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[Despatch Abstract.]

Bubonic Microbe.

Communicating to all Local Boards and Municl. Councils and Municl. Commissioners of Madras the Surgeon General and the Sanitary Commrs. letter from the Govt. of Bombay forwarding copies of Mr. Hankin's two reports on the researches made by him on various articles of produce as regards their susceptibility to ~~and~~ engender or support the _____.

BACK PAPERS.

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

FURTHER PAPERS.

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

Government {1897} of Madras.

LOCAL AND MUNICIPAL.

Recd. } 1897. *Enclosures*
Regd. } *Spare copies*

G.O., No. 395 M., 5th March 1897.

MARCH.

Bubonic Microbe.

Communicating to all Local Boards and Municipal Councils and Municipal Commissioners of Madras, the Surgeon-General and the Sanitary Commissioner letter from the Government of Bombay forwarding copies of Mr. Hankin's two reports on the researches made by him on various articles of produce as regards their susceptibility to engender or support the —.

GOVERNMENT OF MADRAS.

LOCAL AND MUNICIPAL DEPARTMENT.

Read—the following paper :—

From A. M. T. JACKSON, Esq.. I.C.S., Under Secretary to the Government of Bombay, to the Secretary to the Government of Madras, Local and Municipal Department, dated Bombay Castle, 26th February 1897, No. 1600.

I am directed to forward, for the information of the Government of Madras, copies of two reports, dated the 17th and 19th instant, furnished by Mr. E. H. Hankin on researches he has made on various articles of produce as regards their susceptibility to engender or support the bubonic microbe. It will be seen that in his report, dated the 19th instant, Mr. Hankin shows very clearly that the various descriptions of grain and flour usually stored in Bombay contain no trace of the bubonic microbe, and his other report, dated the 17th idem, proves almost to demonstration that grain purposely infected does not permit of the existence of the microbe for more than about four to six days.

2. I am to add that the results of Mr. Hankin's researches have been telegraphed to Dr. Cleghorn at Venice, with a request that they may be communicated to the Sanitary Conference now sitting in that city. Copies of the reports have also been sent to Dr. Cleghorn.

Enclosures.

From E. H. HANKIN, Esq., Chemical Examiner and Bacteriologist, North-Western Provinces and Oudh, to the Secretary to the Government of Bombay, dated Bombay, 17th February 1897.

In accordance with G.O., No. 564—107-P., dated 2nd February, I have the honour to submit the following report on the vitality of the bubonic plague microbe in the chief grain exports from Bombay.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

11. The mouse treated with the yellow rapeseed extract showed no appearances microscopically or otherwise of having succumbed to bubonic infection. There was nothing noteworthy at the seat of inoculation in the back, but there was an extensive blood-stained œdema on the underside of the neck, but appearances suggesting that it had died from some accidental infection.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

28. In these experiments I had kept the grain damp, so that eventually it became covered with mildew. In the experiments now described, by keeping the grain in a dry condition in bags, I imitated more closely the conditions that exists in commerce. Under such conditions the microbe of bubonic plague appears to die out within from 4 to 6 days. In a few instances, my experiments have suggested that it may survive for periods extending up to 13 days. But it must be remembered that my experiments had to be carried out with the aid of mice owing to the impossibility of obtaining a sufficient supply of healthy rats in Bombay at the present time. It is more probable that the death of the mice in these exceptional instances was due to the accidental presence of other microbes than that unusually resistant forms of the bubonic microbe were present.

From E. H. HANKIN, Esq., M.A., Chemical Examiner and Bacteriologist, North-Western Provinces and Oudh, to the Secretary to the Chamber of Commerce, dated Bombay, 19th February 1897.

In reply to your enquiry as to whether I have ever found the bubonic plague microbe in grain and flour, I beg to state that, although I have examined a large number of specimens under different conditions, I have never succeeded in detecting it. My observations have been carried out on both clean and dirty grain, sweepings of granaries under infected dwellings, grain mixed with rats' dejecta taken from near the body of a dead rat, grain that had been carried by rats through a hole in the wall of a granary and stored in a latrine, but in all cases a negative result has been

obtained. I have also examined weevils and other insects parasitic on stored grain without detecting the microbe. I have recently examined flour from a small godown in the fort in which rats had been dying of the plague with a negative result.

2. It is my opinion that the only connection of grain with the plague in Bombay is that, at the beginning of the outbreak, large numbers of rats were affected, and that these animals were especially common in the neighbourhood of granaries.

ORDER—No. 395 M., dated 5th March 1897.

Communicated to all Local Boards and Municipal Councils, the Municipal Commissioners of Madras, the Surgeon-General and the Sanitary Commissioner.

2. The Surgeon-General is requested to communicate these papers to all District Medical Officers and to the Surgeons of Districts in Madras.

(True Extract.)

G. S. FORBES,
Secretary to Government.

To all Presidents of District Boards.
" all Chairmen of Municipal Councils.
" the President, Municipal Commission, Madras.
" the Surgeon-General.
" the Sanitary Commissioner.
Copy to the Public Department.
" " Revenue Department.

Exd. P. Seetharam.

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Government {1897} of Madras.

LOCAL AND MUNICIPAL.

In the Secretary

Proof for approval

2. Please see paras marked

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Recd. } 1897. Enclosures See as above
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G.O., No. 395 M., 5th March 1897.

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ENCLOSURES.

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

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

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

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

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

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

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

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

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

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,,	,,	,,	Yellow rapeseed survived.
,,	,,	,,	Brown ,, died within 24 hours.
,,	,,	,,	Tilseed died within 7 days.
,,	,,	,,	Groundnuts died within 24 hours.
,,	,,	,,	Poppyseed survived.
,,	,,	,,	Wheat (first variety) survived.
,,	,,	,,	,, (second variety) ,,
,,	,,	,,	Flour survived.

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

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

11. The mouse treated with the yellow rapeseed extract showed no appearances microscopically or otherwise of having succumbed to bubonic infection. There was nothing noteworthy at the seat of inoculation in the back, but there was an extensive blood-stained œdema on the underside of the neck, but appearances suggesting that it had died from some accidental infection.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

28. In these experiments I had kept the grain damp, so that eventually it became covered with mildew. In the experiments now described, by keeping the grain in a dry condition in bags, I imitated more closely the conditions that exists in commerce. Under such conditions the microbe of bubonic plague appears to die out within from 4 to 6 days. In a few instances, my experiments have suggested that it may survive for periods extending up to 13 days. But it must be remembered that my experiments had to be carried out with the aid of mice owing to the impossibility of obtaining a sufficient supply of healthy rats in Bombay at the present time. It is more probable that the death of the mice in these exceptional instances was due to the accidental presence of other microbes than that unusually resistant forms of the bubonic microbe were present.

From E. H. HANKIN, Esq., M.A., Chemical Examiner and Bacteriologist, North-Western Provinces and Oudh, to the Secretary to the Chamber of Commerce, dated Bombay, 19th February 1897.

In reply to your enquiry as to whether I have ever found the bubonic plague microbe in grain and flour, I beg to state that, although I have examined a large number of specimens under different conditions, I have never succeeded in detecting it. My observations have been carried out on both clean and dirty grain, sweepings of granaries under infected dwellings, grain mixed with rats' dejecta taken from near the body of a dead rat, grain that had been carried by rats through a hole in the wall of a granary and stored in a latrine, but in all cases a negative result has been

obtained. I have also examined weevils and other insects parasitic on stored grain without detecting the microbe. I have recently examined flour from a small godown in the fort in which rats had been dying of the plague with a negative result.

2. It is my opinion that the only connection of grain with the plague in Bombay is that, at the beginning of the outbreak, large numbers of rats were affected, and that these animals were especially common in the neighbourhood of granaries.

ORDER—No. 395 M., dated 5th March 1897.

Communicated to all Local Boards and Municipal Councils, the Municipal Commissioners of Madras, the Surgeon-General and the Sanitary Commissioner.

2. The Surgeon-General is requested to communicate this to all District Medical officers and to the Surgeons of Districts in Madras. These papers

(True Extract.)

Secretary to Government.

To all Presidents of District Boards.
" all Chairmen of Municipal Councils.
" the President, Municipal Commission, Madras.
" the Surgeon-General.
" the Sanitary Commissioner.

Copy to the Public Department.
" " Revenue Department.

Exd. P. Seetharam.

Revenue (189) Department.

CURRENT No. ~~1800~~ 913 M

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From

The Under Secy to the Govt of Bombay, Rev: Dept

Order No. 395 M. 875-3-97

No. 1600

Communicated to all Local Boards and Municipal councils, the municipal Commissioners of madras, the Surgeon General and the Sanitary Commissioner

Dated 26th Feb.

Received } 1st March. 97

Registered }

Enclosures 2

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V.P 2/3/97

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SUBJECT.

Forwarding, with remarks Copies of Mr. Haukin's two reports on researches made by him on various Articles of produce as regards their Susceptibility to engender or support the bubonic microbe.

To

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GOVERNMENT OF MADRAS
11 MAR 97

LOCAL & MUNICIPAL DEPT.
CURRENT No. 913 M

No. 1600 of 1897.

REVENUE DEPARTMENT.

Bombay Castle, 26 February 1897.

From

A. M. T. Jackson, Esquire, I.C.S.,

Under Secretary to the Government of

BOMBAY.

To

The Secretary to the Government of Madras.

Sir,

I am directed to forward for the information of the Government of Madras copies of two reports dated the 17th and 19th instant furnished by Mr. E.H.Hankin on researches he has made on various articles of produce as regards their susceptibility to engender or support the bubonic microbe. It will be seen that in his report dated the 19th instant Mr. Hankin shows very clearly that the various descriptions of grain and flour usually stored in Bombay contain no trace of the bubonic microbe, and his other report dated the 17th idem proves almost to demonstration that grain purposely infected does not permit of the existence of the microbe for more than about four to six days.

2. I am to add that the results of Mr. Hankin's researches have been telegraphed to Dr. Cleghorn at Venice with a request that they may be communicated to the Sanitary Conference now sitting in that city. Copies of the reports have also been sent to Dr.Cleghorn.

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

A M Jackson

Under Secretary to Government.

Enclosure

2 P

From

E. H. HANKIN,

Chemical Examiner and Bacteriologist,

N. W. Provinces and Oudh,

To

The SECRETARY to GOVERNMENT,

Bombay.

Dated, Health Office Laboratory,

Bombay, 17*th February* 1897.

Sir,

In accordance with Government Order, No. $\frac{664}{107\text{-P.}}$, dated 2nd February, I have the honor to submit the following report on the vitality of the bubonic plague microbe in the chief grain exports from Bombay.

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

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

2 a P

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

4. My first experiment was carried out with the bubonic plague microbe derived from cultures on agaragar. Two cultures, each about a week old, were employed. Of these, one had been derived from a patient in Bombay, the other

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

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

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

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

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

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

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

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

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

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

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

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

11. The mouse treated with the yellow rapeseed extract showed no appearances microscopically or otherwise of having succumbed to bubonic infection. There was nothing noteworthy at the seat of inoculation in the back, but there was an extensive blood-stained œdema on the underside of the neck, but appearances suggesting that it had died from some accidental infection.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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" " " Tilseed survived.
" " " Groundnuts survived.
" " " Poppyseed "
" " " Wheat died within 48 hours.
" " " Flour "

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

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

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

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

28. In these experiments I had kept the grain damp, so that eventually it became covered with mildew. In the experiments now described, by keeping the grain in a dry condition in bags, I imitated more closely the conditions that exist in commerce. Under such conditions the microbe of bubonic plague appears to die out within from 4 to 6 days. In a few instances, my experiments have suggested that it may survive for periods extending up to 13 days. But it must be remembered that my experiments had to be carried out with the aid of mice owing to the impossibility of obtaining a sufficient supply of healthy rats in Bombay at the present time. It is more probable that the death of the mice in these exceptional instances was due to the accidental presence of other microbes than that unusually resistant forms of the bubonic microbe were present.

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

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From

E. H. HANKIN, M.A.,
Chemical Examiner and Bacteriologist,
N. W. P. and Oudh,

To

The SECRETARY to the CHAMBER of COMMERCE,
Bombay.

Dated Health Office Laboratory,
Bombay, 19*th February* 1897.

Sir,

In reply to your enquiry as to whether I have ever found the bubonic plague microbe in grain and flour, I beg to state that, although I have examined a large number of specimens under different conditions, I have never succeeded in detecting it. My observations have been carried out on both clean and dirty grain, sweepings of granaries, under infected dwellings, grain mixed with rats' dejecta taken from near the body of a dead rat, grain that had been carried by rats through a hole in the wall of a granary and stored in a latrine, but in all cases a negative result has been obtained. I have also examined weevils and other insects parasitic on stored grain without detecting the microbe. I have recently examined flour from a small godown in the Fort in which rats had been dying of the plague with a negative result.

2. It is my opinion that the only connection of grain with the plague in Bombay is that, at the beginning of the outbreak, large numbers of rats were affected, and that these animals were especially common in the neighbourhood of granaries.

I have, &c.,
E. H. HANKIN.

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