hankin's report.

Weak solutions of permanganate were found to be reduced by exposure to the action of heat in the autoclave. Some crystals were therefore weighed, and sterilised by exposure to dry heat at 120° C.; by adding these to cold sterile water a solution of known strength was obtained, and from this the solutions of varying strengths were prepared as described under sulphate of iron.

%age.	1 minute.	5 minutes.
0	+	+
·0025		+
·005		+
·01		+
·02		—
·04	—	—
·06	—	—
·08	—	—
·1	—	—

·02 % destroyed vitality in 5 minutes.
·04 % ,, ,, in 1 minute.

Emulsion of agar growth 16 days old, from 6 weeks' broth culture, from 4 days' agar growth, from bubo.

Bromine water is being sold in Bombay as a disinfectant under the title of "Professor Gajjar's Disinfecting Fluid." A specimen of this fluid gave the following results:—

%age.	1 minute.	5 minutes.
0	+	+
·05		+
·1		+
·4		+
·8	—	—
1	—	—
2	—	—

·8 % destroyed vitality in 1 minute.

Emulsion of agar growth 20 days old, from 7 weeks' broth culture, from agar growth 3 days, from body.

Hot water.—Twenty-four test-tubes, each containing 1 cc. of watery emulsion of an agar growth (26 days old, from 6 weeks' broth culture, from agar culture, 3 days from the body) were placed in a beaker of cold water. In a twenty-fifth test-tube containing 1 cc. of water was placed a thermometer. The water in the beaker was gradually heated. At 114° F. a test-tube was removed, and some of its contents inoculated on to sterile nutrient agar; similarly at every increase of one degree of temperature a tube was removed and tested by inoculation. The following results were noted:—

The bacillus suspended in water and heated to 120° F. appears to have its power of growth slightly increased; from 120° F. to 126° F., the vitality appears to be progressively reduced, and after 126° F. it is not possible to obtain a cultivation from it.

Water heated to 127° F. or above appears therefore to destroy the vitality of the bacillus; this temperature can be borne by the hand for a few seconds only.

If the microbe of plague can exist in the surface layers of the earth floor of an infected room (a proposition open to dispute) it seems probable that it could be destroyed by pouring *boiling* water all over the surface.

In summarising these few observations, only those substances will be considered which in a solution of 1 part in 400 or more of water destroyed the bacillus of plague in 5 minutes. The efficient strength of each is quoted.

Perchloride of mercury	1 in 5,000

(in one experiment this proportion was successfully reduced to 1 in 20,000).

Permanganate of potash	1 in 5,000
Chloride of lime	1 in 2,000
"Lysol"	1 in 2,000
Hydrochloric acid	1 in 1,428
Quick lime	1 in 1,000
Sulphate of copper	1 in 1,000
Izal	1 in 1,000
Nitric acid	1 in 500
Sulphuric acid	1 in 500
Phenyle or Lawes' fluid	1 in 400

It will be seen that perchloride of mercury, permanganate of potash, chloride of lime and "lysol" proved to be by far the most potent substances examined. That most classical of germicides—carbolic acid—cannot be included, its efficient strength being but 1 in 50.

The various circumstances under which these different substances act to their best advantage has been described in other reports.

Perchloride of mercury is a thoroughly reliable germicide. As already known, its power is enhanced by the addition of a certain quantity of hydrochloric acid. The drawback to its indiscriminate use is its very poisonous nature.

Permanganate of potash though non-poisonous is speedily rendered impotent in the presence of oxidisable organic material.

Chloride of lime possesses the merits of cheapness and great efficiency. It is not dangerous, in any marked degree, to animal life.

"Lysol" is a potent germicide, but its cost is against its use on a large scale.

Quick lime is a harmless disinfectant, and is readily obtainable in most parts of the country.

Sulphate of copper exerts its germicidal properties in a somewhat irregular and uncertain manner.

Phenyle is fairly cheap. It is harmless to animals.

Very hot or boiling water is an excellent surface disinfectant for plague; its practical application would probably be inconvenient.

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(True Extract.)

for *Secretary to Government.*

J. K.

To all Collectors (A, F, G, H.).
" Surgeon-General.
" Sanitary Commissioner.
" President, Municipal Commission, Madras.
Editors' Table.

Exd. C. Parthasarathy.

Local & Munl. (1898) Department.

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SUBJECT.

Report on the germicidal action of certain disinfectants on the bacillus of plague

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Petit Laboratory,
Bombay, April 22nd, 1898.

SIR,

I have the honour to submit the following observations upon the germicidal action, with reference to the bacillus of Plague, of the following substances:—

Carbolic Acid.	Common Salt.
"Phenyle," and "Lawes' Disinfecting Fluid."	Formic Acid.
"Izal."	Acetic "
"Lysol."	Lactic "
Pure Naphthaline.	Tartaric "
Commercial "	Nitric "
"Naphtho-sublimate."	Hydrochloric Acid.
Corrosive Sublimate (Perchloride of Mercury).	Sulphuric "
Sulphate of Copper (Blue Stone).	Sulphate of Iron (Green Vitriol).
Ammonia water.	Chloride of Lime.
Caustic Potash.	Permanganate of Potash.
Quick Lime.	Bromine water.
Borax (Sodium Biborate).	Hot water.

On pages 16—30 of Mr. Hankin's report upon his investigations into Plague for the year 1897 the effect of most of these substances upon the bacillus is discussed. My results are to a large extent corroboratory of those of Mr. Hankin.

It should be remembered that the action of these various substances upon the Bacillus pestis has been observed in watery media only, a watery solution or suspension of the drug being brought to act upon a watery emulsion of the bacillus. How substances in a solid form (powder) or gaseous form (disinfectant fumes) act upon the bacillus, and how the bacillus is influenced by disinfectants when it itself is contained in a solid medium such as earth, form subjects for enquiry which are now being pursued.

In the observations recorded in this report the methods adopted by Mr. Hankin have to a large extent been imitated, these methods having been varied only when it appeared probable that greater accuracy would be obtained by the change.

It has been considered advisable to use tap water for the making of all solutions to be tested, for the reason that, when applied practically in the disinfection of dwellings, clothing, &c., the solvent of the chemical would be tap water rather than distilled water. Ferrous sulphate alone has, for special reasons, been tested with distilled water. Sterile test-tubes containing sterile solutions, or suspensions, in varying strengths of the chemical are inoculated by means of a capillary pipette with a few drops of a watery emulsion of an agaragar culture of the bacillus. Upon repeating some of the experiments recorded in this report with emulsions of plague cultures of an age different to that of the cultures used in the first experiments, a marked variation in the vitality of the bacillus was observed. This variation appears to be, in the main, that a young culture (and therefore the more virulent pathogenically) is less resistant to a germicide than an old culture. A probable explanation of the greater resisting power of an old bacillus is—that its surface has become denser and less permeable to the poisonous fluid. The discovery of this variation in the resisting power of the Bacillus pestis, whatever may be its complete explanation, has led one to quote after each summary of the action of a drug the age and pedigree of the growth used in testing it.

After allowing the chemical to act for one, five, ten, &c., minutes (the exact period being noted in each case) some of the mixture of chemical solution and bacilli is drawn into a sterile pipette and transferred to nutrient gelatine. A gelatine of 15% strength is used for all the tests; this strength of gelatine is liquid at "room temperature" which throughout all the experiments varied from 80°F. to 86°F. A separate pipette is used for the transference of each strength of inoculated chemical to its gelatine tube.

After the lapse of a week the gelatine tubes so treated, and which have been kept in a dark cupboard at room temperature, are examined; if no growth is evident, the bacillus has been destroyed by its exposure to the chemical. If growth has occurred, it becomes necessary to ascertain whether this is of the plague bacillus or of some other one accidentally introduced during the experi-

ment. For this purpose a drop of the gelatine with growth is first examined directly by the microscope; should the appearance of the bacilli not be absolutely typical, a further specimen of the gelatine growth is inoculated upon agaragar. The growth upon the latter medium is examined both macroscopically and microscopically at the end of two to four days.

It may not be altogether out of place to note here the microscopic appearances of the unstained bacillus as grown in different media. A pure culture, not more than two to three weeks old, grown in 15 % gelatine presents remarkable microscopic characters. If a drop of the culture be examined without dilution with water, the bacterial growth appears as faintly-defined slightly granular masses which can be resolved by the eye into what appear as chains of minute cocci embedded in a homogeneous ground substance; one or two detached bacilli of the usual appearance may be seen floating free, chains of bacilli are conspicuous by their absence.

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A fragment of an agar culture rubbed up in a drop of water and examined directly shows a few clusters of bacilli (especially in old cultures), but the organisms for the most part will be seen to float freely and unconnected with one another. These individual bacilli will be seen, in the case of a young culture, to have a shape resembling an hour-glass with the isthmus of the two bulbs very broad and short. Occupying almost the whole of each bulb of the hour-glass will be seen a dark spot of a flattened lenticular shape the plane surface of which looks towards that of its fellow in the opposite pole. The appearance of two dark lenticular spots with their flat surfaces opposed and close to one another is the first thing which strikes the eye of one examining the normal unstained bacillus. When floating in water two bacilli are frequently seen adhering side to side and their four polar spots being then about equidistant from one another give, at first sight, a tetrad-like appearance. In older cultures the compressed hour-glass form is accompanied by others resembling in shape the compressed [illegible] portion

Commercial Naphthaline—

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0	+	+
·5	+	+
1	+	+
2	+	+
4	+	+

"*Naphtho-sublimate*".—The specimen of this proprietary article tested afforded similarly negative results. The emulsion used for these three substances was of an agar growth 15 days old from an 18-day agar growth directly from the body.

Corrosive Sublimate—

	%age.	1 minute's exposure.
First test... ...	0	+
	·005	—
	·02	—
	·05	—
	·08	—
	·1	—
	·15	—

sterile. The same phenomenon of impaired reproductive power is noticed in the observations upon the action of heat.

Carbolic Acid.—Crystals of pure carbolic acid are dissolved in water contained in test-tubes and the solutions sterilised in the autoclave. Before inoculation with the bacillus the solutions are allowed to cool to room temperature.

In the following tables a + sign indicates the survival of the bacillus, a — sign indicates that its vitality has been destroyed.

A test-tube containing sterile water only, referred to as 0 %, or the "control tube," has in each experiment been inoculated with some of the same emulsion as that used for the tubes containing the chemical. After the transference of some of the contents of the control tube to nutrient gelatine, the appearance of a growth of Bacillus pestis in the latter is taken as evidence of the vitality of the emulsion.

Time of exposure of emulsion to action of drug.

	%age.	1 minute.	5 minutes.	15 minutes.	20 minutes.	1 hour.	6 hours.
Carbolic Acid ...	0	+	+	+	+	+	+
	·5					+	+
	1				–	–	
	2		—	–			
	3		—				
	4	–	—				

2 % solution destroyed vitality in 5 minutes.
4 % " " in 1 minute.

Emulsion of agar growth 6 days old, from agar growth 12 days old from body.

"*Phenyle*".—This preparation makes a remarkably milk-like and slightly alkaline emulsion when diluted with water. Dilute solutions (such as a ·75 %) are comparatively innocuous if drunk; hence a caution in the use of this disinfectant, from its milk-like appearance in water, appears hardly necessary.

	1	—	—
	2	—	—
	5	—	—

·5 % destroyed vitality in 5 minutes.

Emulsion of growth 5 days old, from an agar culture 2 days from bubo.

	% age.	5 minutes.	5 minutes.
Second test ...	0	+	+
	·005		+
	·02		+
	·05		+
	·08		+
	·1	+	—
	·15	+	—
	·25	—	—
	·5	—	—
	1	—	—
	2	—	—
	5	—	—

·15 % destroyed vitality in 5 minutes.
·25 % " " in 1 minute.

Emulsion of growth 19 days old, from a broth culture 7 weeks old, 3 days from the body.

Ammonia Water.—Test solutions of ammonia were prepared by the method adopted for all substances, the efficacy of which would be reduced by heat sterilisation. To test-tubes containing known quantities of sterile water, the appro-

priate quantity of a 5 % solution was added by means of a sterile pipette. The 5 % solution was obtained by the addition of one cc. of strong liquid ammonia (·880) to nineteen cc. of sterile water.

From the accompanying table it will be seen that this powerful volatile alkali has, even in a proportion (5 %) which smells strongly of the gas, no germicidal action upon the B. pestis :—

%age.	5 minutes.
0	+
·25	+
·5	+
1	+
2	+
3	+
5	+

Emulsion of agar growth 3 days old, from an agar growth 2 days, from bubo.

Caustic Potash—

%age.	1 minute.	5 minutes.
0	+	+
·25		+
·5		—
1		—
2	+	—
3	+	+
5	+	+

Emulsion of agar culture 5 days old, from agar culture 2 days, from bubo.

The irregularity of the germicidal effect of the chemical as shown in this table has already been commented upon.

Quick Lime.—Perfectly fresh and hard quick lime, sterilised in a hydrogen flame, was added in measured proportion to water previously freed from carbonic acid gas and sterilised by heat. The ·1 % tube was the only one which did not contain solid slaked lime in suspension.

%age.	5 minutes.
0	+
·1	—
·25	—
·5	—
·75	—
1	—
2	—
3	—

·1 % solution destroyed vitality in 5 minutes.

Emulsion of agar growth 3 days old from agar growth 8 days from bubo.

Borax—Sodium bi-borate—and *Common Salt*—Sodium chloride.—The reputed preservative and disinfectant action of these two substances induced one to test their germicidal power. The result was entirely negative : a 10% solution of either substance failed to destroy the vitality of the plague bacillus even after fifteen minutes' exposure.

Organic Acids :—

Formic Acid—

% age.	5 minutes.
0	+
·25	+
·5	—
1	—
2	—
4	—

·5% destroyed vitality in 5 minutes.

Tartaric Acid—

% age.	5 minutes.
0	+
1	—
2	+
4	—
5	—

4% destroyed vitality in 5 minutes, although germicidal action appears irregular.

Acetic Acid.—Glacial acetic acid was used, and solutions prepared in a similar manner to that used for ammonia.

% age.	1 minute.	5 minutes.
0	+	+
·1		+
·25		+
·5		+ feeble growth.
·75		—
1	—	—
2	+	+
4	—	—

Germicidal action appears irregular.

Lactic Acid—

% age.	1 minute.	5 minutes.
0	+	+
·01		+
·03		+
·05		+
·07		+
·1		+
·2		+
·3		+ feeble growth.
·5		—
1	—	—
2	—	—
4	—	—

·5% destroyed vitality in 5 minutes,
1% „ „ in 1 minute.

The emulsion used for testing formic, acetic, tartaric and lactic acids was prepared from an agar growth 16 days old, from a broth culture 7 weeks old, from an agar culture 2 days, from the body.

Mineral Acids.—Solutions of hydrochloric acid were obtained sterile like those of ammonia; solutions of nitric and sulphuric acids were first prepared and then sterilised in the autoclave.

%age.	Hydrochloric Acid.		Nitric Acid.		Sulphuric Acid.	
	1 minute.	5 minutes.	1 minute.	5 minutes.	1 minute.	5 minutes.
0	+	+	+	+	+	+
·03		—		+		+
·05		+		+		—
·07		—		+		+
·1		—		+		+
·2		—		—		—
·3		—		—		—
·5	—	—	—	—	—	—
·7	—	—	—	—	—	—
1	—	—	—	—	—	—
	·07 % destroyed in 5 minutes. ·5 % „ in 1 minute.		·2 % destroyed in 5 minutes. ·5 % „ in 1 minute.		·2 % destroyed in 5 minutes. ·5 % „ in 1 minute.	
	Emulsion of agar growth 16 days old, from 2 days' agar growth from bubo.				Agar growth 16 days old, from 7 weeks' broth culture, 3 days from body.	

Sulphate of Iron—Green Vitriol.—This substance owing to its power of absorbing oxygen from and thus reducing organic material has long held the reputation of a valuable germicide. The results of both Mr. Hankin's experiments and my own show that this salt even in perfectly unreduced solutions has but little practical utility as a plague germicide. Difficulty was experienced in obtaining sterile solutions of ferrous sulphate unoxidised. The following method proved successful.

A crystal of the salt weighing exactly four grammes held in sterile forceps was dipped into absolute alcohol and then inflamed; when the alcohol had burnt out, the crystal was dropped into a flask containing 100 cc. of cold, distilled, sterile water. From the 4 % solution which thus resulted, measured quantities were removed by means of a sterile graduated pipette and added to measured quantities of sterile distilled water in test-tubes.

% age.	1 minute.	5 minutes.
0	+	+
·1		+
·2		+
1		+
2		+
4	—	—

4 % destroyed vitality in 1 minute.

Emulsion of agar growth 16 days old, from 7 weeks' broth culture from agar growth 3 days from the body.

Oxidising Agents.—Chloride of lime, potassium permanganate, bromine water.

Chloride of Lime.—A solution of this substance when pure and freshly prepared consists of a mixture of the chloride with the hypochlorite of calcium; the latter salt when acted upon by an acid in the presence of moisture is a powerful oxidiser.

%age.	1 minute.	5 minutes.
0		
·005		+
·05		+
·1		—
·25	—	
·5	—	
1	—	

·05 % destroyed vitality in 5 minutes.
·25 % „ „ in 1 minute.

Emulsion of agar growth 16 days old, from 6 weeks' broth culture, from 4 days' agar growth, from bubo.

Permanganate of Potash.—Mr. Hankin states this to be "an extremely potent destroyer of the bubonic microbe"; this certainly seems to be the case, and for its rapidity and certainty of action in dilute solutions one seems justified in placing it on the same footing as perchloride of mercury. The reasons why permanganate cannot be relied upon to disinfect dwelling-houses, drains, &c., have been stated in Mr. Hankin's report.

Weak solutions of permanganate were found to be reduced by exposure to the action of heat in the autoclave. Some crystals were therefore weighed, and sterilised by exposure to dry heat at 120° C.; by adding these to cold sterile water a solution of known strength was obtained, and from this the solutions of varying strengths were prepared as described under sulphate of iron.

%age.	1 minute.	5 minutes.
0	+	+
·0025		+
·005		+
·01		+
·02		—
·04	—	—
·06	—	—
·08	—	—
·1	—	—

·02 % destroyed vitality in 5 minutes.
·04 % „ „ in 1 minute.

Emulsion of agar growth 16 days old, from 6 weeks' broth culture, from 4 days' agar growth, from bubo.

Bromine water is being sold in Bombay as a disinfectant under the title of "Professor Gajjar's Disinfecting Fluid." A specimen of this fluid gave the following results:—

%age.	1 minute.	5 minutes.
0	+	+
·05		+
·1		+
·4		+
·8	—	—
1	—	—
2	—	—

·8 % destroyed vitality in 1 minute.

Emulsion of agar growth 20 days old, from 7 weeks' broth culture, from agar growth 3 days, from body.

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Hot water.—Twenty-four test-tubes, each containing 1 cc. of watery emulsion of an agar growth (26 days old, from 6 weeks' broth culture, from agar culture, 3 days from the body) were placed in a beaker of cold water. In a twenty-fifth test-tube containing 1 cc. of water was placed a thermometer. The water in the beaker was gradually heated. At 114° F. a test-tube was removed, and some of its contents inoculated on to sterile nutrient agar; similarly at every increase of one degree of temperature a tube was removed and tested by inoculation. The following results were noted:—

The bacillus suspended in water and heated to 120° F. appears to have its power of growth slightly increased; from 120° F. to 126° F., the vitality appears to be progressively reduced, and after 126° F. it is not possible to obtain a cultivation from it.

Water heated to 127° F. or above appears therefore to destroy the vitality of the bacillus; this temperature can be borne by the hand for a few seconds only.

If the microbe of plague can exist in the surface layers of the earth floor of an infected room (a proposition open to dispute) it seems probable that it could be destroyed by pouring *boiling* water all over the surface.

In summarising these few observations, only those substances will be considered which in a solution of 1 part in 400 or more of water destroyed the bacillus of plague in 5 minutes. The efficient strength of each is quoted.

Perchloride of Mercury	1 in 5,000

(in one experiment this proportion was successfully reduced to 1 in 20,000).

Permanganate of Potash	1 in 5,000
Cholöride of Lime	1 in 2,000
"Lysol"	1 in 2,000
Hydrochloric Acid	1 in 2,000
Quick Lime	1 in 1,428
Sulphate of Copper	1 in 1,000
Izal	1 in 1,000
Nitric Acid	1 in 1,000
Sulphuric Acid	1 in 500
Phenyle or Lawes' Fluid	1 in 500
	1 in 400

It will be seen that perchloride of mercury, permanganate of potash, chloride of lime and "lysol" proved to be by far the most potent substances examined. That most classical of germicides—carbolic acid—cannot be included, its efficient strength being but 1 in 50.

The various circumstances under which these different substances act to their best advantage has been described in other reports.

Perchloride of mercury is a thoroughly reliable germicide. As already known, its power is enhanced by the addition of a certain quantity of hydrochloric acid. The drawback to its indiscriminate use is its very poisonous nature.

Permanganate of potash though non-poisonous is speedily rendered impotent in the presence of oxidisable organic material.

Chloride of lime possesses the merits of cheapness and great efficiency. It is not dangerous, in any marked degree, to animal life.

"Lysol" is a potent germicide, but its cost is against its use on a large scale.

Quick lime is a harmless disinfectant, and is readily obtainable in most parts of the country.

Sulphate of copper exerts its germicidal properties in a somewhat irregular and uncertain manner.

Phenyle is fairly cheap. It is harmless to animals.

Very hot or boiling water is an excellent surface disinfectant for plague; its practical application would probably be inconvenient.

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