[L. & M. 5,000—5-4-99.]

Local & Munl. (1899) Department.

MISCELLANEOUS.

G.O., No. 758

DATED 20th May 1899

[Despatch Abstract.]

Perchloride of mercury in the presence of Condies

Recording Summary of Dr Marsh's report on the efficiency of perchloride of mercury as a disinfectant when used in the presence of condies

BACK PAPERS.

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

FURTHER PAPERS.

G.O.	No.	Department.	Date.	G.O.	No.	Department.	Date.
CW	1944P	9/99					

Local & Munl. (189) Department.

CURRENT No. 976P

~~Manuscript~~ Abstract **From** The Under Secretary to the Govt of Bombay

Order No. 758 P. Mis. dated 20th May 1899 with S. Comm^r

Recorded. M. N. J.

Requested to issue a circular to Collectors & medical officers on the subject & forward 3 copies of it to the Govt office for record.

No. 3016/P

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SUBJECT.

Forwarding copies of Resol^n No. 3001 P dy 3-5-99 relative to a report by Dr Marsh on the efficiency of perchloride of mercury as a disinfectant when used in the presence of

8.V.99 Cowding

To Surg 15/5/99

The Surgeon-General
" Secretary Comm^r
" Principal, Medical College Madras

Ack^d 15/5/99

Despd. 15/5/99 2015. Encl. No. 1 Hd 8.5.99

Note

Dr Marsh's results are found in the Resolution. One copy of the report may, on receipt be sent to S. G and one to S. C.

This is necessary under Medl Special Comm^r for San Comm^r & Surgeon Genl.

Each one copy with 1 copy [illegible]

BACK PAPERS.

G.O.	No.	Department.	Date.

FURTHER PAPERS.

G.O.	No.	Department.	Date.

Plague.—

Reports.

Report by Dr. E. L. Marsh on the efficiency of mercuric perchloride as a disinfectant when used in the presence of cowdung.

General Department (Plague).
No. 3001-P.

Bombay Castle,
3rd May 1899.

Letter from W. M. Haffkine, Esq., C.I.E., No. 1688, dated the 31st March 1899—

Forwards, with reference to Government Resolution No. 6894 - 6997-P., dated the 16th December 1898, a report by Dr. Marsh on the efficiency of mercuric perchloride as a disinfectant when used in the presence of cowdung, either alone or after the application of electrolysed sea-water; and observes as follows:—

"2. These experiments show that the mercuric perchloride solution must be well acidulated to render it effective under the circumstances found in actual practice locally. If this is done, Dr. Marsh estimates that it is possible, by using the solution freely so that the maximum effect is produced, to reduce the microbes present by 94 per cent. To effect this the 8 gallons have to be used for every 100 square feet of cowdunged floor, and this procedure is effective to a depth of $\frac{1}{16}$ of an inch. It is probable that microbes sheltered by leaves, paper, or hidden in cracks escape.

"3. The use of electrolysed sea-water by itself is proved to be impracticable owing to the large amount required.

"4. The combination of the two methods, *i.e.*, the use of percloride solution after preliminary treatment with electrolysed sea-water appears to afford the best results, as the microbes are reduced by 98 per cent., when the disinfectants are allowed to act under the most favourable conditions. The probable reasons for this are enumerated on page 19 of the report. The amount of liquid required is 5 gallons of electrolysed sea-water and 8 gallons of acid perchloride solution for a floor measuring $13' \times 7\frac{1}{2}'$.

"5. From the summary of results got by counting the numbers of colonies of microbes growing in agar plates, it appears that by perchloride solution only the microbes are reduced 65 per cent. below the average of occupied plague-infected rooms; by perchloride solution + electrolysed water the microbes are reduced 93 per cent. below the average of occupied plague-infected rooms; by perchloride solution + lime-wash + unroofing the microbes are reduced 83 per cent. below the average of occupied plague-infected rooms.

"6. I would suggest that this very careful and laborious research be printed by Government and distributed for information. The Laboratory is now being constantly asked to give advice in such matters as disinfection (*vide* letter attached) and it would be advantageous to have researches like this available for the information of enquirers."

Resolution.—The Report by Dr. Marsh forwarded by Monsieur Haffkine should be printed at the Government Central Press and copies distributed by the Superintendent as follows:—

	Copies.
All Commissioners ... ... (2 each)	8
The Plague Commissioner	1
The Secretary, Indian Plague Commission	10
All Collectors ... ... (5 each)	115
The Deputy Adjutant General, Bombay Command	5
The Political Agents, Kolhápur, Káthiáwár, Cutch, Mahi Kántha and Rewa Kántha ... ... (5 each)	25
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The Inspector General of Police	5
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The Principal Medical Officer, Bombay Command	2
The Chairman, Poona Cantonment and Suburban Plague Committee	2
The Chairman, Belgaum Plague Committee	2
The Chairman, Karáchi Plague Committee	2
The Chairman, Hyderabad Plague Committee	2
The Chief Plague Authority, Poona City	2
Major W. B. Bannerman, M.D., I.M.S., Plague Research Laboratory.	100
The Private Secretary to His Excellency the Governor ...	2
The Government of India...	32
The Government of Madras	10
The Government of Bengal	10
The Government of Burma	10
The Government of the North-Western Provinces and Oudh ...	10
The Government of the Punjáb	10
The Government of Ceylon	10
The Chief Commissioner, Central Provinces	10
The Chief Commissioner, Assam	10
The Resident at Hyderabad	10
The Resident in Mysore	10
The Agent to the Governor General at Baroda	10
The Agent to the Governor General in Central India ...	10
The Editors' Table, Bombay	1
The Editors' Table, Poona	1
The Editors' Table, Belgaum	1
The Editors' Table, Dhárwár	1
The General Department of the Secretariat (Plague)	100

The Surgeon General with the Government of Bombay should be requested to inform the Superintendent, Government Central Press, direct how many copies of the Report he requires for his own use and for distribution to Medical Charges, &c.

J. K. CONDON, Lt.,
Under Secretary to Government.

To

The Secretary, Indian Plague Commission (*care of* India Office, London),
The Plague Commissioner,
The Commissioner in Sind,
The Commissioner, N. D.,
The Commissioner, C. D.,
The Commissioner, S. D.,
All Collectors, including the Collectors and Deputy Commissioners in Sind,
The Municipal Commissioner for the City of Bombay,
The Surgeon General with the Government of Bombay,
The Sanitary Commissioner for the Government of Bombay,

The Health Officer of the Port of Bombay,
The Supervising Medical Officer, Railway Medical Inspection,
The Political Agents, Káthiáwár, Cutch, Kolhápur and Southern Marátha Country, Rewa Kántha and Mahi Kántha,
The Political Superintendents, Pálanpur and Sávantvádi,
The Deputy Adjutant General, Bombay Command,
The Principal Medical Officer, Bombay Command,
The Commissioner of Police, Bombay,
The Inspector General of Police,
The Chairman, Poona Cantonment and Suburban Plague Committee,
The Chairman, Belgaum Plague Committee,
The Chairman, Karáchi Plague Committee,
The Chairman, Hyderabad Plague Committee,
The Chief Plague Authority, Poona City,
The Private Secretary to His Excellency the Governor,
The Superintendent, Government Central Press,
(With the manuscript.)
All Departments of the Secretariat (except the Separate, Legislative and Ecclesiastical Departments),
Major W. B. Bannerman, M.D., I.M.S.,
The Government of India,
The Government of Bengal,
The Government of Madras,
The Government of the North-Western Provinces and Oudh,
The Government of the Punjáb,
The Government of Burma,
The Government of Ceylon,
The Agent to the Governor General at Baroda,
The Agent to the Governor General in Central India,
The Resident in Mysore,
The Resident at Hyderabad,
The Chief Commissioner, Central Provinces,
The Chief Commissioner, Assam.
} By letter.

No. of 1899.

Copy forwarded for information and guidance to

No. $\frac{3016}{P.}$ of 1899.

General Department (Plague).

Bombay Castle, 4th May 1899.

From

Lieutenant J. K. CONDON, I.S.C.,
Under Secretary to Government;

To

The Secretary to the Government of Madr
Local and Municipal Departme

Sir,

I am directed to forward for *the information of the Government of Madras,* 10 copies of this Government Resolution No. 3001-P., dated the 3rd instant, relative to a report by Dr. E. L. Marsh on the efficiency of mercuric perchloride as a disinfectant when used in the presence of cowdung, and to state that the Superintendent, Government Central Press, Bombay, has been directed to furnish you direct with *10* copies of the Report when printed.

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

J. K. Condon.
Under Secretary to Government.

☞This report is to be substituted for the one previously distributed, which should be destroyed.

FROM

E. L. MARSH, M.B., D.P.H.,
Special Plague Medical Officer
attached to the Plague Research Laboratory;

To

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

SIR,

I have the honour to submit my report on the efficiency of perchloride of mercury, in disinfectant solution, as an application to cowdung floors, and on the utility of saturating cowdung (or simple mud) floors with sea water before spraying them with perchloride of mercury. I also enclose and reproduce, as Appendices C and D to the report, letter No. 1992, dated 29th July 1898, from the acting Plague Commissioner, Bombay Presidency, and Government Resolution No. 6894—6997-P., dated 16th December 1898, asking for the information which forms the subject-matter of the present investigations.

I have the honour to be,
Sir,
Your most obedient Servant,
E. L. MARSH.

Plague Research Laboratory,
Bombay, 14*th March* 1899.

Report on the efficiency of Perchloride of Mercury, in disinfectant solution, as an application to cowdung floors; and on the utility of saturating cowdung (or simply mud) floors with sea water before spraying them with perchloride of mercury.

The following report on the efficiency of perchloride of mercury in disinfectant solution, is in answer to a letter from the Plague Commissioner to Dr. Haffkine (*vide* Appendix C), calling for tests as to the degree in which the efficient action of perchloride of mercury is interfered with when used as an application to the cowdung floors of ordinary native houses. The report also includes experiments in answer to the enquiry of Government (*vide* Appendix D) regarding the utility of saturating cowdung (or simple mud) floors with sea water before spraying them with the perchloride solution.

In the experiments of Mr. Hankin and Dr. Pitchford, perchloride of mercury dissolved in sterile water in the proportion of 1 in 5,000 caused the death of a pure agar culture of the plague micro-organism after 1 minute's contact. With a longer exposure the disinfectant only began to be unreliable at 1 in 20,000. Abel, quoted by Mr. Hankin, found that a 1 in 5,000 solution destroyed cultures of the plague bacillus growing on agar within an hour.

These experiments give the efficiency of perchloride of mercury in aqueous solution a high germicidal value when the object is to suppress the plague organism in test solutions *in vitro*. But when the test of disinfection-efficiency includes the organic and other matter commonly present on the floors and walls of native houses, factors of great importance in regard to the retention of the potency of perchloride of mercury in ordinary solution are introduced. The large augmentation of organic and mineral matter received by the floors of ordinary native houses from the use of cowdung as a surface application* occasions a concurrence of chemical and physical conditions that must make the use of a simple solution of perchloride of mercury entirely unsatisfactory for the purpose in hand.

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* According to analyses by Voelker and Leather on the composition of Indian cattle dung published in the *Agricultural Ledger*, No. 3 of 1894, the mean of six analyses of six samples showed a percentage composition of 35·7 organic matter, 27·7 mineral matter, and 36·6 moisture. In the same reference it is stated that for purposes of manure cattle dung at the rate of 120 cwts. per acre per annum is recommended to be applied to land. It is interesting to remark the generous treatment of an acre of domestic flooring. Allowing that 25 grammes represents the amount of moist cowdung commonly applied to 1 square foot of the surface of a mud floor—a moderate estimate according to control results,—and that the average floor space of a native house measures 100 square feet, then 2,500 grammes or $5\frac{1}{2}$ lbs. of cowdung are applied at each weekly dressing, making a total of 285 lbs. a year. It is easy to calculate further and show that for purposes of domestic use cowdung at the rate of 1,100 cwts. per acre is applied to the floors of ordinary native dwellings in India each year.

In many houses the cowdung is mixed with a reddish clayey soil to lighten the colour of the application and improve its cohesion. In Bombay a little of the red soil is often blended with mud from the street in varying proportions, or mud alone may be used. When applied on a basis of beaten earth or mud the air rapidly dries a fresh-spread application giving the surface a firm texture and an impermeability like that of coarse brown paper. The later effects of drying are shown in cracks, fissures, and loosening of the surface in flakes.

It seems doubtful whether the practice is performed more in accordance with the precepts of Hindu sacred law than simply as a measure of utility. The author of *Govinda Sâmanda, or the History of a Bengal Râiyat*, offers the suggestion that its use indicates it to be a disinfectant. In describing and commenting on the practice in Chapter XI, he says: "The women of the household make a solution of cowdung and water, and sprinkle the liquid by the hand on the open yard, which is next swept by a broom. * * The rooms and the verandahs require to be cleansed and washed in another fashion. As the flooring was entirely of earth, there being not a single brick or stone in the house, or a plank of wood either, every inch of the floor of every room was besmeared by means of a bit of rag, with the said solution of cowdung and water, and allowed to dry itself. * * The floor greatly improves by the process. It becomes smooth and glossy, and no cracks are visible. And as for any disgusting smell there is nothing of the sort—the smell, if any, being positively pleasant. Hindu peasants besmear their cottages with a solution of cowdung and water, because cowdung is regarded ceremonially as a purifier; it is, however, a question why Hindu law-givers should have pitched upon cowdung as a purifier. * * From the universal practice of the Hindus of Bengal I should be inclined to think that cowdung was a disinfectant."

Its reputation as a disinfectant seems to be connected with the experience that insects and

Some of the difficulties in the disinfection of native floors have been already detailed in the letter of Lieut.-Colonel R. Boustead, I. M. S., to Government, dated the 20th September 1898 (*vide* Appendix D). An abstract of this reference describes how 'the cowdung naturally contains a considerable amount of albuminous coagulable slime which, when spread over a mud floor, in the usual way, becomes dried and almost impervious to water, but when solutions of sublimate are sprayed over this coating of cowdung the slime in it is coagulated (as is albumin) by the action of the sublimate and forms an actual impervious film which is a sufficient protection to almost all the microbes that are beneath it. It encases them as it were in a water-proof covering. No aqueous solution of sublimate or carbolic acid would be able to effectively penetrate this coagulum and disinfect the microbes below, except perhaps where the coating of cowdung was broken through. Again, in the decomposition of the cowdung on floors (which is continual) ammonia gas is generated; some of it is fixed in organic matter, some of it is free, being given off continually, and this alkali precipitates and renders abortive (useless) the germicidal properties of the sublimate by converting it into simple ammonia chloride, which is useless as a germicide, thus, further, the excellent germicidal properties of the sublimate are destroyed by ammonia within the cowdung.'

As a preliminary investigation on cowdung it was found that the reaction of dung fresh from the food-canal of a healthy animal is alkaline, sometimes neutral. Allowed to dry in the air the cake remains alkaline or neutral. Kept moist with water and exposed to the air, after 3 to 8 days it may become slightly acid, but subsequently again becomes alkaline, and continues slightly alkaline for months. It has a characteristic, somewhat aromatic odour when fresh, which it loses for a faint loamy smell on drying. The faint aromatic odour persists for several months when the sample is kept moist. The presence of saline and albuminoid ammonia* in samples was detectable by Nessler's solution at intervals throughout a period of four months.

A note on the ordinary bacteriology of cowdung is given in Appendix A.

In order to ascertain in what degree (approximately) cowdung—a material of a fairly definite chemical composition—interferes with the germicidal action of perchloride of mercury in relation to the micro-organism of plague the following experiment was undertaken with a solution of the disinfectant of the strength mentioned in the opening statement, namely 1 in 5,000. In this, and in the subsequent experiments, the 'effective strength' of the disinfectant was chiefly tested, the time element being considered of importance only as showing, where negative results were obtained, that the disinfection action continued in operation throughout the 18 to 24 hours of the experiment.

A series of test tubes containing from 0·5 gramme to 5 grammes of *sterilized* fresh cowdung in the form of dust were prepared. A measured quantity of *sterile distilled* water containing perchloride of mercury in solution was added to the

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body-vermin cause less annoyance to the inmates of a freshly cowdunged room. It is claimed for cowdung and bullock's dung as against the dung of buffaloes that while the latter fails to rid the floor of vermin and insects the former not only causes such nuisances to cease, but destroys their breeding ground (Letter of a Native to Dr. Haffkine).

A native work on Aryan Medicine (*Arya Vaidyaka*, p. 83) makes reference to the use of cowdung and other products of the cow in ceremonial processes of purification, and to the value of cowdung as an agent for cleansing and purifying floors and walls. It also mentions its utility when mixed with cow's urine to allay the itching of prurigo, and effect the cure of certain chronic skin diseases. Mixed with salt it is recommended as an application to check excessive sweating of the hands and feet.

According to the *Bombay Gazetteer*, Vol. XVIII, Pt. I, p. 59, the *mantra*, or sacred verse repeated on the exhibition of the products of the cow on the *Shravani* Day, to purify the soul from sin, in translation is, *By the drinking of the five products of the cow the sin which has penetrated into my skin and bones is burnt, like fuel by fire.*

An investigation on the approximate number and varieties of micro-organisms commonly found in cowdung appears as an appendix to this report.

*The organic matter in six samples analysed by Voelker and Leather yielded an average of ·837 per cent. of nitrogen, equal to 1·014 per cent. of ammonia.

The mineral matter averaged 15·58 per cent. of insoluble siliceous matter; 2·36 per cent. oxide of iron and alumina; ·90 per cent. lime; ·36 per cent. magnesia; ·88 per cent. potash; ·30 per cent. soda; ·3 per cent. phosphoric acid, equal to ·7 per cent. phosphate of lime. The *Agricultural Ledger*, No. 3 of 1894.

tubes so that each tube held the test amount of cowdung mixed with 10 cubic centimetres of water containing ·002 gramme of $HgCl_2$ (perchloride of mercury), or a porportion of 1 in 5,000. At the same time each tube received a glass bristle wetted in its lower third by a thin film of living culture obtained from the surface of a three days' old agar growth of plague. The experimental tubes were kept at the temperature of the laboratory for 18 hours. At the end of this interval the glass bristles infected with plague were withdrawn from the paste of cowdung and perchloride solution in the different tubes, washed in a weak solution of carbonate of soda, and inoculated *seriatim* into tubes containing 20 cubic centimetres of nutrient broth. The culture tubes were incubated at the temperature of the laboratory for ten days, and their ability to produce fresh cultures of the test organism was as follows:—

Gramme of $HgCl_2$ in 10 c. c. of water.	Amount of cowdung acted upon.	Growth in Broth after 18 hours' attempted disinfection.
·002	·5 gramme.	—
,,	1·0 ,,	—
,,	1·5 grammes.	—
,,	2·0 ,,	—
,,	2·5 ,,	+
,,	3·0 ,,	+
,,	3·5 ,,	+
,,	4·0 ,,	+
,,	4·5 ,,	+
,,	5·0 ,,	+

— sign denotes the absence of growth.
+ sign ,, presence ,,

Thus, in an experiment done under artificial conditions it would appear that quantities of sterilized fresh cowdung above 2 grammes interfere with the disinfecting action of 10 c. c. of an aqueous solution of perchloride of the strength of 1 in 5,000, and this in an experiment in which the feebly resistant plague micro-organism was used as the indicator, and in which the only variable factor was the sterilized cowdung.

Such a result is due in part to the alkalinity of the cowdung, but (probably) chiefly to the presence of compound ammonia bases and other constituents that interfere with the continued solution of the perchloride of mercury.

The germicidal action of perchloride of mercury may be said to depend on the property of precipitating albumin and albuminoid bodies. Its power to combine with the protoplasm of the plague microbe rapidly and effectively in a solution of less than 1 in 5,000, and thereby kill the organism, is its chief recommendation.

The disadvantages of perchloride of mercury as a common disinfectant are (1) it is easily thrown out of aqueous solution; (2) it acts on metal work; (3) the use of a hard water containing calcium carbonate held in solution by carbonic acid causes a precipitate; (4) it is precipitated by any soluble alkaline carbonate such as carbonate of ammonia (found in cowdung floors soiled with urine); (5) it is decomposed by oxides such as lime with the formation of an insoluble oxide of mercury (hence its use is interdicted for walls, &c., newly lime washed, ; (6) the sulphur and the sulphuretted hydrogen of decomposing organic matter convert it into insoluble sulphides; (7) it acts only as a strictly local disinfectant, and its germicidal efficiency depends on it reaching the organism in such a manner that albuminate of mercury does not form a coating protecting the organism from further action (as for example in the disinfection of mucous discharges like sputa when it may coagulate the exterior of the mass and fail to disinfect the centre).

The weakening by dissociation in (1), (3), (4), (5) and (6) when the basic mercuric chloride is used can be prevented by acidifying the solution with hydrochloric acid, the acid chloride remaining in solution. Hydrochloric acid in slight excess adds to the disinfecting power. The further addition of a chloride of

ammonia or soda helps the keeping power, and aids in the disinfection by disintegrating organic matter like sputum. The use of wooden appliances removes the objection under (2) (the oiling of metal work gives no security, as oleates of mercury are thereby formed).

In order, therefore, to obtain satisfactory results when perchloride of mercury is used as a house disinfectant, the mercury salt must be (1) present in sufficient amount; (2) the permanence of the solution secured; (3) any substance which by precipitation diminishes the available amount of soluble disinfectant present neutralised.

In Bombay, for local disinfection, a solution containing perchloride of mercury, ammonium chloride, and hydrochloric acid is commonly employed. Thus—

Perchloride of mercury	... 21 oz.
Chloride of ammonia	... 15 oz.
Hydrochloric acid	... 100 oz.
Water	... 340 oz.
	476 oz.

For actual use 5 ounces of the above solution are diluted in 1 gallon of water which gives a solution containing the perchloride in the proportion of 1 in 725.

The capacity of hydrochloric acid for combining with mercuric chloride to form an acid chloride of mercury is of importance. As hydrochloric acid belongs to the monobasic acids, it does not, therefore, give true acid salts (like H Na SO_4 or H Na CO_3); nevertheless many metallic chlorides like mercuric chloride are capable of *combining with hydrochloric acid* just as they combine with water. These compounds with hydrochloric acid are more soluble in water than the metallic chlorides themselves. Ammonia chloride also belongs to the class of compounds with which hydrochloric acid can combine. With common salt (chloride of sodium), however, strong solutions of hydrochloric acid cause a precipitate of the soda salt from its solution. It may further be remarked that solutions of ammonia chloride have the advantage of possessing an acid reaction even when prepared from the salt after prolonged washing of the sublimed salt with water.

As strong solutions of hydrochloric acid diffuse more rapidly into dilute solutions of the acid than into plain water it is an advantage in practice to slightly acidulate the water used for diluting the strong stock solution. It may be well to mention that hydrochloric acid combines very energetically with water, and in so doing evolves a considerable amount of heat. The formation between hydrochloric acid and water of two definite hydrates, H Cl, 2 H_2O and H Cl, 6 H_2O, is now accepted upon the basis of many facts. But both of them, inasmuch as they occur in a liquid state, dissociate with great facility into hydrochloric acid and water. According to Roscoe at 75° F. and 104° F. a hundred grammes of water at atmospheric pressure dissolves 70 and 63 grammes of hydrochloric acid respectively. (*Vide* Mendeléeff, *The Principles of Chemistry*, pp. 450—451.)

An experiment on the relative efficiency of two solutions made according to the above formula was carried out. Solution A had been prepared in the laboratory; Solution B was a sample of the disinfectant used by the Plague Officers in one of the districts of Bombay. In this and subsequent experiments some account must be taken of the use of common tap water* as the solvent of the mercury salt, and also of the fact that the composition of the test cowdung and dirt† was rarely constant even in the same series of test. Whenever equivocal results seemed to be obtained, the tests were repeated.

Two series of test tubes containing from 0·2 gramme to 3 grammes of cowdung (weighed dry) were prepared. A measured quantity of the test solution was poured into the tubes so that each tube held the test amount of cowdung wetted by 10 c.c. of the disinfectant solution. Care was taken to thoroughly wet the cowdung with the solution, 10 c.c. of which should moisten dry cowdung in a fine state of division up to 5 grammes. At the end of 18 hours a minute

* A mean of twelve analyses made by Messrs. Treacher & Co., of the filtered and unfiltered water supplied to Bombay during November 1898, gave the following composition: *Total solids* 7·4 grains per gallon; *chlorine* 7 grains per gallon; *free ammonia* ·228 parts per million; *albuminoid ammonia* ·168 parts per million. *Hardness* 3 degrees.

† The samples collected from the floors of native houses consisted of particles of sand, chalk, clay, carbon and crystals of salts in varying proportions. Also fragments of vegetable *débris*, seeds, spores, starch grains; the dead remains of insects; dried sputum, cowdung, hairs, living insects, and a quantity of questionable material.

quantity of the cowdung was abstracted and inoculated into nutrient broth. The results obtained were as follows:—

Gramme of $Hg Cl_2$ in 10 c.c. of water.	Amount of cowdung acted upon.	Growth in Broth after 18 hours' attempted sterilization.	
		Solution A.	Solution B.
·014	·2 gramme.	—	—
,,	·3 ,,	—	—
,,	·4 ,,	—	—
,,	·5 ,,	— — —	— — +
,,	·8 ,,	— — +	+ +
,,	1·0 ,,	+ +	+
,,	1·5 grammes	+	+
,,	2·0 ,,	+	+
,,	3·0 ,,	+	+

NOTE.—Spores of moulds and of Bacillus megatherium, B. subtilis, and B. mycoides were obtained from the unsterilized cowdung.

Thus it would appear that 0·5 gramme of ordinary cowdung can be efficiently sterilized by 10 c.c. of solution A, and that 0·8 gramme of cowdung cannot be efficiently sterilized of spore-bearing micro-organisms by either of the test solutions.

While the above results represent the optimum efficiency of the solution of perchloride of mercury when used as a sterilizing agent, much less chemical energy would be required of the solution to disinfect the same material of a comparatively feebly resisting micro-organism such as the plague bacillus. As it is of importance from a practical point of view to ascertain the degree of efficiency of the disinfectant solution in relation to the micro-organism of plague, the above experiment was repeated in all its details with living cultures of the plague bacillus as the test-organism. Each tube received, in addition to the cowdung and disinfectant solution, a glass bristle infected with a culture of the plague microbe recovered from a broth culture 15 days old.* Care was taken to wash the bristles in sterile water to remove the adherent broth before inoculating them into the test tube. On withdrawing the bristles at the end of the experiment they were first washed in sterile water made faintly alkaline with carbonate of soda, then agitated in melted agar, and finally plated from a second tube of melted agar for observation and recovery of the test organisms. Although this method of differentiating and identifying the test organism is not a delicate one, still, in the absence of animal inoculation tests, it appeared the most satisfactory one to employ. The use of broth is very unsatisfactory owing to the rapid vegetation of the common saprophytic bacteria. In those instances where the results seemed to fall short of accurate demonstration the tests were repeated. The results obtained were as follows:—

* The glutinous property of a culture of the plague micro-organism is utilized in this method. The possession of this property enables the organism to retain its hold of the surface of the bristle throughout the experiment and thereby facilitates the recovery of the organism at the end of the test. It might be objected that this method introduces a fallacy into the tests, in that it (possibly) prevents the disinfectant solution getting into sufficiently intimate contact with the organism to kill it. As the same difficulty exists in natural conditions—inoculated into sterile garden mould the organisms effect the same tenacious hold of the earth particles—the adoption of the bristle method only copies the difficulties peculiar to disinfection against plague.

Gramme of Hg Cl2 in 10 c.c. of water, &c., (*vide* formula).	Account of cowdung acted upon.	Growth of Plague bacillus after 18 hours' attempted disinfection.	
		Solution A.	Solution B.
·014	·2 gramme.	—	—
,,	·3 ,,	—	—
,,	·4 ,,	—	—
,,	·5 ,,	—	—
,,	1·0 ,,	—	—
,,	1·5 grammes.	— —	—
,,	1·75 ,,	— — —	— — —
,,	2·0 ,,	— — —	+ — +
,,	2·2 ,,	+ — +	— + —
,,	2·5 ,,	— + —	+ + +
,,	2·8 ,,	+ + +	+ + +

The results show a small success of Solution A over Solution B, the latter being capable of a germicidal action on 1·5 grammes of cowdung experimentally infected with the organism of plague, the former a similar action on 2 grammes.

In the succeeding experiments, Solution B was further tested. Instead of ordinary cowdung the test material employed was dried mud and cowdung dust, —test material of complex and varying chemical composition—scraped from the floors of native dwellings. The results obtained were as follows:—

Experiment on the germicidal value of a sample of the perchloride of mercury solution used in Bombay for disinfecting native houses:—

Gramme of $Hg Cl_2$ in 10 c.c. of water containing ammonia chloride and hydrochloric acid.	Amount of earth and cowdung acted upon.	Growth in Broth after 18 hours' attempted sterilization.
·014	·2 gramme.	— —
,,	·3 ,,	— —
,,	·4 ,,	— — +
,,	·5 ,,	+ + +
,,	·6 ,,	+ +
,,	·7 ,,	+ +
,,	·8 ,,	+ +
,,	·9 ,,	+ +
,,	1·0 ,,	+ +
,,	1·5 grammes.	+ +

NOTE.—Spores of moulds, and of B. megatherium, B. subtilis and B. mycoides were commonly obtained from the unsterilized material.

Thus it would appear that an amount of earth and cowdung from the floor of a native dwelling over ·3 gramme is not efficiently sterilized of spore-bearing organisms by an acid solution of perchloride of the strength of 1 in 725.

The above experiment was repeated with living cultures of the plague micro-organism as the test organism. A series of test tubes containing from 0·1 to 1 gramme of earth and cowdung dust obtained from the floor of a native house were prepared. To each tube a glass bristle infected with living culture from a nine days' old agar growth of plague, and 10 c.c. of the sample disinfectant solution were added. The results obtained were as follows:—

Gramme of $Hg Cl_2$ in 10 c.c. of water containing ammonia chloride and hydrochloric acid.	Amount of earth and cowdung acted upon.	Growth of the test organism after 18 hours' attempted disinfection.
·014	·1 gramme.	— —
,,	·2 ,,	— —
,,	·3 ,,	— —
,,	·4 ,,	— —
,,	·5 ,,	— —
,,	·6 ,,	— —
,,	·7 ,,	— —
,,	·8 ,,	— — +
,,	·9 ,,	+ — +
,,	1·0 ,,	+ + +

Thus it would appear that an amount of earth and cowdung from the floor of a native dwelling over ·7 gramme is not efficiently disinfected by a solution containing perchloride of mercury in the proportion of 1 in 725 when experimentally inoculated with plague.

As has been already mentioned, the alkaline reaction of cowdung and cowdunged earth is one of the factors largely responsible for the impotency of the perchloride solution as a disinfectant in relation to such material. How considerable a factor it is, is indicated in the following experiment in which the reaction to litmus of the disinfectant solution (containing nearly three-quarter per cent. hydrochloric acid) was examined before and after contact with various quantities of earth and cowdung. It was found that while a control sample of the disinfectant solution always gave a strongly acid reaction, samples which had been in contact with earth and cowdung gave reactions modified according as the earth and cowdung were increased in amount. Thus—

Amount of earth and cowdung acted upon by 10 c. c. of acid disinfectant solution.	Reaction of Solution.	
	Within 2 minutes of contact.	Within 24 hours of contact.
Control.	*Strongly acid.*	*Strongly acid.*
·1 gramme	acid	acid.
·2 ,,	acid	acid.
·3 ,,	Faintly ,,	Very faintly ,,
·4 ,,	Very ,, ,,	Neutral.
·5 ,,	Neutral	,,
·6 ,,	,,	Faintly alkaline.
·7 ,,	Faintly alkaline	Very ,, ,,
·8 ,,	,, ,,	Alkaline.
·9 ,,	Alkaline	,,
1·0 ,,	,,	,,

In a particular investigation in which 8 samples of dirt and cowdung from a room were examined bacteriologically *before disinfection*, and 12 samples from the same room *immediately after disinfection*, and in which care was taken that the quality and amount of perchloride of mercury used and its method of application allowed a 1 in 725 acid solution to produce its maximum effect, a comparison of results showed that while 4,057,800 was the average number of colonies of bacteria recovered on agar from 1 gramme of each sample before disinfection, the average number after disinfection was 228,000—a reduction of 94 per cent.*

* "The mere presence of even considerable numbers of micro-organisms in a sample of earth does not necessarily show that there is danger to health (in living on such a locality) and therefore the enumeration of the number of colonies which can be grown on a nutrient medium from a given weight of earth is a very fallacious test." ** Certain forms indeed are distinctly beneficial, since by their life processes oxidation (nitrification) of nitrogenous organic matter in the soil is brought about, while other forms, as agents of putrefaction and fermentation, play a very important part in the economy of nature. * * The only use of these results is in enabling us to judge of the approximate number of micro-organisms present in the sample examined, and not in forming an opinion as to whether the soil is of such a nature as would be likely to harbour organisms of a dangerous nature.' Stevenson and Murphy, *Hygiene and Public Health*, pp. 388—389.

Statement of conclusions on the efficiency of perchloride of mercury, in expert hands, as a disinfectant of dirt and cowdung from the floors of native houses experimentally infected with the micro-organism of plague.

1. That 10 cubic centimetres of an acid solution of the strength of 1 in 725 (ammonium chloride formula) efficiently disinfects 1·5 grammes of cowdung dust experimentally infected with the organism of plague. It also reduces but does not entirely suppress the more resistant saprophytic bacteria.

2. That 10 c.c. of the 1 in 725 acid solution efficiently disinfects 0·7 gramme of cowdung floor experimentally infected with organism of plague. It also reduces the number but does not entirely suppress the more resistant saprophytic bacteria.*

3. That as the effective limit of the 1 in 725 acid solution in regard to disinfection against plague is represented by the action of 10 cubic centimetres of the solution on 0·7 gramme of cowdung floor, and as 0·7 gramme of cowdung floor is the amount commonly recoverable from 4 square inches of the earthen floor of a native house when the immediate surface for a depth of $\frac{1}{10}$th inch is removed, then 100 square feet would yield an amount of surface material that would require approximately 8 gallons (3 bucketfuls †) of the 1 in 725 acid solution to vitally damage any contaminating plague micro-organisms.

4. That as the perchloride of mercury solution has only a surface action, if the contaminating material were below the immediate surface under disinfection, or on the surface protected by leaves, bits of rag, &c., then with such an application it could hardly be hurt at all.

Experiments on the efficiency of sea water (electrolysed) as a disinfectant of cowdung floors either alone, or as an application before spraying with perchloride of mercury solution.

In the foregoing experiments on the efficiency of a solution containing perchloride of mercury, hydrochloric acid, and ammonium chloride, the advantage of associating a common chloride such as ammonium chloride with the mercuric chloride in acid solution is detailed and exhibited. The remarks on the susceptibility of mercuric chloride to chemical changes, and its special liability to these changes when applied to cowdung floors will indicate the necessity of employing an acid such as hydrochloric acid, and a common chloride whenever perchloride of mercury is used for purposes of floor disinfection. While the presence of a small percentage of acid is an obligatory condition for the efficient action of a disinfectant solution of perchloride of mercury, it is not so essential that a common chloride should be in such intimate association with the mercury salt. In fact, the advantage of such a chloride *may be* obtained in the manner suggested to Government by Lieutenant-Colonel R. Boustead, I.M.S. (*vide* Appendix D), namely, by saturating the cowdung (or simple mud) floor with sea water, or with a strong solution of common salt before spraying it with the perchloride of mercury solution. But the use of ordinary sea water would not be in any special way more advantageous than the addition of a chloride to the solution of the mercury chloride as in the association which has already been prescribed and used (*vide* formula, page 4). Indeed, the use of ordinary sea water has certain disadvantages owing, firstly, to it being an appreciably alkaline solution and as such would add to the factors against which the perchloride of mercury solution would have to contend; and secondly, to it containing an abundance of organisms the destruction of which would in part engage the energy of the disinfectant solution. The use of a solution of ordinary salt or artificial sea water‡ would remove these objections, but as has already been stated, it is more certainly advantageous to employ the chlorides of mercury and ammonia, or soda (common salt), in the same solution.

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* The necessity, however, of killing the ordinary saprophytic bacteria is not very obvious since it is (presumably) an advantage to preserve alive and keep in action agents that accomplish by microphytic processes the purification of the soil.

† Buckets of a capacity of two and three-quarter gallons. Many of the wooden buckets used in Bombay have a capacity of two and a quarter gallons only.

‡ Artificial sea water can be made by dissolving 27 grammes of common salt and 4 grammes of magnesia chloride in a litre of water.

Sea water may be used with special advantage, however, if it be electrolysed beforehand. This procedure has the double recommendation of (1) sterilizing the sea water of the 10 to 60,000 micro-organisms which certain samples of Bombay sea water were found to contain per cubic centimetre, and (2) making available and active the oxidising and disinfecting agents that are potentially present in the sea water.

By passing a galvanic current through sea water between electrodes of platinum certain of the chlorine compounds in solution in sea water decompose with the evolution of the chlorine and the formation of chlorides and hypochlorites. The magnesium hypochlorite thus formed further dissociates into (1) magnesia, which deposits on the walls and floor of the electrolyser, and (2) free hypochlorous acid which remains in solution according to the following equation :—

$M_g(OCl)_2$	+	$2\,H_2O$	=	$M_g(OH)$	+	$2\,H\,O\,Cl$
Magnesium hypochlorite		Water		Magnesia		Hypochlorous acid.

The process thus practically resolves itself into the production of a chemically active solution of hypochlorous chlorine.

In contact with organic matter the hypochlorous acid splits up into hydrochloric acid and oxygen, and it is on the liberation of oxygen that its value as a disinfectant largely depends.

The amount of available chlorine liberated from sea water by ordinary electrolytic methods varies from 0·5 to 1 gramme per 1,000 grammes, or one litre of sea water ('available chlorine' meaning chlorine which is either free or belongs to a hypochlorite). This chlorine *in the presence of water* acts directly as an oxidising agent.

The above remarks will make it clear that the utility of such a solution depends partly upon the energy of the nascent oxygen set free from its combination with hydrogen in water, *e.g.*

H_2O	+	Cl_2	=	$2\,H\,Cl$	+	O
Water		Chlorine		Hydrochloric acid		Oxygen.

and partly upon the activity of chlorine in breaking up ammonia, sulphuretted hydrogen, and organic matter generally. Thus the sulphuretted hydrogen gas of putrefaction is resolved into sulphur and hydrochloric acid—

H_2S	+	Cl_2	=	$2\,H\,Cl$	+	S
Sulphuretted hydrogen		Chlorine		Hydrochloric acid		Sulphur.

—the ammonia is decomposed into ammonium chloride and nitrogen—

$8\,N\,H_3$	+	$3\,Cl_2$	=	$6\,N\,H_4Cl$	+	$2\,N$
Ammonia.		Chlorine.		Ammonium chloride.		Nitrogen.

—and lastly the nitrogenous organic matter of germs, and the albuminoid ammonia and the nitrites forming their surroundings are oxidised into the highly mineralized form of nitrates.

Therefore, and for other reasons which will be mentioned later, the use of electrolysed sea water is likely to be of chemical and material assistance in the disinfection of cowdung floors by perchloride of mercury or carbolic acid.

In the following experiments it is proposed to investigate the utililty of electrolysed sea water both as a disinfectant alone and as an application to cowdung floors before treating them with perchloride of mercury.

The electrolysis of the sea water employed in these experiments was effected in the Municipal Workshops in Bombay by a current of 1200 amperes at a pressure of 13 volts. The strength of the resultant electrolysed fluid in chlorine, or its equivalent, as determined by the arsenious acid process by Captain Bourke, I. M. S., Chemical Analyser to Government, is 0·771 gramme of chlorine per litre, or 0·077 per cent., *i.e.*, 0·15 per cent. above Pettenkofer's limit of human endurance *of the vapour in the air*. Exposed to light and air, and to a high atmospheric temperature, electrolysed sea water gradually changes in chlorine

strength as is illustrated in the following analyses made by Captain Bourke, I. M. S., at varying times with the same sample :—

Strength within 24 hours of electrolysis = 0·771 gramme chlorine per litre.
" " 48 " " = 0·749 " " "
" " 5 days " = 0·561 " " "

The sample under examination was kept in a corked quart bottle. A similar sample openly exposed as a column in a 300 c.c. measure-glass showed at the end of 5 days a strength of 0·447 gramme chlorine per litre.

A reference to the results obtained for a number of micro-organisms by different observers will indicate the possibilities of a disinfectant holding chlorine in the above proportions. The details, which are taken from various published statements, but chiefly from Dr. Rideal's book on *Disinfection and Disinfectants*, are :—

0·004 parts of available chlorine per 1,000 reduced the organisms in sewage *effluent* from innumerable to 10-50 per cubic centimetre. *Kanthack.*

0·005 " " " " had a marked germicidal action on the organisms in sewage, but no appreciable effect on the organic matter.* *Rideal.*

0·018 " " " " affected the organisms of sewage *effluent*, so that samples taken after 14 minutes showed no growth at room temperature in three-and-a-half days. *Rideal.*

0·17 " " " " destroyed typhoid and cholera germs in 2 hours. *Bolton.*

0·2 " " " " destroyed diphtheria and cholera germs in 1 minute, and typhoid bacilli in 5—10 minutes. *Lancet Report on the Hermite electrolytic process.*

0·25 " " " " sterilized the fæcal matter in sewers. *Piton.*

0·33 " " " " destroyed anthrax bacilli in 1 minute. *Nissen and Bolton.*

0·4 " " " " destroyed typhoid bacilli and cholera germs in 5 minutes. *Nissen and Bolton.*

0·5 " " " " destroyed non-sporing germs in 5 minutes. *Klein.*

0·66 " " " " destroyed staphylococcus pyogenes aureus in 30 minutes. *Nissen and Bolton.*

1·0 " " " " destroyed bacilli and B. Enteriditis in 1½ hours. *Klein.*

1·0 " " " " destroyed all cultivations in broth except B. subtilis and B. anthracis. *Boyce.*

2·0 " " " " destroyed pure vaccine virus. *Baxter.*

3·0 " " " " destroyed anthrax spores in 70 minutes. *Nissen and Bolton.*

3·55 " " " " disinfected and deodorised sewers, garbage, &c. *Reports of American bacteriologists.*

Rideal (*loc. cit.* p. 74) considers that the minimum amount required for sterilization is about 0·5 per 1,000, but must vary greatly with the conditions. This authority argues that as its use introduces the principle of disinfection by chemical oxidation, whenever there is a large amount of oxidisable matter present, so large a proportion of the agent becomes thereby partially or entirely wasted on the inert matter present that it has no opportunity of attacking the bacteria. In such a condition the best it does is to remove the food of the

* The reason for the small action on the organic matter is elucidated in Dr Rideal's book (p. 70) :—"The sewage was found to require for complete oxidation about 11 parts of oxygen per 100,000, whilst the amount of 'available chlorine' added was only about 0·55 parts per 100,000. As 35·5 parts of hypochlorous chlorine are equal in oxidising power to 16 parts of oxygen, it will be seen that the amount of fluid added must be increased nearly fifty-fold to effect complete oxidation." Rideal further states that this result only emphasises the fact that although many disinfectants, *if used in sufficient quantity*, can kill bacteria and destroy offensive matter, the amount required is far in excess of what is prescribed, and becomes almost prohibitive in cost. Chlorine and hypochlorites, and indeed other chemical disinfectants, practically fail when applied to the solid matters of sewage. Although the chlorine, when brought in contact with the germ, is capable of killing it, it does so mainly by oxidising the organic matter in the germ; but on the other hand in presence of a large amount of organic matter, the chlorine often expends itself on the latter before it has time to sterilize the organisms.

organisms, but the amount they require is so small and so widely diffused that such a consummation is hardly practicable. Therefore as a method *per se*, it is costly and unsound.

As will be seen from the present record of experience with electrolysed sea water on the cowdung and dirt of the floors of native houses the results agree with the quoted statement that the chlorine available as a disinfectant is not in sufficient amount for this purpose. Its use, however, enables a subsequent application of perchloride of mercury solution to act with increased efficiency.

The first experiments have to do with the germicidal efficiency of a sample of electrolysed sea water (containing 0·771 per 1,000 of 'available chlorine') on the plague microbe.

A series of test tubes containing 10 c.c. of the sea water were prepared. Into each tube a glass bristle wetted in its lower third by a thin film of living culture obtained from the surface of a 9 days' old agar growth of plague was inserted. After the short intervals enumerated below the infected glass bristles were withdrawn and inoculated into nutrient broth. The broth tubes were incubated at the temperature of the laboratory for 10 days. By this time any living test organisms would have had ample time and opportunity of producing easily visible growth. The results obtained were as follows :—

Interval.	Growth in Broth.
½ minute.	+ +
1 "	+ —
2 minutes.	— —
3 "	—
4 "	—
5 "	

Thus an exposure of from one to two minutes was sufficient to kill the test plague micro-organism. The same 'time efficiency' of the solution for sterilization was shown when cultures of the plague micro-organism growing on agar were treated by the addition of 10 c.c. of electrolysed sea water to each of the culture tubes.

When various dilutions of the electrolysed sea water with sterilized tap water were tested, the following results were obtained :—

Strength of test solution in 'available' chlorine gramme per 1000.	Growth of plague organism after 18 hours' attempted sterilization.
0·01	+
0·02	—
0·03	—
0·038	—
0·05	—
0·06	—

When a culture of the plague micro-organism growing on agar was treated by the addition of 10 c.c. of a solution of electrolysed sea water of the strength of 0·02 gramme 'available' chlorine per 1000 the culture was not sterilized.

The next two experiments have to do with the efficiency of a sample of the electrolysed sea water (0·771 per 1,000 strength) as a disinfectant in relation to the organisms in mud and cowdung from the floor of a native house.

A series of test tubes containing from 0·1 to 3 grammes of mud and cowdung (weighed dry) from the floor of a native house were prepared. A measured quantity of electrolysed sea water was poured into the tubes so that each tube held the test amount of mud and cowdung wetted by 10 c.c. of the electrolysed sea water. Care was taken to thoroughly wet the mud and cowdung with the fluid. At the end of 24 hours a minute quantity of the dirt and cowdung was abstracted from the various test tubes and inoculated into a series of tubes containing nutrient broth. The broth tubes were incubated at the ordinary temperature of the laboratory for 8 days. The results obtained were as follows :—

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Gramme of 'available chlorine' in electrolysed sea water per 1000.	Amount of mud and cowdung acted upon.	Growth in Broth after 24 hours' attempted sterilization.	
		Non-spore-bearing organsims.	Spore-bearing organisms.
0·771	·1 gramme	—	+[2]
,,	·2 ,,	—	+[2]
,,	·3 ,,	—	+[2]
,,	·4 ,,	—	+[3]
,,	·5 ,,	—	+[3]
,,	·6 ,,	+[1]	+
,,	·7 ,,	+	+[4]
,,	·8 ,,	+	+[4]
,,	·9 ,,	+	+[4]
,,	1·0 ,,	+	+[4]
,,	2·0 grammes	+	+[4]
,,	3·0 ,,	+	+

NOTE.—[1] Growths of Bacterium vulgare and B. acidi lactici obtained.
[2][2] No appearance of growth for 48 hours when B. megatherium began to vegetate.
[3][3] No appearance of growth for 24 hours when B. megatherium and B. subtilis began to vegetate.
[4] &c. Growths appeared within 24 hours.

Thus 10 c.c. of the electrolysed sea water are incapable of sterilizing 0·1 gramme of the test material of the more resistant organisms present but are able to destroy vegetative forms (comparable to the plague micro-organism) up to 0·5 gramme. As the presence of chlorine when tested for by potassium iodide and starch at the end of the above tests was not evidenced, it seems probable that all the available chlorine had been used up in acting on the organic matter present in the test material. The retardation of the growths indicates that the organisms were only partially devitalised by exposure to the fluid. The results obtained under the subheading 'non-spore bearing forms' will give a fair idea as to what would have been the fate of any plague micro-organisms had they been present in the test samples.

When the above experiment was repeated with 20 c.c. instead of 10 c.c. of the electrolysed sea water, 0·1 gramme of the test material (mud and cowdung from the floor of a native house) was completely sterilized in two out of three observations—the Bacillus mesentericus vulgatus being permitted to survive the addition of the electrolysed sea water in the non-sterilized sample.

Calculating from the data that 10 c.c. of electrolysed sea water of 0·771 gramme 'available chlorine' strength per 1,000 (*i.e.*, per litre) are required to disinfect ·5 gramme of mud and cowdung from the floor of a native house against vegetative micro-organisms like the plague micro-organisms, then to similarly disinfect the 2,520 grammes of surface material recoverable from 100 square feet of floor space in native houses would require 11 gallons of electrolysed sea water of the above strength. While if 20 c.c. are required to *completely sterilize* 0·1 gramme, then to similarly sterilize the surface material on this amount of floor space would require 110 gallons of the electrolysed sea water.

So large a quantity of sea water as 11 gallons is an impracticable amount to use at one time on 100 square feet of floor space, and contra-indicates the use of electrolysed sea water for its disinfecting action. When the effect of using a more convenient quantity, namely 5 gallons, of the electrolysed sea water was tested bacteriologically with samples collected half-an-hour *after* its careful application to the surface of a cowdung and earth floor measuring 13′ × $7\frac{1}{2}$′, it was found that 6 cultures made from 6 samples within four hours of collection yielded after 24 hours' incubation on agar an average of 10,704,600 colonies per gramme of sample, or nearly twice the average number, namely, 5,417,950, obtained from 6 cultures from 6 samples collected from the same floor half-an-hour *before* treatment with the electrolysed sea water. The richness of the former samples in micro-organisms indicates that the addition of the sea water to the dirt and cowdung had the effect of breaking down the particles, so that groups of micro-organisms which had previously given rise to only one colony became broken up into smaller groups or even into individual organisms, and as such were abundantly multiplying throughout

An experiment on the combined efficiency of electrolysed sea water *and perchloride of mercury solution* as disinfectants of samples of mud and cowdung from the floors of native houses was next carried out.

A series of test tubes containing electrolysed sea water acting on mud and cowdung in the proportion of 10 c.c. of sea water to from 0·1 to 5 grammes of mud and cowdung (weighed dry) from the floor of a native house were prepared. Under agitation the sea water was allowed to act in intimate association with the particles of test material for 30-40 minutes. The excess of sea water was then pipetted off and 10 c.c. of a 1 in 725 acid solution of perchloride of mercury were added to each tube. At the end of 18 hours a minute quantity of the dirt and cowdung in each tube was transferred to tubes containing nutrient broth. The culture tubes were incubated at the ordinary temperature of the laboratory for 10 days. The results obtaind were as follows :—

NOTE.—In order to contrast in parallel columns the results obtained in the present experiment with those obtained when a solution of perchloride of mercury had been used alone, use is made of the experiment recorded on page 6.

Amount of mud and cowdung acted upon.	Growth in Broth after 18 hours' attempted sterilization.	
	a. Perchloride of mercury solution 1: 725 alone.	*b.* Electrolysed sea-water (0·771 Cl strength) and perchloride 1: 725.
·1 gramme.	—	—
·2 ,,	—	—
·3 ,,	—	—
·4 ,,	+—	—
·5 ,,	+	—
·6 ,,	+	—
·7 ,,	+	—
·8 ,,	+	—
·9 ,,	+	—
1·0 ,,	+	—
2·0 grammes	+	+ —
3·0 ,,	+	+ +
4·0 ,,	+	+
5·0 ,,	+	+

Thus it would appear that an amount of earth and cowdung from the floor of a native dwelling under 1 gramme is efficiently sterilized after treatment by 10 c.c. of electrolysed sea water followed by 10 c.c. of an acid solution containing perchloride of mercury in the proportion of 1 in 725: whereas the use of an acid solution of perchloride of 1 : 725 strength alone is only capable of sterilizing an amount of earth and cowdung under 0·4 gramme.

In continuation of the investigation which has already been recorded (*vide supra*) in which 6 samples of dirt and cowdung from the surface of a floor (13′×$7\frac{1}{2}$′) in a native house were taken *before treatment* for disinfection, and 6 samples from the same floor half-an-hour *after treatment* with 5 gallons of electrolysed sea water, a further collection of 6 samples obtained from the floor half-an-hour *after the further treatment* with 8 gallons of an acid solution of perchloride of mercury of the strength of 1 in 725 was made. A comparison of results showed that, while 5,417,950 and 10,704,600 were the average numbers of colonies of micro-organisms recovered from 1 gramme of each sample, respectively, before and after treatment with the electrolysed sea water, the average number after the further use of perchloride of mercury solution was 78,480—a reduction of over 98 per cent. from the original estimation.

The value of electrolysed sea water as an adjunct of perchloride of mercury in the disinfection of native floors is, therefore, not so much because of any germicidal action it may exert as for its utility in oxidising and neutralising the chemical ingredients of the mud and cowdung which interfere with the efficiency of perchloride of mercury.

In summary and conclusion it may be stated that the advantages of employing electrolysed sea water are —

1. That its application makes the surface of the floor under treatment friable, and, therefore, more easily acted upon by the perchloride solution.

2. That it oxidises and neutralises the organic and mineral matters that interfere with the efficient action of a perchloride of mercury solution.

3. That it enables spore-bearing micro-organisms to vegetate (important in disinfection against anthrax and glanders) and thereby become more easily killed by the action of a perchloride of mercury solution.

Further account of bacteriological investigations on the results following the application of various disinfection procedures to native houses.

The investigations include an account of the approximate number and varieties of micro-organisms commonly found in samples of the surface soil and cowdung dust from the rooms of native dwellings before and after the disinfection conducted by District Plague Officers in Bombay. The examination of samples was undertaken before disinfection to afford a basis of comparison with the results obtained after treatment by disinfectants. The samples were collected, and the examination was made in the third and fourth quarters of the year 1898, and in the first quarter of the current year.

Method of procedure.—Samples to the number of from 6 to 14 from the surface of the earth floor, and from the dust and dried mud on the walls were taken from the room under examination. These samples were conveyed to the laboratory in sterile glass tubes—the time occupied in the collection and transport of the samples to the laboratory varying from two to four hours. A quantity of the powdered sample (1—100th gramme) was immediately added to a test tube containing 10 c.c. of melted agar. By shaking it was distributed as evenly as possible through the agar, which was then spread over the surface of a Petri plate to solidify. The plates were incubated at the temperature of the room for 24—48 hours when the number of colonies which had developed in the agar on the plate, and in the agar still remaining in the emptied test tube were counted. Agar was employed because of its suitability as a solid nutrient medium at the comparatively high temperature of the laboratory (76°—86° F.). For this reason the number of colonies counted is probably much lower than might have been obtained with gelatine had it been practicable to use that material. Again, only aerobic bacteria and rapidly-growing organisms are enumerated by this method, and agar is not always a favourable culture medium even for some of the aerobic species present in the soil. Moreover, as the determination of the bacterial population of samples from the rooms was made from scrapings of the exposed surfaces of the walls and mud floors, the number of colonies obtained is likely to be less than the yield of samples recovered from the moister and more retentive soil a little below the surface. Therefore the results obtained do not give a quantitative estimation with certainty: they are probably incomplete and have only a relative value, or an importance such as that described by Flügge (*Die Mikroorganismen*, p. 509), namely, that a greater or less contamination of the soil by bacteria is of importance in the connection that the presence of a large percentage of saprophytes makes the soil less able to conserve pathogenic bacteria, "for pathogenic bacteria are in strict contrast to some saprophytic species which may grow with nutritious material of a very low quality and which, therefore, have the possibility of developing strongly in the soil."

Summary of Results.—The number of colonies of micro-organisms present calculated for 1 gramme of the different samples varied as follows:—

Unoccupied Rooms. Two examined.

5,608,700 highest number of colonies. House No. 2. Sample 1.*
8,600 lowest " " House No. 1. Sample 11.
1,474,200 average of 22 cultures from 22 samples.

Occupied Plague-infected Rooms. Two examined.

8,753,300 highest number of colonies. House No. 1. Sample 4.
126,000 lowest " " House No. 1. Sample 2.
3,199,580 average of 20 cultures from 20 samples.

Disinfected Rooms: treated by perchloride of mercury solution. Two examined. The samples were collected from within a few hours to four days after treatment.

4,546,000 highest number of colonies. House No. 1. Sample 11.
Nil lowest number (sterile). House No. 2. Samples 9 and 11.
1,108,800 average of 25 cultures from 25 samples.

* *Vide* Appendix B.

Disinfected Room: treated by perchloride of mercury solution, limewashing and fresh air (by unroofing). One examined. The samples were collected six days after the commencement of treatment.

1,844,500 highest number of colonies. Sample 2.
2,400 lowest " " Sample 1.
519,000 average of 5 cultures from 5 samples.

Disinfected Room: treated by electrolysed sea water and perchloride of mercury solution One examined. The final samples were collected from within a few hours to six days after treatment.

5,417,950 average number of colonies before treatment.*
10,704,630 " " after using electrolysed sea water.
228,500 " " after following up the use of electrolysed sea water with perchloride of mercury.

Comparative observations. On agar culture media.

349,900 average number of colonies obtained from 1 gramme of a sample of soil from the top of Lohogad Fort.
481,100 " " obtained from 1 gramme of the mud at the bottom of a dried well, Lohogad Fort.
49,200 " " obtained from 1 gramme of the soil on the floor of a badly ventilated, uninhabited rock cave, Bhaja.

On gelatine culture media.

750,000 average number of colonies obtained from 1 gramme of the soil of Montsouris, Paris. *Miquel.*
8,326 " " " " " of virgin sand. *Houston.*
475,280 " " " " " of virgin peat. *Houston.*
115,044,400 " " " " " of the soil from the trench of a sewage farm. *Houston.*

Thus, from the above summary of results got by counting the number of colonies of microbes growing on agar plates, it appears that by disinfecting with perchloride of mercury solution (ammonium chloride formula) alone the microbes are reduced in practice 65 per cent. below the average of occupied plague-infected rooms; by perchloride solution + limewash + unroofing the microbes are reduced 83 per cent. below the average of occupied plague-infected rooms; by perchloride solution + electrolysed sea water the microbes are reduced 93 per cent. below the average of occupied plague-infected rooms.†

The proportion between moulds and bacteria present is of some interest from the observation that the ratio of bacteria to moulds increases with vitiation of the air, because moulds come principally from the outside, bacteria from the floors, walls, &c., of rooms. It is considered that in a properly ventilated tenanted space the bacteria should not exceed moulds more than 30 to 1 (Davies, *Handbook of Hygiene*, page 20). In occupied room No. 2, the bacteria stood to moulds in the ratio 240 to 1. In the five samples from the disinfected, limewashed and unroofed house the ratio was 75 to 1.

Varieties of Bacteria.—The majority of the bacteria recovered from the various samples were obligate saprophytes—dealing with oxidation, nitrification and formation of carbonic acid of the dead and decaying matter in the filth of the houses. A large number of the samples contained species of the more sensitive facultative parasites. The abundance of chromogenic bacteria in the unoccupied and unroofed dwellings was in marked contrast to the sparseness of the number obtained from the worst of the disinfected houses.

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* Not detailed in Appendix.

† *Vide ante*, pp. 7, 13 for results obtained by using the perchloride solution and perchloride solution + electrolysed sea water, so that their maximum *immediate* effects are produced.

Divided into spore-bearing and non-spore-bearing forms the following, account may be given :—

Spore-bearing : B. subtilis, B. megatherium, B. mycoides, B. vulgatus, B. mesentericus B. butyricus,—in order of frequency.

Non-spore-bearing. Bac. vulgare and other species of proteus, Staphylococci.

B. coli communis and other species of the colon group.

Bac. acidi lactici, Sarcinæ, Bac. fluorescens. Micrococcus candicans, Micrococcus luteus, Bac. putidum, Bac. prodigiosum, M. agilis, M. pyogenes (various species), Bac. latericium, Bac. syncyaneum, Bac. pyocyaneum, Streptococcus pyogenes, Bac. janthinum. Diplococci. A number of undifferentiated species. Moulds. Yeasts.

The spore-bearing species were ubiquitous, and in a great preponderance, especially in the samples obtained from disinfected houses.

APPENDIX A.

NOTE ON THE APPROXIMATE NUMBER AND VARIETIES OF MICRO-ORGANISMS COMMONLY FOUND IN INDIAN COWDUNG.

Method of obtaining the micro-organisms.—The excrements of cows and buffalo dropped on the floors of byres and on the public streets were selected for examination. Material was taken from the sample either immediately after it had left the bowel or at intervals of from two to twenty-four hours subsequent to that event. A small portion from the interior of the sample was in each instance removed on the looped end of a sterile platinum needle. This quantity was inoculated into sterile salt tubes. From the salt tubes agar plate cultures were made at once. These were incubated at the ordinary temperature and at 37°C. For purposes of enumeration a loopful of the dung weighing 1-100th gramme was inoculated into melted agar and plated.

Results.—In every case micro-organisms or their spores were present in the dung in large numbers. The number of colonies obtained per gramme of the different samples varied as follows :—

90,800,000 highest number, Sample 4. Examined 18 hours after passage from the food-canal.

5,402,000 lowest number, Sample 1. Examined immediately after passage from the food-canal.

35,266,000 average of 8 cultures from 6 samples of cowdung.

Comparative observation by Warington :—

165,000,000 estimated number of micro-organisms from a gramme of dung of an English cow fed on hay, the estimation being made from cultivations on gelatine.

In fresh normal dung the micro-organisms were commonly represented by the spores of Bacillus subtilis, Bacillus vulgatus, and other micro-organisms of the same group. Samples collected from the floors of byres or from the street two hours or more after defœcation contained large numbers of vegetative bacilli and micro-cocci. The bacilli commonly found were the ordinary saprophytes represented in the fresh dung by their spores. From one sample of cowdung the Bacillus enteritidis sporogenes—believed by Klein to be the cause of infantile diarrhœa—was obtained. Two samples of dung from bullocks suffering from diarrhœa yielded almost pure cultures of the Bacillus coli communis.

The following details may be given in illustration :—

1. Dung of a cow byred in a private compound and fed upon hay and green food.—The cultures yielded the Bacillus subtilis, B. mesentericus vulgatus, and B. megatherium in large numbers. Also colonies of B. coli communis, a white coccus, and a yellow staphylococcus.
2. Dung of a buffalo byred in a dairy compound and fed upon hay and oil-cake.—The cultures yielded a moderate number of colonies of B. coli communis and a few colonies of Proteus Zenkeri. A considerable number of colonies of B. subtilis, B. mycoides, B. megatherium, and B. acidi lactici were also obtained.
3. Dung of a bullock byred in a ghari stable.—B. Subtilis, B. coli communis, a yellow staphylococcus, sarcinæ and moulds were obtained.
4. Dung of a cow from the street.—About 80 per cent. of the colonies consisted of B. subtilis, B. mesentericus vulgatus and similar common saprophytes. In addition a number of lactic acid bacilli, staphylococci, sarcinae, and moulds were obtained.

APPENDIX B.

UNOCCUPIED HOUSE No. 1.—*Servants quarters' Bungalow, Malabár Hill. House old, ventilation fair: room with earth floor (cowdunged—last application one month ago) on ground floor. Unoccupied for six months. Samples taken 18th October 1898.*

Number of sample.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.*	Remarks.
1	Sample of dust clinging to laths in wall.	145,600	92,000	B. subtilis, micrococci, and sarcinæ common. Moulds. Altogether eight different species of micro-organisms present.
2	Sample of dust from ledge of sconce in wall.	18,900	...	Same as above. Moulds again present.
3	Sample from heap of dust sweepings.	34,700	26,300	Large number of cocci and sarcinæ. Also B. mycoides. B. subtilis very common. Six different species counted
4	Scraping from surface of earthen floor near corner of room. Wall damp from monsoon wet.	2,966,000	268,000	B. subtilis and B. megatherium mostly. Also Bacterium vulgare (mirabilis). Nine different species of bacteria. Two varieties of mould.
5	Sample of dust sweepings from floor.	26,000	...	B. megatherium. Cocci very common; eight different species present.
6	Scraping of dust and cowdung from wall.	290,000	87,000	B. subtilis and megatherium. Cocci and sarcinæ. Fluorescent colonies visible. Nine different species of bacteria present.
7	Scraping of dust and cowdung from window ledge.	89,000	...	B. subtilis, B. mesentericus. B. mycoides in large numbers. Two species of mould.
8	Scraping from surface of floor (cowdunged) near wall (damp).	3,600,000	1,826,000	B. subtilis, B. megatherium, cocci, Bacterium fluorescens (non-liquefying). Bacterium vulgatus. Nine distinct species of micro-organisms present. The spores were chiefly those of B. subtilis and B. megatherium.
9	Scraping of surface of earth under site of hand-mill.	4,200,800	1,099,800	Colonies were counted at the end of 48 hours. B. mycoides, B. subtilis, B. megatherium abundant. Eight distinct species counted. Spores of B. mycoides, B. mesentericus, B. megatherium largely present.
10	Scraping from surface of earthen-floor (cowdunged) near corner of room.	2,966,300	...	B. mycoides, B. subtilis common. B. fluorescens visible. B. coli communis and several protean forms obtained.

* The occasional estimation was undertaken by adding $\frac{1}{10}$th gramme of the sample to 10 c.c. of melted agar, which was heated to 80° C. for 10 minutes, and then poured into a Petri plate.

UNOCCUPIED HOUSE No. 1—*continued.*

Number of sample.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
11	Scraping of dried cowdung on front door-step.	8,600	...	B. subtilis, B. megatherium, cocci, most common. Sarcinæ in two distinct species.
12	Sample scraped from surface of floor (cowdunged) where it is partly cemented.	1,193,400	...	B. subtilis. Sarcinæ. Two varieties of mould. Few colonies of Bact. vulgare.
13	Sample scraped from cowdunged wall.	84,000	60,700	B. subtilis, B. megatherium, sarcinæ. Four varieties of cocci.
14	Sample of moist cowdung, on back door-step.	1,183,700	28,600	B. mycoides, B. subtilis, B. megatherium. Some protean forms. Micro. candicans. Mould.

UNOCCUPIED HOUSE No. 2.—*Old House, ventilation bad. Room on ground floor unoccupied for four months. Earth floor, cowdunged, very neglected. Samples taken 7th November 1898. Address—No. 15, Gilder Street.*

Number of sample.	Description of the samples.	Number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
1	Scraping from surface of floor near damp well.	5,608,700	18,900	Bact. coli communis, Bact. vulgare. Bact. fluorescens common. Eight different species of micro-organisms.
2	Scraping from centre of floor (cowdunged).	2,552,500	826,000	Bact. acidi lactici, Bact. vulgare. Many cocci. Altogether nine different species of micro-organisms.
3	Scraping of bottom of damp wall near floor.	1,290,000	...	Micrococcus candicans. Yellow staphylococcus abundant. Ten different species of bacteria counted.
4	Sample of dust from a lamp niche in wall.	190,700	160,800	B. subtilis, B. mycoides, B. mesentericus. Five different species of bacteria counted. Moulds.
5	Sample of dust from surface of laths exposed in wall.	189,400	...	B. subtilis, B. prodigisous, B. mycoides. Eight different species of bacteria counted.
6	Samples of dust from surface of old fire place.	190,900	...	B. mycoides and B. megatherium mostly present. A number of cocci and sarcinæ.

UNOCCUPIED HOUSE No. 2—*continued.*

Number of sample.	Description of samples.	Total number of Bacteria in 1 gramme of sample.	Number of Spores of Bacteria in 1 gramme of sample.	Remarks.
7	Scraping from the surface of cowdunged floor near door.	2,644,000	...	Varieties of the colon bacillus obtained. Also Bac. vulgare and a fluorescent organism. Twelve distinct varieties of bacteria counted. Several moulds.
8	Scraping from the surface of cowdunged floor near window.	2,959,000	196,800	Colonies typical of B. coli communis present. Bac. vulgatus, B. subtilis very common. Eleven different species of micro-organism present.

OCCUPIED HOUSE No. 1. *Address—No. 6 Street, Kámáthipura. Description similar to Occupied House No. 2.*

Number of sample.	Description of samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
1	Scraping from floor near wall.	3,176,000	...	Colonies of B. coli communis, B. vulgatus, Bacterium vulgare, Bact. mesentericus. Also B. mycoides, subtilis and megatherium. Fourteen distinct species counted.
2	Scraping from wall coated with cowdung.	126,000	36,800	B. megatherium, B. subtilis, coloured cocci numerous. Eleven distinct species counted.
3	Scraping from floor near back wall.	934,000	...	Colonies of B. coli communis and protean forms. Sarcinæ.
4	Scraping from floor near window.	8,753,000	...	Numerous colonies of B. coli communis. Bact. Zopfii isolated. Other protean forms numerous.
5	Scraping from floor near one of the four corners of the room.	5,920,000	...	B. megatherium common Fifteen distinct species of bacteria counted.
6	From surface of heap of dirt behind door.	1,243,600	183,900	B. megatherium, B. mycoides, and other spore-bearing forms common. Eleven distinct species counted.
7	From surface of platform in dark recess.	7,739,300	226,900	B. subtilis, B. megatherium, B. vulgatus in a preponderance. Number of cocci, and sarcinæ. Moulds. Ten different species of micro-organisms counted.

OCCUPIED HOUSE No. 1—*continued.*

Number of sample.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
8	Scraping from surface of floor near passage wall.	962,400	...	Eight different species counted. B. megatherium numerous.
9	Scraping from centre of floor.	1,026,400	908,000	B. mycoides, B. megatherium, B. subtilis common. Thirteen different species counted.
10	Scraping from floor near small recess.	894,300	...	B. coli communis, Bac. vulgare numerous; also B. megatherium. Ten distinct species counted.
11	Scraping from surface of floor where plague corpse had lain.	413,200	256,000	B. subtilis numerous. Also cocci. Six d istinct spe cies counted.
12	Dust from surface of ledge near front wall.	360,400	299,000	B. megatherium, B. mycoides, B. vulgatus common. Eleven distinct species counted.

OCCUPIED HOUSE No. 2. *Address—No. 8, Kámáthipura (11th Street).—Samples taken 20th February 1899. Man died of plague on 19th February 1899. Bubo in right axilla.*

Description of Room—Ground floor, badly lighted and ventilated: entrance off a narrow dark passage. Size 8′ × 8′ × 8′; floor of beaten earth, floor and sides of walls covered with cowdung.

Number of sample.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
1	Sample of dirt from irregular surface of beam supporting wall.	658,600	...	Bacillus subtilis, yellow staphylococcus, white staphycococcus. Streptococcus. Eleven distinct species of bacteria counted.
2	Sample of dirt from plinth at front door.	1,433,000	...	B. subtilis, B.¶ acidi lactici. Moulds very common. Twelve species of micro-organisms recovered.
3	Surface of earthen floor near corner.	5,482,700	984,600	B. coli communis, B. mycoides, Bacterium vulgare. Several varieties of mould-fungi. Ten different species counted.
4	Scraping from surface of earthen floor near centre of room.	4,499,800	130,000	Bacterium vulgare (mirabilis). B. mycoides. Eleven distinct species counted.

OCCUPIED HOUSE No. 2—*continued.*

Number of sample.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
5	Scraping from surface of sodden floor near cooking place.	6,082,000	...	Numerous colonies of B. fluorescens non-liquefying, B. coli communis, and protean forms. Eight different species counted.
6	Scraping from surface of floor near one of the corners of the room.	3,006,000	...	Many protean forms. Also B. mycoides, B. acidi lactici.
7	Scraping from surface of floor near inner wall.	7,175,000	...	Numerous colonies of B. coli communis. Also Bac. mesentericus, Bacterium vulgare.
8	Scraping from dirt behind door.	4,126,600	...	B. mycoides predominating. Number of colonies of Bacterium vulgare.

DISINFECTED HOUSE No. 1. *Address—No. 15, Fanaswady, District C, Ward B. Samples taken 7th December 1898. "Man died of Plague on 2nd December 1898. The house has been flushed as usual, and has a mud floor." Note from District Officer, dated 4th December 1898.*

Description of House.—One of a row of one-storey houses: front and back window and door: tiled roof—rafters directly under tiles. Tiles partly removed and light comes in from the roof. Size of house 15′ × 12′ × 9′ for length, height and breadth, respectively. There is a narrow plinth in front of house about 1 foot above level of ground. Plinth at back about 2 feet above level of common ground. Floor of beaten earth covered with cowdung. Cowdung smeared on walls, which are cracked and dirty. Floor cracked and neglected. A large number of insects on the walls and floor.

Number of sample.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
1	Scraping from wall where it was covered with dry expectoration. December 7th, 1898.	764,366	...	Colonies of B. megatherium, B. subtilis, and sarcinæ very numerous. Sample showed no evidence of $HgCl_2$ on testing with HCl and H_2S, nor any evidence of mercury when HNO_3 added and the metal tested for as mercurous nitrate.
2	Dried cowdung adhering to wall near corner.	1,946,400	88,900	Colonies of B. mycoides, B. subtilis, B. megatherium most abundant. Chemical examination showed no evidence either of the salt ($HgCl_2$) or of the metal (Hg).

DISINFECTED HOUSE No. 1—*continued.*

Number of samples.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
3	Scraping from ledge of small recess in wall.	986,546	...	.....
4	Scraping from surface of moist earth near back door (inside of house).	1,226,700	...	Colonies of Bacterium vulgare, B. coli communis, B. mycoides numerous. Chemical examination showed no evidence of perchloride, but the presence of the metal (Hg) demonstrated as follows: Took sample earth suspended in sterile distilled water, added HNO_3 to form soluble mercurous nitrate: filtered and evaporated off excess of HNO_3 in water bath: dissolved residue in distilled water and tested for mercurous nitrate by adding sulphuretted hydrogen. Got black sulphide in soluble in excess of HNO_3 Confirmed by KHO and $K_2Cr_2O_7$ tests.
5	Scraping from surface of floor near back window.	1,646,000	...	Colonies of B. mycoides, B. megatherium, B. vulgatus most numerous.
6	Scraping from surface of floor in centre of room.	1,487,000	299,800	Colonies of B. vulgatus and a fluorescent organism most common.
7	Scraping from surface of floor near wall at back part of room.	4,205,000	...	Colonies of B. vulgatus, B. mycoides, B. subtilis most common. B. coli communis frequent.
8	Scraping from wall in dark corner of room.	368,000	...	B. subtilis, B. mycoides most common. Cocci. Sarcinæ. Few moulds. Eleven species counted.
9	Scraping from floor near front window.	2,376,900	...	Ten different species of bacteria counted.
10	Scraping from floor near dark corner of room.	927,000	820,000	Spores of B. mycoides common.
11	Scraping from floor near back door.	4,546,000	...	B. fluorescens. Bac. vulgare. A few colonies of B. coli communis.
12	Scraping from floor in small recess off front part of room	164,000	...	Six distinct species counted.
13	Scraping from floor near partition wall.	4,338,000	296,900	B. coli communis obtained. Also B. fluorescens and Bac. vulgare. Thirteen distinct species counted. Spores of B. megatherium common.

DISINFECTED HOUSE No. 2. *Address same as Occupied House No. 1. Samples taken 20th December 1898. Samples taken within twelve hours of disinfection. 12 gallons of 1 : 725 solution used, viz., 8 gallons for floor, 4 gallons for walls.*

Number of samples.	Description of the samples.	Total number of Bacteria in 1 gramme of sample.	Number of spores of Bacteria in 1 gramme of sample.	Remarks.
1	Scraping from floor near wall.	377,600	299,900	Spore-bearing forms mostly. A few cocci.
2	Scraping from wall coated with cowdung.	130	26	B. subtilis. Few colonies of B. megatherium.
3	Scraping from floor near back wall.	3,620	...	B. vulgatus and other spore-bearing forms common.
4	Scraping from floor near window.	970,700	...	Spore-bearing forms in the majority. Few Bact. vulgare colonies and colonies of cocci. Six different varieties of bacteria counted.
5	Scraping from floor near one of the four corners of the room.	929,000	347,800	B. fluorescens visible. Also a few colonies of B. coli communis. Majority of colonies belong to B. megatherium. Moulds.
6	From surface of heap of dirt behind door.	436,000	...	B. mycoides common. Seven varieties of bacteria counted. Sarcinæ and moulds present.
7	From surface of platform in dark recess.	1,600	480	Four species of bacteria counted. B. subtilis common.
8	Scraping from floor near passage wall.	15,600	...	B. megatherium very abundant. Three distinct varieties of micro-organisms counted.
9	Scraping from centre of floor.	Sterile.	...	.....
10	Scraping from floor near small recess.	2,542	...	All spore-bearing forms.
11	Scraping from floor where plague corpse had lain.	Sterile.	...	.....
12	Dust from surface of ledge near front wall.	2,656	1,820	B. subtilis and others of the same spore-bearing group.

APPENDIX C.

Letter from A. Cumine, Esquire, I.C.S., Acting Plague Commissioner, to Monsieur W. Haffkine, C. I. E., No. 1922, dated 29th July 1898 :—

"When Plague appears in a place we endeavour to get all the people to come out of it, and we disinfect the houses with perchloride of mercury and we make openings in the roofs and walls to admit the light.

"The good results of getting all the people in an infected area to come out of it are patent to us. But we have not to the same extent ocular proof of the good results of 'perchloriding' the houses and admitting light and air into them. Only microscopical examination could tell us what is in practice the effect

(1) of the perchloride, and

(2) of the light

on the germ-infected cowdunged floor of an ordinary native house, and microscopical examination is not within the reach of the District Officers.

"I should, therefore, be very much obliged to you if you could kindly conduct some experiments on these two points and let me know the result. What I am anxious to know is what the perchloride and the light do, not in the laboratory, but in the actual circumstances in which we use them. If it be found that the perchloride in the circumstances and of the strength that we use it is neutralized by cowdung in the floor then could we cure that defect by making the solution stronger?

"I should also be glad if you could kindly tell me what the effect is in practice of the perchloride on a house which having been once infected has been lime-washed and then in consequence of being re-infected 'perchlorided.' Does the lime neutralize the perchloride?

"I shall be much obliged if you will kindly make these experiments for me."

APPENDIX D.*

Letter from Lieut.-Colonel R. Boustead, I.M.S., dated the 20th September 1898 :—

"I have the honour to submit that, in the *Overland Mail* of the 10th September, page 1181, which—through my moving about—I have only now received, I observe a paragraph headed

'Cowdung and the Microbes of Plague,'

in which it is stated that Mr. Hankin and Lieutenant Storrocks, R.A., have carried out a series of experiments with especial reference to 'Disinfection against Plague.'

"They discovered that it is extremely difficult to disinfect an ordinary mud and cowdung floor, and that instead of eradicating the microbes the ordinary solutions of sublimate, *i.e.*, perchloride of mercury, and carbolic acid, are found to lead to an apparent increase to the number of microbes present, and that the resistance to disinfection depends on some substance in the cowdung which protects the microbes from the action of the disinfectants. That substance has not been discovered, and to leave it undiscovered, would be to grant immunity to one of the most fertile sources of plague propagation.

"May I venture to suggest that there are manifest reasons why those powerful germicides may have failed to destroy the microbes of plague.

"First, the cowdung naturally contains a considerable amount of albumenous coagulable slime which, when spread over a mud floor, in the usual way, becomes dried and almost impervious to water, but when solutions of sublimate are sprayed over this coating of cowdung the slime in it is coagulated (as is albumen) by the action of the sublimate and forms an actual impervious film which is a sufficient protection to almost all the microbes that are beneath it. It encases them as it were in a waterproof covering. No aqueous solution of sublimate or carbolic acid would be able to effectively penetrate this coagulum and disinfect the microbes below, except perhaps where the coating of cowdung was broken through. Again, in the decomposition of the cowdung on floors (which is continual) ammonia gas is generated, some of it is fixed in organic matters, some of it is free, being given off continually, and this alkali precipitates and renders abortive (useless) the germicidal properties of the sublimate by converting it into the simple ammonia chloride of mercury, which is useless as a germicide, thus, further, the excellent germicidal properties of the sublimate are destroyed by ammonia within the cowdung.

"But I would suggest that, if the cowdung (or simple mud) floors of plague-infected dwellings were first saturated with sea water or with an equally strong or stronger solution of common salt and then well sprayed with the sublimate disinfectant,

* Extract from Government Resolution No. $\frac{6849}{6097}$ P., dated 16th December 1898.

most of the sublimate would be carried in solution through the film, as sublimate is held in perfect solution by common salt and it will permeate the film through the pores and passages which the salt water has caused by dissolving the slimy albumenous film of the cowdung.

"Moreover, the chlorine of the sea water—in addition to its rendering the sublimate into a perfect condition for the destruction of microbes—is a disinfectant itself, and it also considerably neutralizes the ammoniacal gas in the ground—whether free or in combination—producing chloride of ammonium, a substance which also holds the sublimate in perfect solution in water and renders it more active as a germicide in earthy matter—floors, woodwork, drains, etc.

"The sea water—or salt and water for up-country use—is, I think, equally necessary for a first application to cowdung floors when carbolic acid solutions are used, as it renders the floor more permeable to the disinfectant.

"Where disinfection by spraying is undesirable or insufficient it would, perhaps, be advisable to take an inch, or more, deep of the cowdung covering off the floors and burn it to calcination, then dig from eight inches to a foot of the floor, replacing it with fresh earth, well beaten down level, then sprayed with the sublimate and finally well smeared with plenty of coal tar from the gas-works, followed when nearly dry with half an inch layer of dry sea sand which when quite dry could be swept of all superfluity that had not adhered. This would make an impervious solid and perfectly asceptic floor which would be difficult, if not impossible, for any microbes to enter, and ought not to be expensive at all.

"The walls of infected dwellings might be sprayed with a one per cent. solution of a 30 Vol. saturation of Formaldehide, or with a 1-500 solution of sublimate.

"I cannot but think that among other causes of infection insect life has an important share in propagating the plague by direct inoculation—the virus is most potent. Mosquitoes and flies, bugs and vermin (which abound in the East) by puncturing and feeding upon plague-stricken persons and subsequently settling upon and puncturing healthy persons with their poison-laden organs, they deposit the virus as effectively as if it were done by the point of a lancet or by an hypodermic syringe and disseminate the disease just as Purulent Ophthalmia is carried broadcast by flies, &c., and, I think, it would be a wise precaution against such a terrible disease that all mosquito and other bites should be touched as quickly as possible with a 1-500 solution of sublimate, especially by nurses and attendants on plague patients.

"I shall endeavour to forward a copy of this to the Surgeon General with the Government of Bombay next mail."

RESOLUTION.—The foregoing letter from Lieut.-Colonel R. Boustead, I.M.S., should be communicated to the Surgeon General with the Government of Bombay, the Sanitary Commissioner for the Government of Bombay, the Commissioner in Sind and the Commissioners of Divisions, the Municipal Commissioner for the City of Bombay, and Monsieur W. M. Haffkine for information.

2. The Surgeon General and Monsieur Haffkine should be requested to favour Government with their opinions, without delay, on the suggestion contained in the letter regarding the utility of saturating cowdung (or simple mud) floors with sea water or with a strong solution of common salt before spraying them with the sublimate, with any other remarks they may have to offer.

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