A study of the evidence as to the source of the infection which caused the cases of tetanus at Mulkowal, Punjab, India, during inoculation against plague in October, 1902 / by James Cantlie.

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A Study of the Evidence as to the Source of the Infection which caused the Cases of Tetanus at Mulkowal, Punjab, India, during Inoculation against Plague in October, 1902,

BY

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A STUDY OF THE EVIDENCE AS TO THE SOURCE OF THE INFECTION WHICH CAUSED THE CASES OF TETANUS AT MULKOWAL, PUNJAB, INDIA, DURING INOCULATION AGAINST PLAGUE IN OCTOBER, 1902.

ALTHOUGH it is over four years since the lamentable accident occurred at Mulkowal by which nineteen persons contracted tetanus as the result of injection with plague prophylactic, it is only now that the particulars of the accident are presented to us in a sufficiently precise and complete form to enable us to come to a scientific conclusion as to the source of the infection which caused the accident.

There have been several cases of an allied nature, one in Italy, one in the United States, and one in the Philippines.

In no instance has any searching enquiry been published as to how these accidents took place. This is a matter much to be regretted from every point of view, for it is probable that an investigation of any one of them would have furnished us with important facts for the guidance of bacteriologists and operators in the preparation and administration of sera and vaccines in general.

It must be remembered that prophylaxis by these new methods is still largely on its trial, and in some cases in its infancy, and mishaps are, perhaps, inevitable; but should they occur, a thorough investigation and a careful study and analysis of the facts is of vital consequence to scientists and to the public. From a medico-legal point of view, if from no other, the matter has great scientific value; for the methods of investigation as to the origin of contamination causing such accidents have not hitherto been dealt with in a manner which can be regarded as of any real value.

The present instance—the Mulkowal accident—affords us, for the first time, an opportunity of studying such a case as a whole, for we have not merely expressions of opinion to guide us, but facts, the result of rigorous investigations, which are indisputable and conclusive. Any one reading the evidence must be impressed with the accurate manner in which the details of the subject have been studied. Merely, therefore, as a scientific work, this investigation will remain as a model of how thorough and complete questions of the kind can and should be worked out at the present day; and there can be no question as to the importance of publishing as fully as the Government of India have done, the steps taken to elucidate such a subject.

Beyond the mere academic interest, however, attaching to this question, the great practical importance of this publication is obvious.

The report issued by Government, in a supplement to the *Gazette of India*, Calcutta, December 1st, 1906, is very voluminous; the larger part of it is, however, occupied by the discussions of the relative merits of two methods of preparing Haffkine's prophylactic. Both of these methods, the broth-cultivation one, the so-called *standard* method, and the agar cultivation process, are in use in various laboratories, and their description and details have only a distant bearing on the case. It has, therefore, been decided in the pages of this Journal to reproduce the portions of the report which refer to the accident at Mulkowal and the origin of the tetanus contamination. As the reader will notice, even that portion appears to entail a considerable amount of close reading; but the gist of the matter is really simple.

As a guide to the reader it may serve a useful purpose to summarise as briefly as possible the points at issue:—

(1) The accident was confined to the contents of a particular bottle, and to the use of a particular syringe.

(2) The subject was investigated by two scientific bodies: (*a*) a Commission in India, and (*b*) the Lister Institute in London.

(3) Assuming that the accident was caused by the syringe, the contamination could only have originated locally in the Punjab; but if the contamination was in the bottle, it may have come from the laboratory at Bombay, or have been introduced at Mulkowal at the time of the inoculations, when the bottle was opened.

(4) The evidence shows that the bottle, which was prepared forty-one days, and issued from the laboratory twenty-six days, before the accident, was free from contamination up to at most two or three days before its contents were used, and readers will be able to judge for themselves that the evidence on this point is of a very exact nature. The bottle was odourless at the time of opening, whereas tetanus growing in a medium gives out a strong, pungent smell, and when, experimentally, the bacillus was introduced into bottles of plague prophylactic, it was found that the smell becomes pronounced and unmistakable within three days.

Secondly: The syringe used on the nineteen people was rinsed out with carbolic lotion after the inculpated bottle had been emptied, and afterwards, with the same syringe, other people were inoculated by fluid from other bottles. Now it has been proved experimentally that a syringe, into which a tetanus culture grown in a bottle of prophylactic even for a few days only has been drawn, becomes so grossly contaminated that after the rinsing, as practised at Mulkowal, and filling it with harmless fluid, the syringe caused tetanus to seventeen out of nineteen animals on which the experiment was tried. In the case of the inoculation at Mulkowal, however, not one of the people injected with the syringe after the rinsing exhibited any abnormal symptoms. In other words, the prophylactic in the incriminated bottle could not have contained a developed culture of tetanus.

Thirdly: Numerous experiments have shown that the prophylactic, in its agar-grown variety as used in the Punjab, was an extraordinarily suitable medium for the growth of tetanus bacilli, and that when tested after a few days' growth, the fluid, contaminated with the Mulkowal tetanus bacillus, gave tetanus to animals in doses of $\frac{1}{2,400}$ cc. and $\frac{1}{48,000}$ cc., whereas with an ordinary cultivation medium a dose of $\frac{1}{10}$ cc. was required. It is evident that if the patients had been injected with $1\frac{1}{2}$ cc. of such an extraordinarily toxic fluid, the incubation period of the disease and the duration of the latter would be

greatly curtailed. Now, in both these respects, the Mulkowal patients exhibited clinical manifestations coinciding with those of average, not acute, cases in India, arising from an invisible infection introduced during an accident or operation, and running a relatively slow course.

Fourthly: The bottle was recovered and examined fifteen days after the accident, and instead of the rich and toxic culture above referred to, it was found to contain a poor culture of tetanus, of reduced toxicity, and mixed up with a common micrococcus. Now tetanus bacilli, being anaerobes, give the rich and toxic culture mentioned above when they are introduced into a bottle full of prophylactic. When they find themselves in a few drops of fluid left in a bottle, and are thus exposed to the air, they do not grow at all, or else give a poor culture and of little toxicity if aerobic microbes present in the fluid partially use up the oxygen. This again points to the tetanus microbes found in the Mulkowal bottle not having been there whilst the bottle was full of fluid, but having been introduced at a time when the bottle was at once emptied of most of its contents.

Lastly, as will be mentioned subsequently, the bottle was dealt with at Mulkowal in an extraordinarily faulty manner.

(5) The Indian Commission started, however, with the assumption that the contamination of the syringe, or the contamination of the bottle at the time of inoculation, was not likely to cause nineteen cases of tetanus, and upon this assumption they rejected all the evidence brought out by the enquiry, and pronounced that the bottle was contaminated in Bombay.

(6) The Lister Institute ascertained by experiment that the Commission's assumption was not maintainable, and that the accident *could* have been caused locally; but it abstained from expressing an opinion and giving a pronouncement on the actual origin of the accident. When pressed subsequently for a definite and precise statement the Institute authorities, on November 9th, 1905, replied in the following words:—

From Dr. Charles J. Martin, M.B., D.Sc., F.R.S., Director.
To The Under-Secretary of State for India.

"In reply to your letter of November 2nd, 1905 (R and S, 2658), I am directed to state that the Institute is of opinion that the fluid used at Mulkowal probably became contaminated with tetanus before the time of opening the bottle at Mulkowal, but it also agrees with the Commission (extracts from the Report of the Punjab Plague Inoculation Commission, p. 2547,[1] 5th line) that the possibility of contamination having effected an entrance at Mulkowal cannot be excluded."

The reader will observe that the opinion of the Lister Institute authorities is directly opposed to the fundamental assumption and the finding of the Indian Commission. In yet another communication from the Lister Institute, dated May 5th, 1906, they refer to the subject in the following terms:—

"In further reference to your letter, R and S 409, of March 9th, 1906, I have now the honour, by direction of the Governing Body of the Lister Institute, to submit to you the following observations on the letters from Mr. Haffkine, of February 14th, 1906, and March 10th, 1906.

"The Governing Body of the Institute, while of opinion that the probabilities were in favour of the view that the tetanus impurity was primarily in the fluid, did not feel justified in asserting this as a proved fact in view of the possibility of contamination at Mulkowal.

"The Governing Body regret that in their report of November 24th, 1904,[2] they inadvertently referred to their conclusion as the same as that of the Commission. The sentence in the Government report that gave rise to this idea was, "That it (the contamination) might have affected an entrance at Mulkowal, cannot be disputed; thus, by way of illustration, the stopper or the forceps might have been dropped on the ground and applied to the mouth of the bottle with contamination adhering, or spores settled between the rubber stopper and the rim of the bottle might have dropped in as the stopper was pulled out." The Governing Body overlooked the fact that the Commission subsequently considered this possibility as one cancelled by other considerations. But in this respect the Governing Body are bound to say that they cannot fully concur, and while holding to their view of the probability, they think Mr. Haffkine has a right to claim the benefit of the doubt, especially in view of the fact elicited that the forceps used for drawing the cork at Mulkowal were dropped on the ground, and subsequently re-used by the operator on the same cork.

"The Governing Body also regret that in the first paragraph of the section of their report dealing with the probable origin of the tetanus virus (p. 3)[2] to which Mr. Haffkine draws attention in his letter of February 14th, the words "the fact" were drafted into the sentence. The words should have been omitted and the sentence should have run, "But that a bottle, presumably tightly corked, should contain enough tetanus growth to destroy nineteen people, and yet not be accompanied by sufficient smell to arouse the suspicion of Dr. Elliot, who, according to his evidence, remembers smelling this particular bottle, is difficult to comprehend." Mr. Haffkine is certainly entitled to this correction, though the argument in his favour was already clear from the context."

It is evident that the Lister Institute refrained from a declaration of the conclusions which were obvious and must appear to every one inevitable, viz., that the bottle of prophylactic was not originally contaminated as it left the laboratory; that the contamination is directly attributable to the opening of the bottle by a forceps that had been dropped on the ground during that operation; and that the accident was due to the contaminated forceps not having been flamed before being applied again to the bottle.

J. C.

[1] P. 41 in this Journal.

[2] P. 45.

THE DOCUMENTS ON THE MULKOWAL ACCIDENT.

SUMMARY OF MR. HAFFKINE'S EVIDENCE.

No. 258, *dated February* 4, 1903.

From W. M. Haffkine, Esq., C.I.E., Director-in-Chief, Plague Research Laboratory, Parel, Bombay, to the Secretary, Commission of Enquiry into the Mulkowal Tetanus Case.

In connection with my evidence to the Commission on the case under consideration, I have the honour to [forward attached papers, and to] summarise their contents as follows:—

* * * * * * * *

Referring to the Mulkowal misfortune, I enumerated in my evidence the facts which go against attributing the tetanus contamination to the Laboratory.

As to what was the real origin of the tetanus there, it is not possible for me to say at present. Speaking in the abstract, it might be a needle contaminated on the outside, or having a contaminated nozzle, and which escaped effective sterilisation, and was used for those nineteen persons alone; or a contaminated portion of carbolic lotion used for washing the needle in; or a particular pad which was contaminated, and used only on those persons. Contamination might have got into the bottle either from the needle, or by a gust of wind suddenly raising a heavy dust, or by an assistant stoppering the bottle with a contaminated finger for shaking it, &c. *The objection I mentioned against a tetanus culture having been drawn into the syringe is maintainable in regard to such a culture as found by Major Semple.*[1] *If, on the other hand, contamination was introduced just before the syringe was filled, the amount of it may have been such as to admit of the rinsing of the syringe rendering it innocuous;* and so on.

It is impossible for me to make more than guesses on all these points. It would be very important, if that is possible, to find out which of the enumerated or other possibilities actually occurred, or to think out, as we are always trying to, which of such possiblities may occur, so as to add to the technique of the operators such precautions as may obviate similar occurrences. At the same time, the objections mentioned in my evidence, to admitting that the tetanus contamination occurred in the Laboratory, must stand independent of our ability or otherwise to find out how it actually occurred.

The absence of smell at the time of testing the bottle in Mulkowal, and the presence of that smell when examined in Kasauli, point to the contamination having occurred after the bottle was opened in Mulkowal.

Regarding the possibility of conferring tetanus by minute quantities of tetanus virus, I beg to refer to Vaillard and Rouget's experiments (*Annales de l'Institut Pasteur*, 1902, pp. 406 and 417): cultures made of tetanus-producing earth revealed the presence of some two or three tetanus microbes in a particle of earth sufficient to cause tetanus to a guinea-pig.

From the same publication (pp. 415 and 416), it will be seen that several animals infected with such contaminated earth may exhibit a disease of *equal* severity. The fact, therefore, that in the cases in Mulkowal the incubation period did not differ more than it did, does not render necessary the conclusion that the disease was caused in them by absolutely equal doses of virus or toxin.

[1] *Vide* p. 57 under (9).

EVIDENCE GIVEN BEFORE THE COMMISSION OF ENQUIRY BY DR. A. M. ELLIOT, INOCULATING OFFICER.

At Mulkowal I had two syringes and four needles in use.

I had used one of the syringes the previous evening; I had used both on the 27th. I cannot tell of which of the two I had used first at Mulkowal. I had used these four needles on the 27th and 29th. I disinfected the four needles every day, and used two out of the four.

At the close of the operations of the 27th I gave both the syringes and the four needles to the compounder, who was supposed to clean them out. I did not see him clean them out. When we first started inoculating on October 10th I saw him clean them out. I had to teach him the routine I wished to be observed, and I did the cleaning at first myself.

My procedure[1] was as follows: I removed the needle from the syringe, and put it in the carbolic lotion which had been prepared that day; it was different from that used to swab the patients' arms.

This carbolic lotion had only been used during the day to dip the lint in for putting on the seat of inoculation, placing the spare needles in it, and rinsing the syringe.

Then I filled my syringe with carbolic lotion, and put the needle I had just been using on the syringe again, and I squirted the lotion through the needle once. Both syringes were treated in the same way, and the needles.

Then the needle was removed from the nozzle of the syringe, and it was placed in its case. I removed the needle with my hand. I then took the needles from the lotion with my hand, and placed them in the case.

The syringe was also placed in the case. This is how I taught the compounder to do the work.

The cases containing the syringes and needles were replaced in the box.

On October 30th I got syringes and needles which on the previous day of use had been presumably treated in that manner by the compounder.

I have a distinct recollection of the 30th. I did my work then in the open under trees on the outskirt; it was surrounded by houses on three sides; it was about 30 feet by 40 feet; the backs of the houses looked over it; there were cattle on the other side.

There were no animals excepting my horse, which was being walked about; I had ridden up, the compounder had walked across; the square was hard ground; I do not remember seeing droppings of animals.

My bottles[2] and instruments were placed on a table brought over from my camp; it was carried by coolies . . . One coolie carried it. By the time I arrived everything was ready; this was done by the compounder.

Everything was lying on the table, the syringes and needles in their cases ready for me to take them out; the cases were not open. The oil was in a small metal vessel . . . and a spirit lamp

[1] At the close of each day's operations.

[2] Apparently bottles of carbolic lotion, *vide* below.

. . . and probably one or two bottles of the lotion. The lotion would be poured out into the vessels which had previously been in the box in a special partition.

When I arrived the lamp was lit and the oil was placed over it; then I took a case with a syringe, took out the syringe, filled it with carbolic which had been prepared by the compounder, then I took my case of needles and placed them in the oil; I did not wait until the oil was heated. The syringe with the lotion was in the bowl or leaning against it, and it so remained until I put the needle on. The other needles and syringe were treated in the same way.

I squirted out each syringe twice, once with and once without the needle. After the second rinsing out, the syringes with the needles on them were placed in the bowl.

There were people there, and as soon as they were ready I filled syringe No. 1 with prophylactic.

For that purpose I told the compounder to give me a bottle; I looked at it and gave the brew number and dose to the clerk. I passed it back to the compounder who opened it with forceps which was lying in the same bowl of carbolic; the forceps had before that been placed in the hot oil to remove the needles.

I remember looking at the stopper and paper; both were intact; there was no sign of leakage; before that I had noticed bottles with the stoppers prised out.

When the compounder opened the bottle he handed it to me to smell; I am sure it was the compounder and not a villager; a villager has never assisted me to open the bottle; I would not trust him; I would not trust the compounder without his being under my eye.

I remember smelling this bottle; I perceived nothing; my sense of smell is acute; prior to October 30th I had discarded a bottle on the ground of bad smell.

(We test Dr. Elliot with certain bottles, including the Mulkowal bottle.)

I cannot specialise the smell.

I did not perceive a smell like any of these. Prior to this the compounder had shaken the bottle before opening. He did not drop the cork. He did not shake the bottle with his finger on the mouth; I was watching him.

I never told him to put the mouth of the bottle in a flame, or in hot oil, or carbolic lotion before removing the stopper. I cannot tell whether on this occasion he put the mouth in carbolic.

After smelling I passed the bottle back to him and I took my syringe out of the lotion and filled it with the fluid. Then he restoppered the bottle—whether he had previously swished the stopper through carbolic or not I cannot tell—then placed the bottle on the table and the forceps in the carbolic.

I then operated on the first man almost immediately, and he was followed by thirty or forty persons without any substantial break. With the first syringeful I must have inoculated about twelve people; I did them with the same needle. I probably dipped my needle into the carbolic after each inoculation. When the syringe was empty I went on continuously; I told him to open the bottle. He did so, and I filled my syringe with the same needle on; I did not change my needle between each syringeful.

Between the syringefuls I did not draw any carbolic into the syringe.

I filled the syringe a second time in the same way, and followed the same procedure. I cannot account for the compounder giving a different version; at Shadiwal some days before, owing to pressure of work the Deputy Commissioner suggested that the compounder should fill a syringe; I allowed him to do so, but it was done so badly that I squirted it out. On no other occasion did the compounder fill the syringe.

The second syringeful exhausted practically all that remained in the bottle.

When I finished it I filled my syringe with carbolic from the basin and put it down, leaving it full; it remained full until the second bottle was finished. That would be about three or four minutes.

At the third bottle I took up syringe No. 1 again: I squirted the lotion on the ground and filled my syringe from bottle No. 3, using the same needle; I am positive I used the same needle. I then used the whole of bottle No. 3 with syringe No. 1; there would be about sixteen people so inoculated. Of these sixteen none exhibited tetanus. Some inoculated with bottle No. 2 exhibited hysterical tetanus; it was fright that caused these symptoms.

I was told there had been no tetanus in the village. I did not wipe the needle with a piece of lint before each inoculation; I swished it in the lotion.

The arm of each patient was washed by the chaprassi, with lint dipped in carbolic 1 in 20; he used the same piece of lint probably the whole day.

A villager may have placed the lint on the arm after inoculation.

I think the tetanus contamination must have been in the prophylactic.

I think the carbolic acid which was in the syringe for three or four minutes would sufficiently wash out the syringe to account for the freedom from attack of those inoculated with the third bottle. I attribute this to the mechanical action of the rinsing; for this purpose it would be equally efficacious to rinse out with sterile water.

My syringes had been in use since October 10th. I used my Mulkowal needles on the 27th; none of the persons inoculated on the 27th showed tetanus symptoms.

The needles were in the oil about one minute after it began to smoke. It begins to smoke at about 150° C. It was olive oil; a new lot.

I found loose stoppers at Shadiwal; I discarded the bottles.

I think looking after the syringe and needle is the most important.

I have often seen the compounder swish the mouth of the bottle in carbolic.

I saw all the cases but three; there is no doubt it was tetanus.

I got the Mulkowal needles about the 20th; they were not rusty.

It was by the restriction on the corks that I judged they had been prised out.

I have never heard a stopper come out with a pop.

MR. HAFFKINE'S OBSERVATIONS ON DR. ELLIOT'S EVIDENCE.

(*Appendices* (2) *and* (3) *to letter dated February* 14, 1906, *p.* 54.)

The Odour of a Tetanus Culture.

The French say about this odour, "Odeur infecte; tout à fait désagréable; très caractéristique."

Lieutenant-Colonel Semple ascertained for Sir L. Jenkins's Commission whether, indeed, tetanus, notably the particular specimen received from Mulkowal, growing in the Bombay water-agar prophylactic bottles (as distinct from usual laboratory cultivation media), gave out a smell. He examined his bottle *seven days* after contamination. The result of one of his trials is given in the Commission's report, Appendix A, Experiment 5.[1]

In the Mulkowal bottle the tetanus bacillus was found together with a micrococcus. This condition was realised in another experiment of Lieutenant-Colonel Semple's, viz., Experiment 6, same Appendix.

In another instance the air was rigorously excluded from the culture; Experiment 7, same Appendix.

In another, instead of associating with tetanus a micrococcus, a different variety of bacteria, a coccobacillus, was taken; Appendix B, bottle No. 1.[1]

The Lister Institute ascertained whether the smell was present after *three days;* the result is stated in Appendix D[2] to their report, which refers to experiments with full bottles of prophylactic, so as to exclude air, the contamination being effected with tetanus alone. Then they made experiments with full and with half-full bottles, the contamination being done with tetanus microbes associated with another bacillus.

Then (Appendix E) they made experiments with 1 cc. of prophylactic left as a remnant in a bottle of 30 cc., so as to see whether the characteristic smell would develop in a bottle contaminated at the time of use and emptied directly afterwards of most of its contents, as would correspond to the Mulkowal circumstances.

In every instance a contaminated bottle opened seven, four, and three days after contamination exhibited the characteristic tetanus smell.

The compounder who assisted the inoculating officer at Mulkowal said on evidence, when describing their procedure: "Dr. Elliot took out a bottle from the box and gave it to me, that was the first thing in the morning. I was to open the bottle . . . I then took out the cork with the forceps. I kept the cork with the forceps in my hand and Dr. Elliot smelt the bottle."

The Inspecting Inoculation Officer, in his report on the accident, said: "Dr. Elliot, European Inoculating Officer, inoculated 107 persons at this village on October 30th, in the forenoon. He seems to have taken great care in performing the operation and even went so far as filling his syringe with carbolic acid solution after using up each bottle. This is a precaution I have not known others resort to.

The Judicial and General Secretary to the Government of the Punjab, in reporting the accident to the Government of India, in the Home Department, expressed himself as follows: "Dr. Elliot, the inoculator, adopted every necessary precaution to guard against accident, and he had no reason to suspect the fluid on account of its smell or appearance, or on any other account whatsoever. He was so sure of its harmlessness that he, in a practical way, signified to the people his own readiness to be inoculated with it . . . The Lieutenant-Governor desires to place on record the admirable manner in which Dr. Elliot has conducted himself throughout."

The following was the Punjab Chief Plague Medical Officer's statement: "Before commencing to inoculate at Mulkowal on October 30th, he (Dr. Elliot) offered to be inoculated himself in order to gain the confidence of the people, and had actually bared his arm for the operation, when the people said they would be inoculated without this . . . When the news of the catastrophe was received, Dr. Elliot did all in his power for the unfortunate sufferers, and his devotion has done much to convince the villagers that the calamity was the result of an accident only, and to prevent any outburst of feeling against the Government."

The details regarding the condition of the bottle at the time of using it were, in Dr. Elliot's description, the following: "I went to Mulkowal on Thursday, October 30th. I took the precautions I had already described as being my general method. I am sure I did not omit any of them. I began my operations that morning with 53 N; I smelt the bottle and noticed nothing; nothing interfered with my power of smelling. I was on the look-out, as I had discarded a bottle from the same box the previous week . . . I have a good sense of smell, and I am positive the bottle did not smell."

The above is from Dr. Elliot's evidence given by him in the Punjab. Subsequently he and his compounder were asked to come down to Bombay and appear before the Commission again. I was present at their giving evidence,[3] and I find in the records correctly recorded Dr. Elliot's saying: "When the compounder opened the bottle he handed it to me to smell . . . I remember smelling the bottle; I perceived nothing. My sense of smell is acute. Prior to October 30th I had discarded a bottle on the ground of bad smell."

But after this passage an abbreviation of the records follows which seems to me to render possible a misunderstanding and which, as a matter of fact, has led to one.

The lines which follow in the records of Dr. Elliot's above declaration are these:—

("We test Dr. Elliot with certain bottles, including the Mulkowal bottle.")

"I cannot specialise the smell."

"I did not perceive a smell like any of these."

[1] P. 43.

[2] Pp. 45 *et seq.*

[3] Pp. 35 and 38.

The words are authentic, but by the omission of detail, this part of the record has acquired, I think, an effect divergent from that which it had; and this circumstance is reflected in the Commission's subsequent pronouncement on this matter.

I reproduce herewith the corresponding part of the evidence, and vouch for the faithful accuracy of every portion of my narrative. The following were the details:—

In answering questions put to him, Dr. Elliot informed the Commission that he knew the slight odour[1] which the *ordinary* prophylactic had. When on plague duty in the Bombay Presidency and in Sind, prior to his engagement in the Punjab, he worked with that prophylactic, and was well familiar with its properties. On the other hand, the new preparation used in the Punjab was odourless. The bottles he dealt with at Mulkowal had no smell of any kind. He remembered the first bottle distinctly. He was going to be inoculated from it himself. He was in no way in a hurry at the time; the work had not yet begun, and he made his preparations at leisure.

The Commission then placed before him three bottles, and asked him to examine them and say what he thought of them.

He picked up one, smelt it, and did not say whether it was good or bad, but put it aside. Then he took up another, and said he thought he might pass that bottle; returned to the first, and said he was not sure of that one, as it had some sort of smell. Then he took up the third bottle, smelt it twice, and said that was a bad bottle.

The President asked him whether he was sure of this; would he reject a bottle of that kind? And the reply was, he was sure he would. The further question was, could he specialise the smell in that bottle? To this Dr. Elliot replied that he could not. The President then asked him whether he was familiar with the smell of a tetanus culture, and Dr. Elliot said that he was not. The further question was whether he could say that the Mulkowal bottle did not smell like the one just before him, and he replied that in the Mulkowal bottle he found no smell of any kind, neither like that in the bottle he thought bad, nor in the one which he was not quite sure about; and then he smelt the condemned bottle again, and said he was certain he would have never used on any man, nor on himself, a bottle like that one.

The President then told him that the bottle he thought bad was the Mulkowal bottle. To this Dr. Elliot made no reply; and it appeared that the two other bottles were, one of broth prophylactic, in this Dr. Elliot had indicated a slight odour; and the other, of water-agar prophylactic.

It seems to me that the above part of the evidence has been inadequately rendered in the two sentences put on record, viz.:—

"I cannot specialise the smell.

"I did not perceive a smell like any of these."

It will be also seen that this portion of the evidence can be restored by taking as witness any average person and making him go through the same examination with similar bottles as gone through on the above occasion by Dr. Elliot.

The misunderstanding which I have alluded to above is this: had the Commission preserved on record the above details, the following two points would, amongst others, have been kept before their consideration:—

(1) The missing details put in evidence the difference between perceiving—detecting the presence of—a disagreeable smell, on the one hand, and on the other, "specialising" it, *i.e.*, defining the particular fermentation by which it is produced.

(2) They indicate the difference between the water-agar prophylactic used in the Punjab, and which has no smell, on the one hand, and the ordinary peptone broth prophylactic, in which the slight odour proper to the peptonised meat is mingled with that of a ½ per cent. carbolic acid added to that prophylactic. Dr. Elliot showed that, had he been dealing with the latter variety, he would have differentiated a tetanus contaminated bottle, such as bottle 53 N was at the time of his examining it in Bombay, from amongst normal bottles. But, in reality, when working in the Punjab, notably at Mulkowal, he was not dealing with that variety of prophylactic, and, as he said, expected a total absence of odour in every bottle which he was scrutinising.

EVIDENCE GIVEN BEFORE THE COMMISSION OF ENQUIRY BY NARINDAR SINGH, DR. ELLIOT'S COMPOUNDER.

The needle never fell. I never opened a bottle without Dr. Elliot's orders. I never smelt the bottle; Dr. Elliot always did so.

I never noticed a stopper come out with a pop.

I noticed loose corks. Dr. Elliot used to see them.

The prophylactic in those bottles was used.

The cork at Mulkowal was not loose.

I remember it was a very tight cork, and the forceps fell out of my hand on to the ground.

I cannot say whether Dr. Elliot saw it.

I swished the forceps in the lotion, and then pulled out the cork with it.

I never mentioned this to any one before.

The circumstance was recalled when I was asked whether the cork was tight.

I am certain it happened to the first bottle at Mulkowal.

I never gave it a thought before.

I am certain I dropped the forceps; the forceps also fell on to the ground in Gujarat itself; it was at the fourth bottle.

The forceps has fallen down on many other occasions.

I do not remember being asked whether the cork was dropped.

The cork used to fall very often; and it was put in the antiseptic.

I did not drop it at Mulkowal.

Forceps may have fallen at other places besides Mulkowal and Gujarat, but I have no recollection. I do not know any one now alive who saw the forceps drop.

The cork had begun to move when the forceps dropped.

[1] Due to the carbolised peptone or broth medium.

MR. HAFFKINE'S OBSERVATIONS ON THE CIRCUMSTANCES IN WHICH THE MULKOWAL ACCIDENT TOOK PLACE.

(*Letter of February* 14, 1906, *p.* 49.)

The circumstances of the operation as carried out on the occasion in question were directly conducive to an accident; and the latter was rendered possible by the technique prescribed to the Punjab operators, which substituted a process of momentary submersion ("dipping") in carbolic lotion for sterilisation by boiling and burning as prescribed by the Laboratory.

Tetanus germs are derived in Nature from the soil and from manure of herbivorus animals, such as cattle and horses. The two schools of writers on the subject, the *telurists* and *equinists*, are only divided on the point as to whether the soil primarily infects forage and the intestinal tract of animals, or animal manure infects the soil. A culture of tetanus goes on, however, in both these media. The presence of tetanus germs has been detected in a certain number of instances also outside their breeding ground; but under the effect of light and oxygen, *e.g.*, when carried with dust into the air, pathogenic germs rapidly lose their specific properties; and one has to go to the particular medium or animal in which a microbe has its appropriated habitat to find it in Nature. Thus, glanders is practically never contracted outside stables, or without contact with diseased animals; anthrax, outside wool and hide factories; the risks of tetanus, in common life, are only thought of when, in a riding, bicycle, or motor-car, accident, a wound is inflicted soiled with earth or stable manure; and a surgeon thinks of these risks when he is obliged to operate in the open, or in a place exposed to gusts and to freshly raised earth particles.

The technique prescribed by the Laboratory for guarding against accidents was contained in a set of printed instructions enclosed in each box of prophylactic bottles. The portion indicating how the latter must be opened without contaminating their contents was recapitulated again upon a strip of coloured paper, pasted around each bottle in such a way as to render it necessary for the operator to deal with that strip before getting at the stopper. I think it desirable to reproduce here the corresponding part of these instructions.

According to these the hypodermic needles are to be sterilised either together with the syringe, after being fixed on the body of that instrument, notably by means of hot oil, as recommended by the Government of India; or else separately from the syringe, by boiling in a cup of water or in a test-tube for ten minutes before use. The instructions prescribe that this "procedure is to be repeated every time a needle happens to fall on the ground during the operations, as the ground dust contains dangerous micro-organisms. After boiling, the needle is not to be touched with the fingers, but taken out with a pair of forceps, the points of which have just been heated in a lamp.

"Dissecting forceps are to be used for removing the stopper. The branches of the forceps are heated in the flame of a spirit lamp immediately before use, and guarded from contact with any unsterilised object afterwards.

"The stopper and neck of the bottle are then passed several times through the flame of a lamp, so as to cause very slight singeing of the stopper; and the latter is withdrawn with the heated forceps. After the bottle is opened any contact between its mouth and other, unsterilised, objects is to be carefully avoided; and if contact inadvertently occurs, the mouth is to be heated again in the flame to disinfect it. The contents, or part thereof, to be then absorbed into the syringe. The bottle is kept as nearly horizontal as possible during the whole time the cork is out. During the time the stopper is withdrawn from the bottle it should be held in the sterilised forceps, and care taken not to let it touch the table or other unsterilised object. It may, however, be deposited in a dish of carbolic solution, if assistance is not to be had, until replaced in the bottle."

The *Punjab Plague Manual, 1902*, issued to the operators shortly before commencing operations, and regarding which I was not consulted, entrusted the handling of the fluid to Indian compounders and partly to villagers; and substituted for the above Laboratory instructions, notably for that portion which pertained to the manipulations entrusted to these assistants, the following simplification, in which, as stated, dipping in carbolic is prescribed in place of sterilisation by burning:—

INSTRUCTIONS REFERRING TO THE INOCULATOR.

"Having arranged his apparatus, the inoculator sterilises all the needles he intends to use by dropping them into oil heated to 160° C.

"After immersing his needles in the heated oil, the operator should place them in carbolic lotion until required for use."

INSTRUCTIONS REFERRING TO THE COMPOUNDER OR VILLAGER.

"The compounder in proceeding to open a bottle must shake it well, then dip its neck into carbolic lotion, 1 in 20, and then with a pair of dissecting forceps, which, when not in use, are to be kept in the carbolic lotion, remove the cork, holding the bottle as horizontally as possible without spilling its contents; the cork is to be kept in carbolic lotion while the syringe is being filled, and is to be replaced immediately after. If there is great stress of work, the duty of actually opening the bottles may be entrusted to any intelligent person, care being taken to see that he observes the above instructions carefully." (*Report on Plague and Inoculation in the Punjab from October 1st, 1902, to September 30th, 1903*, by Major E. Wilkinson, I.M.S.; p. 32.)

Unfortunately, while microbial germs and their spores are devitalised by heat long before organic matter, which constitutes their substance, begins to get singed, not alone dipping in carbolic of 1 in 20, but a ten hours' submersion in that antiseptic has been shown to leave the spores the faculty of causing tetanus when injected into animals.

The technique of the *Manual* appears to have been from the first corrected in actual practice by many of the inoculators, in some cases in essential, in others in less essential details. In certain instances, however, even the *Manual's* prescriptions were considered superfluous (*vide* the Punjab inoculators' evidence, Sir L. Jenkins's Commission).

The circumstances in which the inoculations were done at Mulkowal have been described by Dr. Elliot, the inoculating officer, and his assistant in the following words :—

Dr. ELLIOT'S evidence: "I did my work in the open, under trees, on the outskirt; it was surrounded by houses on three sides; it was about 30 feet by 40 feet, the backs of the houses looked over it; there were cattle on the other side. There were no animals excepting my horse, which was being walked about . . . The square was hard ground. I do not remember seeing droppings of animals. My bottles and instruments were placed on a table brought over from my camp. By the time I arrived everything was ready; this was done by the compounder. When the compounder opened the bottle he handed it to me to smell. I am sure it was the compounder, and not a villager; a villager has never assisted me to open a bottle; I would not trust him; I would not trust the compounder without his being under my eye."

When Dr. Elliot was opening a bottle personally, he, in accordance with the prescriptions of the *Manual*, "did not heat the neck of the bottle before opening; he placed it in carbolic solution."

The compounder, on his part, stated: "I opened it according to the instructions in the *Manual*. I took off the label with a forceps; I always did so. I shook the bottle and put the neck into carbolic lotion. I then took out the cork with the forceps."

While he thus followed the *Manual's* technique and was entrusted with the handling of the fluid (chapter iv., paragraph 32 [8] of the *Manual*: "The opening of the bottles of prophylactic and the filling of syringes are the chief duties of the compounder"), he showed his concern about the dangers of soil contamination in general, and described the occurrences at Mulkowal in the following words :—

"I noticed loose corks—Dr. Elliot used to see them.[1] The prophylactic in these bottles was used. The cork at Mulkowal was not loose. I remember it was a very tight cork, and the forceps fell out of my hand on to the ground. I cannot say whether Dr. Elliot saw it. I swished the forceps in the lotion and then pulled out the cork with it. I never mentioned it to any one before. The circumstance was recalled when I asked whether the cork was tight. I am certain it happened to the first bottle at Mulkowal. I never gave it a thought before. I am certain I dropped the forceps; the forceps also fell on the ground in Gujarat itself; it was at the fourth bottle. The forceps has fallen down on many other occasions.[2] I do not remember having been asked whether the cork was dropped. The cork used to fall very often; and it was put in the antiseptic. I did not drop it at Mulkowal. Forceps may have fallen at other places besides Mulkowal and Gujarat, but I have no recollection.[3] I do not know any one alive who saw the forceps drop.[4] The cork had begun to move when the forceps dropped."

. . . . Besides the Mulkowal accident, and prior to it, 10 instances of fatalities following close upon inoculation, and one of which was a death from tetanus, as well as a certain number of minor accidents, notably abscesses, attended the Punjab inoculations of that month. . . . These fatalities occurred in single individuals inoculated from different bottles of prophylactic. In none of the cases did any other person inoculated from the same bottle suffer. . . . All these cases took place prior to the revision of the inoculation technique; and no fatality coincident with the injection, and at the same time standing in no possible connection with the kind and quality of fluid supplied from Bombay, came to light in the subsequent 370,000 inoculations of that season.

REPORT OF THE INDIAN COMMISSION OF ENQUIRY ON CERTAIN INOCULATION CASES WHICH PRECEDED THE MULKOWAL ACCIDENT, AND ON THE ORIGIN OF THE LATTER ACCIDENT.

"In the letter of reference it is said: 'From the information before the Government of India, the first intimation of danger appears to have come from the Laboratory itself, the authorities of which telegraphed to recall a consignment of fluid sent to Ludhiana. During the next few days the contents of numerous bottles were discovered by operators in several different districts to be putrid, and at least four deaths and several instances of abscesses occurred after inoculation in circumstances which gave rise to grave suspicion that they were due to the employment of contaminated fluid. In one instance, at least, an inoculated person died of tetanus.' As this passage is relevant to the question of contamination, we deal with it at this stage: (*a*) In the first place the consignment of fluid to Ludhiana was recalled merely because it was found by the clerical staff that there were two brews of the same number. (*b*) Judging from the practical tests, to which inoculating officers were subjected in our presence, their pronouncement as to putridity must be accepted with considerable reserve. (*c*) It was admitted by the Punjab officers, and seems to be the case, that the four deaths and several instances of abscesses, to which the letter alludes as giving rise to grave suspicion that they were due to the employment of contaminated fluid, could not be attributed to that suggested cause. There no doubt have been several instances of abscesses beyond those to which the letter alludes, but from the facts before us it is impossible to connect them with the fluid rather than with defective inoculation. . . . . (*d*) We are satisfied that the isolated case of death from tetanus cannot be ascribed to contaminated fluid.

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[1] Dr. Elliot judged by the "restriction on the cork" that a certain cork "had been prised out." The restriction was seen when a cork from a bottle with a larger neck was used, on a subsequent occasion in one with a narrower neck.

[2] The details of this reply were as follows: On his having made the statement above, the Commission asked him whether he was aware of the gravity of what he was saying, and knew that by having picked up the forceps from the ground and used it in the way he did, he might have been the cause of the death of nineteen people. To this he replied: "Why should I be the cause of the death of these people? The forceps fell on many other occasions and nobody died."

[3] The Commission pressed the witness to substantiate his statement by giving the names of the villages and the number in the order of bottles at which mishaps occurred, and he replied that these particulars he could not recall (not that he could not recall whether forceps had fallen at other places).

[4] He said the lambardar (head villager) who assisted him at Mulkowal was dead, and he did not know who else might have seen the forceps drop.

As a prelude to our discussion of the thirteenth and fourteenth issues[1] we will briefly state the salient and undisputed facts relating to the death of the nineteen persons after inoculation at Mulkowal. They were the first to be inoculated on the morning of October 30th and the only persons inoculated from a bottle marked 53 N. The operation was performed by Dr. Elliot, his assistant being the compounder Narindar Singh: in all tetanus symptoms were manifested on November 4th or 5th; and all died between the 6th and 9th inclusive. There is a conflict of evidence on the point, but after careful consideration we have come to the conclusion that the nineteen persons were all inoculated with one and the same syringe.

The two broad alternatives with regard to the seat of the specific contamination are that it either was in the prophylactic fluid, or it was not; for, notwithstanding the form of the thirteenth issue, the second of these two hypotheses cannot be regarded as outside theoretical possibility. If not in the fluid the contamination may have been (*a*) on the needle, (*b*) on the arms of the persons inoculated, or (*c*) introduced in the process of washing those arms. In support of this alternative instances were cited in which a number of successive persons or animals are reported to have been affected with tetanus by the repeated use of the same instrument. An objection common to all these suggestions is the precise concurrence of the incidence of the attack with the use of the fluid of this particular bottle. Thus to deal first with the theory that the needle may have been the seat of the mischief, it is a remarkable circumstance that the contamination should have survived only so long as bottle 53 N was in use, and then have ceased to manifest itself. To proceed with the needle, it was placed in smoking-hot oil before operations commenced; this should in the ordinary course have sufficed to completely sterilise it. Then Lieutenant-Colonel Semple has made an experiment in which a similar needle dipped in a tetanus culture, and used immediately afterwards on a guinea-pig, failed to impart tetanus; this shows that a contaminated needle would not necessarily convey tetanus in all cases. The contamination could not have been transferred from some infected material to the needle between the several inoculations, as the evidence establishes no such material was ever used. We think the possibility of the mischief having been on the arms of the nineteen persons, or imparted by the material with which the arms were washed, too remote to be accepted in the circumstances of the case. We have dealt with these hypotheses as fair samples of what might be advanced in support of the alternative that the mischief was not in the fluid, and not as an exhaustive catalogue of possibilities; but we regard them as so typical as to render further enumeration needless. There is also the important circumstance that on the examination of the bottle at Kasauli it was found to contain tetanus bacilli, and though not absolutely conclusive on the point, we have always regarded this fact as strongly suggestive of the inference that the contamination was in the fluid. We are now confirmed in this view by the experiments set out in Appendix B (bottle No. 4, Experiments 7 and 8)[2] which establish that, though there were tetanus germs in the ½ cc. of the fluid left in the bottle No. 4, the conditions in it when thus emptied did not permit of their growth, and this points to the conclusion that the bacilli found by Lieutenant-Colonel Semple in the Mulkowal bottle were the result of a growth that had taken place in the fluid.

If the matter had rested here, we should have regarded it as the only reasonable inference, that at some time or other the specific contamination had been introduced into the fluid. But the evidence shows that this same syringe was used after an interval of a few minutes and that the persons inoculated by its means from another bottle are not known to have manifested any symptoms of tetanus. On this fact has been founded the argument that the freedom from tetanus of those subsequently inoculated is inconsistent with the theory that the specific contamination could ever have entered the syringe[1]; for if so contaminated, it is urged, its sterilisation could not have been completed by being merely rinsed out with carbolic acid of a strength of 1 in 20. The experiments that have been made by Lieutenant-Colonel Semple, appended to this Report, show, we think, that the argument is so far well founded, that, where a fluid containing a culture of tetanus is used, the syringe, even after it has been washed out with a 1 in 20 solution of carbolic acid, may retain enough contamination to affect fluid with which it is subsequently filled. The extent, however, of its influence varies according to the richness of the growth between fatal results and absence of all manifestations. There are no means of forming an opinion as to the precise richness of the growth in the bottle 53 N beyond Lieutenant-Colonel Semple's view that it was a poor growth in comparison with that which he used in his Experiments Nos. 1 and 2 in Appendix B.[2] This view is based on (1) microscopical specimens, and (2) the rapidity of the fatal effects on guinea-pigs. We, therefore, are unable to accept the argument that the freedom from tetanus of those persons subsequently inoculated shows that the specific contamination could not have entered the syringe, and after full consideration we think the contamination was in the fluid. Mr. Haffkine's experiments, Appendix C,[1] in no way disturb this conclusion.

If the contamination was in the fluid, then it must have found its way there either before or after the bottle was opened at Mulkowal. That it might have effected an entrance at Mulkowal cannot be disputed; thus, by way of illustration, the stopper or the forceps might have been dropped on the ground and applied to the mouth of the bottle with contamination adhering, or spores, settled between the rubber stopper and the rim of the bottle, might have dropped in as the stopper was pulled out.

In favour of the view that the mischief happened at Mulkowal, reliance has been placed on (*a*) failure to observe a smell, (*b*) the absence of explosive noise, or, to adopt the language of one of the witnesses, the fact that the stopper did not come out with a pop, and (*c*) the tardy manifestation of the tetanus symptoms. No doubt the presence of tetanus, at any rate in a pure culture, may be detected by one experienced and on the alert, by its characteristic smell; our personal

[1] Concerning the Mulkowal accident. [2] P. 44.

[1] *Vide* App. C, p. 44. [2] P. 43.

observation, however, leads to the conclusion that all bottles of plague prophylactic have a peculiar and not agreeable smell, so that Dr. Elliot, under the circumstances in which he was working, may well have failed to specialise a tetanus smell. The failure to perceive a smell is undoubtedly a fact that calls for consideration, but we do not regard it as so conclusive in favour of tetanus having been introduced at Mulkowal as to outweigh all other considerations. To the absence of explosive sound we attribute no importance, so that it only remains to consider the value of the inference based on the tardy manifestation of the disease. It is contended that had the contamination been introduced in the laboratory and growing for five weeks, it would have formed toxins, which would have caused a more rapid onset of the symptoms of the disease. It must be conceded that in experiments on animals the rapidity of the onset is influenced by the amount and virulence of the toxin introduced, and *a priori* we see no reason for saying that similar results might not follow the injection of the tetanus microbe with its toxin into the human body. Manifestly there can be no appeal to clinical experience which is based on observations of cases where it must be supposed the microbe alone has been introduced, and has only commenced the elaboration of toxin—the actual cause of the symptoms—after it has established itself in the injured part. Though therefore the ordinary incubation of tetanus in clinical experience in India is a period which substantially corresponds with what was manifested at Mulkowal, it is impossible to say in the absence of experiment what the sequel would be where a ready-formed toxin is injected into the human system. It is therefore clear, accepting all that this train of thought implies, we cannot estimate the exact import of the duration of the incubation in the Mulkowal cases. This concludes the considerations favouring the view that the contamination was introduced at Mulkowal. Against it there is the fact that the specific contamination permeated the whole fluid in the bottle, as is shown by the uniform results on the sufferers, and without a series of suppositions opposed to all reasonable probability, these results can only be attributed to a growth of some duration, though not necessarily of great vigour, a view strongly supported by the experiments mentioned above. We regard this circumstance of such moment as to outbalance the arguments on the other side.

It follows from this that in our opinion the specific contamination was introduced before the bottle was opened at Mulkowal, and we see nothing in the quality of the agency employed, taking it as a whole, which imposes any antecedent improbability in the way of accepting this conclusion. Though our finding does not in form follow the terms of the thirteenth issue, in substance it does; for the possibility of contamination entering the bottle after it was issued, but before it was opened, could only be attributed to defective operations in the laboratory.

How the contamination was introduced cannot be ascertained; we can only endeavour to narrow down the possibilities, and for this purpose it is necessary to state certain relevant facts. The prophylactic contained in the Mulkowal bottle was part of a brew known as 53 N. This brew was inseminated on September 17th, 1902. On September 19th it was examined and emulsified, sterile water being syphoned down aseptically and the film of plague growth washed off the agar surface by shaking the flask. On the same day, Stephen, who then for the first time commenced practical work, decanted by syphoning the contents of the flask into five issuing bottles, at the same time syphoning a sample of the material into an agar test tube. The test tube growth was four days later examined for purity, and found pure, and the five bottles were tied up in a separate bag and sent for sterilisation by heat. On September 23rd one of the five bottles taken as a sample was examined for sterility by insemination into a tube of sterile broth. On the 25th this tube was found to be sterile, and after dosing, the five bottles, were handed over to the despatching department. Four of them were sent out on September 30th, in box 202, to the plague medical officer at Jullunder, and on October 4th the fifth bottle was sent in box 205 to the Superintendent, Central Disinfection Depot, Jullunder. It was this last bottle that was used at Mulkowal, and as a result of the evidence and what we saw at the laboratory, we hold that this was not the sample bottle manipulated by De Souza. We think it improbable that contamination was introduced into the fluid prior to the stage at which it was handed over to the decanting clerk, (1) by reason of the tests which cover that period, one of which was anaerobic as well as aerobic; (2) because the manipulations up to that time had been performed by highly trained bacteriologists; and (3) because, as we have discovered, the remaining bottles of brew 53 N were used without producing tetanus symptoms, or, so far as we could learn, any bad results. For the reasons already stated, the possibility of contamination at the sampling stage may be excluded, so that limiting ourselves to what is reasonably probable the source of contamination is narrowed down to a choice between the following possibilities:—

(*a*) The bottle may have been insufficiently sterilised before it came into the decanter's hands.

(*b*) The decanting may have been performed with defective precautions.

(*c*) The final sterilisation may have loosened the stopper, with the result that the specific contamination entered, either in the cold bath, or afterwards, before the bottle was opened at Mulkowal.

To make an exclusive selection from these possibilities with any show of reason is impossible.

L. Jenkins.
G. Bomford.
D. Semple.

Bombay,
April 16th, 1903.

APPENDICES TO THE INDIAN COMMISSION'S REPORT.

Appendix A.

No. 1,375, *dated March* 6, 1903.

From Major D. Semple, M.D., R.A.M.C., Director Pasteur Institute of India, Kasauli, to the Secretary to the Government of India, Home Department.

I have the honour to forward herewith the results

of a series of experiments carried out by me with the object of throwing more light on several points discussed in the answers of the Commission to issues 13 and 14 in connection with the Mulkowal tetanus cases.

(2) Before leaving Bombay I discussed these experiments (or the general trend of them) with Sir Lawrence Jenkins and Lieutenant-Colonel Bomford, I.M.S., and I am now sending a copy of the results to them.

In order to clear up several points touched upon by the Commission in their report when answering issues 13 and 14 in connection with the specific contamination which gave rise to the Mulkowal tetanus cases, the following experiments were carried out by me at the Pasteur Institute of India, Kasauli, on my return from Bombay.

Experiment 1.—A needle dipped in a tetanus culture, and used immediately afterwards to prick a guinea-pig, failed to give the animal tetanus.

Experiment 2.—A 1 cc. syringe filled with a tetanus culture, then emptied and washed out once with 1 in 20 carbolic acid solution, then filled with sterile broth and the contents inoculated hypodermically into a guinea-pig, failed to give the animal tetanus.

Experiment 3.—A guinea-pig inoculated hypodermically with $\frac{1}{20}$ cc. of a tetanus culture did not contract tetanus.

The result of this experiment does away with the necessity for Experiments 1 and 2, and at the same time proves that a considerable quantity of this particular tetanus is required to infect a susceptible animal.

Experiment 4.—A guinea-pig inoculated hypodermically with $\frac{1}{10}$ cc. of a tetanus culture contracted tetanus on the third day.

Experiment 5.—Tetanus planted in a bottle of water-agar plague prophylactic, and incubated at 37° C. for seven days, grew well, and gave the characteristic smell of tetanus when opened.

There was no explosive noise of any kind when removing the cork from the bottle. Numerous tetanus bacilli were present in stained specimens.

Experiment 6.—Tetanus and micrococci planted in a bottle of water-agar plague prophylactic, and incubated at 37° C. for seven days, grew well, and gave the characteristic smell of tetanus when opened.

There was no explosive sound of any kind when removing the cork from the bottle. Numerous tetanus bacilli and also micrococci were present in stained specimens.

Experiment 7.—Tetanus planted in water-agar plague prophylactic, and incubated under anaerobic conditions for seven days, grew well and gave the characteristic smell of tetanus when opened. Numerous tetanus bacilli were present in stained specimens.

These experiments, with the exception of No. 4, were done on February 19th. Experiment No. 4 was done on February 23rd, because Experiment No. 3 failed to contract tetanus.

The guinea-pigs used were two-thirds grown, healthy animals.

The water-agar plague prophylactic used was obtained from the Plague Research Laboratory, Bombay, and had been returned from the Punjab last November.

The bottles were tested for sterility (including an anaerobic test for tetanus) before planting tetanus into them.

The tetanus used in all cases was a pure culture isolated by me from the Mulkowal bottle (53 N) on November 15th, 1902, when it was sent to Kasauli for examination.

Appendix B.

Further Experiments with Tetanus to Elucidate Certain Points touched upon by the Commission in Connection with the Specific Contamination which gave rise to the Mulkowal Tetanus Case.

Bottle No. 1.—A sterile bottle of water-agar plague prophylactic (capacity 30 cc.; Brew, No. 58 B i.; date October 20th, 1903; Dose, 1·5 cc.) was inoculated on February 19th, 1903, with one platinum loop of a pure broth culture of Mulkowal tetanus bacilli, and also with a non-pathogenic cocco-bacillus. The bottle was then incubated at a temperature of 37° C. for seven days, and examined microscopically on February 26th, 1903, when a rich growth of tetanus germs was found, also other bacilli. The characteristic smell of tetanus was observed, but no explosive noise of any kind, on removing the cork. It was then put aside in a dark cupboard at room temperature (about 18° C.).

Experiment 1. March 31.—Three guinea-pigs received hypodermically from bottle No. 1, $\frac{1}{20}$; $\frac{1}{10}$; and $\frac{3}{10}$ cc. respectively. All three died from tetanus next day; the $\frac{3}{10}$ cc. one at about 8 a.m.; the $\frac{1}{10}$ cc. one at 11 a.m.; and the $\frac{1}{20}$ cc. one at about 3 p.m. This experiment proves that bottle No. 1 contained a rich and virulent growth of tetanus.

Experiment 2.—A sterile 20 cc. syringe was filled once from bottle No. 1, and allowed to remain full for two minutes, after which the contents were slowly expelled through the needle. It was then filled with a 1 in 20 solution of carbolic acid, and after two minutes (without any shaking) this was also slowly expelled through the needle. The syringe was then filled with sterile peptone broth, and 1½ cc. injected hypodermically into nineteen guinea-pigs (a second refilling to the extent of 8½ cc. being necessary to complete the nineteen inoculations of 1½ cc. each).

Results: April 3.—Three show symptoms of tetanus.

April 4.—Ten show symptoms of tetanus.

April 5.—Fifteen show symptoms of tetanus.

April 6.—Seventeen show symptoms of tetanus.

April 15.—Since the 6th, seven have died from tetanus, symptoms slight in the others, which are likely to recover.

Bottle No. 3. April 1.—A large platinum loop from bottle No. 1 (a rich growth of tetanus and other bacilli) was inoculated into a sterile bottle of water-agar plague prophylactic containing 30 cc., and then well shaken up.

Experiment 5. April 1.—Two guinea-pigs received hypodermically 1 cc. and 1½ cc. respectively from bottle No. 3.

Result.—Both contracted tetanus on the fourth day.

Remarks.—This experiment shows that a platinum loop of tetanus from a rich growth in water-agar plague prophylactic is sufficient to permeate a 30 cc. bottle to such an extent that 1 cc. conveys the disease to a guinea-pig.

Bottle No. 4. April 6.—A small piece of thin straw about ¼ cm. square was dipped into bottle No. 1 (a rich growth of tetanus and other bacilli) and allowed to remain in for two minutes, and moved about so as to contaminate it. It was then removed with a sterile needle and allowed to dry in a sterile glass dish at room temperature for one hour, then placed in a sterile bottle of water-agar plague prophylactic containing 30 cc. for five minutes, and well shaken up several times, after which it was removed.

Experiment 6. April 6.—Five guinea-pigs received hypodermically 1 cc. each from bottle No. 4.

Result.—All five animals remained free from tetanus.

Experiment 7. April 6.—The contents of bottle No. 4, with the exception of ½ cc., were poured out, the bottle corked up and placed in the incubator at 37° C.

Result.—No growth of tetanus took place in this bottle.

Experiment 8. April 6.—An anaerobic cultivation was made from the ½ cc. remaining in bottle No. 4.

Result.—Typical growth of tetanus was present on the third day.

Remarks.—Experiments 6, 7 and 8 prove (1) that the amount of tetanus bacilli which adhered to the small piece of straw when dipped in a rich culture of tetanus was sufficient to permeate a 30 cc. bottle of plague prophylactic, but not to such an extent that 1 cc. of its contents would convey the disease to guinea-pigs. (2) That 0·5 cc. of water-agar plague prophylactic thus infected and allowed to remain in a 30 cc. bottle would not admit of a growth of tetanus even when incubated at 37° C. (the most favourable temperature for the growth of this bacillus). The aerobic conditions in the bottle would account for absence of growth.

D. Semple, Lieut.-Col., R.A.M.C.

April 16th, 1903.

Appendix C.

(Demi-official.)

(*No.* 292, *dated February* 8, 1903.)

From W. M. Haffkine, Esq., C.I.E., Director-in-Chief, Plague Research Laboratory, to Major D. Semple, M.D., R.A.M.C., Secretary to the Punjab Inoculation Commission.

I.

I shall be obliged for your kindly examining and submitting to the Commission accompanying specimens.

(1) On the 2nd inst. Kapadia and I placed into a sterile prophylactic bottle containing 30 cc. of sterile broth a minute bit of straw exactly similar to the one now forwarded in a sample tube. (It is surrounded there by a square of red ink). The bit was taken, aseptically, from the interior of a freshly dropped ball of horse dung. It was about 1 mm. in length, ½ mm. in breadth, and of a macroscopically unmeasurable thinness. As soon as the straw was placed in the bottle the latter was shaken lightly (it was plugged with a sterile cotton plug), and a sterile 20 cc. syringe was filled from it. The contents of the syringe was then injected in doses of 1½ cc. into thirteen test tubes containing melted agar, which was allowed to solidify in a sloped position. As soon as the syringe was empty it was refilled with the remaining 10 cc. of broth, and another six agar tubes were treated in the same way. Thus in a few minutes nineteen agar tubes, representing nineteen patients, were injected with sterile material contaminated by the bit of straw above described.

(2) After that I filled the syringe with sterile broth, rinsed it by shaking it, and ejected the contents; filled the syringe with sterile broth again, shook it again, and ejected this too, to imitate Elliot's (*a*) first squirting out a syringeful of carbolic lotion, and (*b*) then filling the syringe with lotion again and emptying it when taking up bottle No. 3.

(3) After that I filled the syringe with sterile broth, and injected the latter in doses of 1½ cc. into thirteen agar tubes. The latter would correspond to the patients inoculated by Elliot from bottle No. 3.

I forward herewith the agar tubes in question. The first series are marked as Nos. 1 to 19 inclusive; the second as Nos. 20 to 32. In the first series, apart from a few larger colonies, there is a number of minute ones in the depth of the agar (up to fifty colonies and above per tube). I am sending in a magnifying glass for their examination. In order to make it certain that these are colonies of microbes, I have cut out, as you will see, a piece of agar from tube No. 1. The piece cut out is in the second of the two sample tubes which I am sending. I made a microscopic slide from one of the suspected colonies, and am sending the slide on. It shows a flourishing mass of stout cocco-bacilli, some in pairs, but mostly single; sometimes in fours.

In the second series of tubes I find, in some of them, certain single points or a couple of points per tube, resembling the minute colonies of the series Nos. 1 to 19. In one there is one colony fairly well developed, apparently of another species. I have not made slides from these tubes yet, but the difference in the number of colonies per tube, assuming that the second series contains real colonies, is great.

II.

(4) You will find in the parcel a third series of agar tubes marked Nos. 33 to 42. These were inseminated as follows: Dr. Gibson prepared a moderately turbid emulsion of the yellow sarcina in sterile broth. I filled a sterile syringe with that emulsion, and gave it to Kapadia to squirt out the contents, at dose of 1½ cc., at an interval of about one minute. When the 20 cc. of the contents were squirted out, 10 cc. more of the same emulsion were introduced and squirted out in the same way, to imitate contamination of a syringe by a fairly rich culture of (tetanus) microbes injected into nineteen patients. After that the syringe was twice rinsed with sterile broth, to imitate "sterilisation" by rinsing. The syringe was then filled with sterile broth and 1½ cc. of the latter

squirted out into each of the agar tubes, Nos. 33 to 42, to correspond to the inoculation of patients with No. 3 Mulkowal bottle. Apart from the larger colonies grown on the surface of the agar, there are numberless small ones in the mass of it.

The result of the above two experiments illustrates, it seems to me, the following line of reasoning: If, at the time of filling the first syringe at Mulkowal, bottle 53 N contained a fairly rich culture of tetanus bacilli, similar to, or even much less rich than, Dr. Gibson's emulsion, rinsing should have left the syringe very badly contaminated, and one would be justified in expecting tetanus cases in those inoculated from bottle No. 3. If, on the other hand, contamination occurred at the time of the operation from, say, a bit of dirt in the needle, or from one dropped in during the opening of the bottle, a number of contaminating microbes would be injected into *each* of the first nineteen patients, and only single microbes, or hardly any, injected into those who had bottle No. 3."

THE REPORT OF THE LISTER INSTITUTE DATED NOVEMBER 24, 1904, AND ADDRESSED TO THE UNDER-SECRETARY OF STATE FOR INDIA.

"(3)[1] PROBABLE ORIGIN OF THE TETANUS VIRUS.

From consideration of the evidence and in the light of experiments (*vide* Appendix F), the Institute agrees with the Commission that in all probability the tetanus was at the time of the inoculation in the fluid contained in the bottle, but the fact that a bottle presumably tightly corked (*vide* Dr. Elliot's evidence) should contain enough tetanus growth to destroy nineteen people, and yet not be accompanied by sufficient smell to arouse the suspicion of Dr. Elliot who, according to his evidence, remembers smelling this particular bottle, is difficult to comprehend.

The Commission expresses itself (Extract, p. 4)[2] as confirmed in the view that the tetanus contamination was in the fluid contained in the bottle 53 N, by the results of Colonel Semple's experiment to show that although there were tetanus organisms in the .5 cc. remaining in the bottle when examined by him, the conditions obtaining were not such as to permit of growth.

We have made experiments to determine whether tetanus might not grow in a nearly empty bottle of water-agar emulsion if associated with the growth of some ordinary saprophytic organisms.

As may be seen from the results (Appendix E, Experiments 1 to 8), although these conditions did not permit of the growth of a pure culture of tetanus, in those cases where it was sown in association with aerobic saprophytic organisms, growth occurred. We think, therefore, that Colonel Semple's experiments cannot be held to exclude the possibility of contamination at the time of the opening of the bottle.

Apart from the circumstances mentioned above, we do not think that any other evidence discussed in the Commission's report and including Mr. Haffkine's experiments, seriously militate against this view of the Commission. The precise concurrence of the incidence of the attack with the use of the one particular bottle of vaccine, appears to us as it did to the Commission, to suggest in the strongest way that the tetanus was derived from the bottle, and the argument that the specific contamination never entered the syringe,[1] founded on the fact that those subsequently inoculated escaped from tetanus, is, we consider, unsound. Whether the inoculation of a fluid containing tetanus organisms into the body of an animal is or is not followed by the disease depends, as was shown by Semple, upon the number of organisms introduced under the particular circumstances.

Our own experiments (Appendix F (*a*), Experiments 1 and 2), show that $\frac{1}{100000}$ of the quantity of a tetanus culture grown under imperfect anaerobic conditions which is required to kill[2] a guinea-pig, could be detected by cultural methods.

An ordinary 20 cc. syringe leaves about $\frac{1}{8}$ cc. of content unexpelled at the end of the stroke of the piston. Assuming $\frac{1}{4}$ cc. to have been the dead space in the syringe used by Dr. Elliot, and that the growth was a fairly uniform one, as must be supposed from the similarity and fatal issue of the disease in the nineteen cases, the individuals inoculated from a fresh bottle after the rinsing of the syringe with carbolic solution, would have only received $\frac{1}{32000}$ of the dose of the tetanus virus injected into the persons who were inoculated from the contaminated bottle.

With regard to Mr. Haffkine's experiments (Appendix C of Extracts),[1] we would remark that because a high dilution of a culture of a saprophytic organism still gives growth when sown upon an artificial nutrient medium,[3] it does not follow that a similar dilution of tetanus will give rise to the disease when inoculated into a man possessing a considerable capacity of resistance to invasion.

Although of opinion that the evidence points strongly to the infection being in the bottle at the time of the inoculation, we agree with the Commission that it is quite impossible to determine at what stage in its history and in what way bottle 53 N became contaminated. . . . .

(4) The conclusions of the Institute coincide with those of the Commission, that in all probability the tetanus was at the time of the inoculation in the fluid contained in the bottle, but that it is impossible to determine at what stage in its history or in what way bottle 53 N became contaminated.

CHARLES J. MARTIN, *Director*."

APPENDICES TO THE REPORT OF THE LISTER INSTITUTE.

"D.—*Experiments to show that water-agar is a suitable medium for the growth of the tetanus bacillus.*

Experiment 1.—Two bottles of 30 cc. capacity were filled with water-agar prophylactic fluid. One was inoculated from a pure culture on glucose broth of *B. tetani*; the other with a mixed growth of *B. tetani* and *B. subtilis*. They were stoppered with rubber corks and placed at 37° C. In three days both bottles had a growth. Both bottles had about equally the characteristic odour of tetanus. Microscopical examination revealed tetanus bacilli.

Experiment 2.—A bottle was half filled with water-

[1] Sections 1 and 2, and Appendices A, B and C, deal with subjects other than the Mulkowal case.
[2] P. 41.

[1] Comp. App. C., Ind. Com.'s report, p. 44.
[2] *Vide* dose required for manifestation of symptoms, p. 47.
[3] Comp. Ind. Com.'s rep., App. B, exp. 2, p. 43.

agar prophylactic fluid and inoculated with *B. tetani* and *B. subtilis*.

In three days a growth with characteristic odour. Microscopically, tetanus bacilli.

These experiments show that the tetanus bacillus grows on water-agar fluid either alone or in the presence of an aerobic organism such as the *B. subtilis*, and that a full bottle is not necessary to obtain the growth.

E.—*Experiments on the growth of tetanus in bottles containing small quantities of water-agar emulsion.*

Experiments 1 *to* 8.—To ascertain whether the tetanus bacillus is capable of growing in a small quantity of water-agar prophylactic emulsion in corked bottles of 30 cc. capacity. The series consisted of eight bottles of 30 cc. capacity. Each had 1 cc. of water-agar emulsion measured into it. A four days old glucose broth culture of *B. tetani* was employed as the inoculating material.

Experiment 1.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of undiluted culture. A sub-culture was then made with the same loop on sulphindigotate broth. A microscopical examination was made immediately after the inoculation. Several microscopical fields showed no bacilli. The sub-culture made before corking showed no growth after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C.

After four days in the incubator the bottle was opened. It had no tetanus smell. Microscopical examination showed no tetanus bacilli. Sub-cultures did not grow.

Experiment 2.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of culture diluted to 1 in 10 in neutral broth. A sub-culture was then made with the same loop on sulphindigotate broth. The sub-culture made before corking showed no growth after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C. After four days in the incubator the bottle was opened. It had no tetanus smell. Microscopical examination showed no tetanus bacilli. Sub-cultures did not grow.

Experiment 3.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of culture diluted to 1 in 100 in neutral broth. A sub-culture was then made with the same loop on sulphindigotate broth. The sub-culture made before corking showed no growth after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C. After four days in the incubator the bottle was opened. It had no tetanus smell. Microscopical examination showed no tetanus bacilli. Sub-cultures did not grow.

Experiment 4.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of culture diluted to 1 in 1,000 in neutral broth. A sub-culture was then made with the same loop on sulphindigotate broth. The sub-culture made before corking showed no growth after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C. After four days in the incubator the bottle was opened. It had no tetanus smell. Microscopical examination showed no tetanus bacilli. Sub-cultures did not grow.

Experiment 5.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of undiluted culture. It was then inoculated with the Hay bacillus and a *Staphylococcus aureus*. A sub-culture was then made with the same loop on sulphindigotate broth. A microscopical examination was then made immediately after the inoculation. Several microscopical fields showed no tetanus bacilli. The sub-culture made before corking showed no growth of tetanus bacilli after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C. After four days in the incubator the bottle was opened. It had a marked tetanus smell. Microscopical examination showed numerous typical tetanus bacilli. The sub-culture gave a growth of the tetanus bacillus.

Experiment 6.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of culture diluted to 1 in 10 in neutral broth. It was then inoculated with the Hay bacillus and a *S. aureus*. A sub-culture was then made with the same loop on sulphindigotate broth. The sub-culture made before corking showed no growth of the tetanus bacillus after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C. After four days in the incubator the bottle was opened. It had a marked tetanus smell. Microscopical examination showed numerous typical tetanus bacilli. The sub-culture gave a growth of the tetanus bacillus.

Experiment 7.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of culture diluted to 1 in 100 in neutral broth. It was then inoculated with the Hay bacillus and a *S. aureus*. A sub-culture was then made with the same loop on sulphindigotate broth. The sub-culture made before corking showed no growth of the tetanus bacillus after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C. After four days in the incubator the bottle was opened. It had a marked tetanus smell. Microscopical examination showed numerous typical tetanus bacilli. The sub-culture gave a growth of the tetanus bacillus.

Experiment 8.—A bottle containing 1 cc. of water-agar emulsion was inoculated with a 2 mgm. loopful of culture diluted to 1 in 1,000 in neutral broth. It was then inoculated with the Hay bacillus and a *S. aureus*. A sub-culture was then made with the same loop on sulphindigotate broth. The sub-culture made before corking showed no growth after four days in the incubator under anaerobic conditions.

The bottle was corked with a rubber cork and incubated at 37° C. After four days in the incubator the bottle was opened. It had a marked tetanus

smell. Microscopical examination showed numerous typical tetanus bacilli. The sub-culture gave a growth of the tetanus bacillus.

This series of experiments, Nos. 1 to 8, shows that growth of the tetanus bacillus can take place in a corked bottle containing a very small quantity of a water-agar prophylactic emulsion if other aerobic organisms such as *B. subtilis* and *Staphylococcus aureus* are associated with it.

F (*a*).—*Experiments to show the relation between the quantity of tetanus organism necessary to kill to the amount capable of giving a growth on artificial media.*

Experiment 1.—Dilutions were made of a culture grown under imperfect anaerobic conditions (for seven days in sulphindigotate of soda broth) such as would exist in a bottle of vaccine. This accounts for the low toxicity[1] of the culture, for the same bacillus was that employed in Experiments F (*b*) 2 and 7, where an average toxin formation occurred.

The dilutions made were: —

1 in 10	Growth.
1 in 100	,,
1 in 1,000	,,
1 in 10,000	,,
1 in 100,000	,,
1 in 1,000,000	,,
1 in 10,000,000	No growth.

Cultivation made with these dilutions showed growth of the tetanus bacillus up to 1 in 1,000,000.

Experiment 2.—The same quantities (?) as used in the previous experiment for cultures, and from the same dilutions, were mixed with 1 cc. of Haffkine's fluid and injected into guinea-pigs of about 400 grams weight.

Guinea-pig	
No. 1.—	1/10 cc. plus 1 cc. Haffkine's prophylactic; death, sixth day; tetanus.
No. 2.—	1/100: Slight local tetanus.
No. 3.—	1/1,000 ,, ,, ,,
No. 4.—	1/10,000: No tetanus.
No. 5.—	1/100,000 ,,
No. 6.—	1/1,000,000 ,,
No. 7.—	1/10,000,000 ,,

Experiments 1 and 2 F show that $\frac{1}{100000}$ of the quantity required to kill a guinea-pig could be detected by cultivation methods.

F (*b*).—*Experiments to show the pathogenic characteristics of the bacillus used.*

Experiment 1.—To show the toxicity of a filtrate from the culture employed in these experiments on mice. In this case the bouillon medium was deprived of free oxygen by the passage of hydrogen through it, *i.e.*, the anaerobic conditions were good.

Mouse	
No. 1.—	1/1,000 cc.: Dead of tetanus on the third day.
No. 2.—	1/2,000 ,, ,, ,, ,, fifth ,,
No. 3.—	1/4,000 ,, ,, ,, ,, seventh ,,
No. 4.—	1/8,000: Lived; no tetanus.

Experiment 2.—To show the toxicity of a filtrate from the culture used in the experiments on guinea-pigs under similar conditions to previous experiment.

Guinea-pig	
No. 1.—	1/100 cc.: Dead on the fourth day of tetanus.
No. 2.—	1/200 ,, ,, ,, fourth ,, ,,
No. 3.—	1/300 ,, ,, ,, fifth ,, ,,
No. 4.—	1/400 ,, ,, ,, sixth ,, ,,
No. 5.—	1/500 ,, ,, ,, seventh ,, ,,
No. 6.—	1/600 cc.: Recovered.

Experiment 3.—To show that tetanus spores alone will not produce tetanus.

A month old broth culture was heated to 80° C. for one hour to destroy all or nearly all the toxin.

A guinea-pig received subcutaneously 1 cc. of this. No tetanus.

Experiment 4.—To show that tetanus spores and 1 cc. of Haffkine's fluid may not in certain doses be enough to produce tetanus.

A guinea-pig received subcutaneously 1 cc. of the above heated culture plus 1 cc. of Haffkine's fluid. No tetanus.

Experiments made from the heated culture gave abundant growth of tetanus in four days.

Experiment 6.[1]—A month old culture of the same batch as that used in the previous experiment had all the supernatant fluid filtered off from the bacillary deposit. It was made up to its original volume with fresh broth. A series of guinea-pigs were inoculated with quantities of this and 1 cc. of Haffkine's fluid.

Guinea-pig No.	Haffkine's Fluid.	
1.—	1 cc. plus 1 cc.:	Slight tetanus, recovery.
2.—	1/10 ,, ,, ,,	,, ,, ,,
3.—	1/100 ,, ,, ,,	,, ,, ,,
4.—	1/1,000 ,, ,, ,,	No tetanus.
5.—	1/10,000 ,, ,, ,,	,,
6.—	1/100,000 ,, ,, ,,	,,
7.—	1/1,000,000 ,, ,, ,,	,,

This experiment shows that tetanus spores and Haffkine's fluid may not when injected together in certain doses produce tetanus.

Experiment 7.—An emulsion of sporing tetanus bacilli, which had been grown on agar, was made with sterile broth, a piece of straw, 3 by 10 mm. in length, which was picked up outside the laboratory, was then soaked in this emulsion, placed in a sterile tube and allowed to dry in the 37° C. incubator for one hundred and twenty minutes. This piece of straw was then added to 21 cc. of carbolised Haffkine's fluid, well shaken for two or three minutes, and the following mice injected with ½ cc. of this contaminated Haffkine:—

Mouse No.	
10.	Alive fourteen days later.
11.	(Not whole of ½ cc.) found dead day after.
12.	Found dead.
13.	Died three days later; no sign of tetanus.
14.	Alive fourteen days after.
15.	,, ,,
16.	,, ,,
17.	,, ,,
18.	,, ,,
19.	Not inoculated; found dead the following day.
20.	Died two days later; no sign of tetanus.

The experiment shows that a series of susceptible

[1] Comp. (4), p. 54.

[1] Experiment 5 is omitted in the original Report of the Institute.

animals inoculated with Haffkine's fluid, which has been seriously contaminated immediately before injection with sporing tetanus do not necessarily all contract tetanus. Of the animals that died none showed the symptoms of tetanus[1] which are so characteristic in the mouse. Of eleven mice in the series five died, but two of these had not been inoculated. A number of cases of mouse diphtheria occurred at this time.

G.—*Experiments with a syringe contaminated with tetanus.*

Experiment 1.—A syringe of 20 cc. capacity was filled with an eight days old broth culture of *B. tetani*. The contents were syringed out gently in quantities of 1cc. Cultures were made from the first and last cc. of the twenty. The syringe was then filled with 1 in 20 carbolic acid, which remained in the syringe for four minutes. The carbolic solution was then ejected and the syringe filled with Haffkine's fluid. This was injected by separate cc. into a series of sulphindigotate broth tubes. All the tubes were put under anaerobic conditions at 37° C. In four days the tubes inoculated during the first syringing before the carbolic solution was drawn into the syringe all gave good growth. The series of tubes inoculated after the carbolic solution showed a certain irregularity of growth. 1, 5, 6, 8, 9, 12, and 16 showed growth; 2, 18 and 20 showed no growth.

The numbers refer to the sequence of quantities of 1 cc. each expelled from the syringe; the numbers missing are those which were discarded and not used to inoculate tubes of media.

Experiment 2.—The needle of a syringe of 20 cc. capacity was dipped in a sporing broth culture of tetanus and allowed to dry for three hours in the incubator. It was filled with Haffkine's prophylactic fluid and successive doses of 1 cc. ejected. Some of these were injected into guinea-pigs and others into test tubes, as shown in the schedule:—

1st	cc. into	guinea-pig	...	Slight tetanus.
2nd	,,	test tube	...	Growth tetanus.
3rd	—	—	...	—
4th	,,	guinea-pig	...	Slight tetanus.
5th	,,	test tube	...	Growth tetanus.
6th	—	—	...	—
7th	—	—	...	—
8th	,,	guinea-pig	...	Well.
9th	,,	test tube	...	—
10th	—	—	...	—
11th	—	—	...	—
12th	,,	guinea-pig	...	Well.
13th	—	—	...	—
14th	—	—	...	—
15th	—	—	...	—
16th	,,	guinea-pig	...	Well.
17th	,,	test tube	...	Tetanus growth.
18th	—	—	...	—
19th	—	—	...	—
20th	,,	test tube	...	No tetanus.

The syringe was then filled with carbolic solution 1 in 20 for three minutes, which was then ejected, and the syringe was again filled with Haffkine's prophylactic fluid. This was ejected in the same way into tubes and guinea-pigs.

(*a*)	Guinea-pig	...	No tetanus.
(*b*)	Test tube	...	No growth of tetanus.
(*c*)	—	...	—
(*d*)	Guinea-pig	...	Well.
(*e*)	Test tube	...	No growth.
(*f*)	—	...	—
(*g*)	—	...	—
(*h*)	Guinea-pig	...	—
(*i*)	Test tube	...	—

This experiment shows that a seriously contaminated needle (dried) under the test conditions does not give uniform infective results, either before or after the use of the carbolic solution. The infective material tends to disappear at the end of the injection of the first syringeful, and has disappeared after the carbolic washing."

———

PRESS COMMUNIQUE ISSUED BY THE GOVERNMENT OF INDIA IN CALCUTTA ON DECEMBER 13, 1904.

"At the Health Exhibition organised by the Bombay Sanitary Association, under the authority of the Local Government in connection with the Industrial and Agricultural Exhibition about to be held in Bombay, a series of demonstrations will be given by the Director of the Plague Research Laboratory of the method by which the fluid used for inoculation against plague is now being manufactured.

The primary object of these demonstrations is to enable the public to ascertain by personal observation and by enquiry from the experts in charge of the manufacture the precise nature of the precautions which are now taken to guard against the contamination of the fluid by disease-bearing germs. The Director has been instructed to give full explanations of the process to any one who is interested in the subject.

The system of inoculation against plague with a fluid prepared from the sterilised virus of the disease was introduced into India by Mr. Haffkine early in 1897.

. . . . .

On November 6th, 1902, nineteen persons who had been inoculated on October 30th in the village of Mulkowal from a single bottle (labelled 53 N) of the new fluid were found to be suffering from tetanus and all of them subsequently died, and a Commission consisting of Sir Lawrence Jenkins, K.C.I.E., Chief Justice of Bombay, Lieutenant-Colonel Bomford, M.D., C.I.E., Principal of Medical College, Calcutta, and Major Semple, M.D., R.A.M.C., Director of the Pasteur Institute at Kasauli, was appointed by the Government of India to enquire into the disaster. They found that the germ of tetanus had been introduced into the fluid before the bottle was opened at Mulkowal, and they thought it probable that this might have occurred owing either to insufficient sterilisation or to the process of filling the bottle from a larger flask having been performed with defective precautions. . . . .

Experiments undertaken in India by two independent enquirers appeared to confirm this view, and their conclusions, together with the data on which they were based, were submitted with the report of the

[1] Comp. remark under *Exp.* 1, F. (a), p. 47.

Commission for examination and for a further experiment to the Lister Institute in London. . . . . Their report on these points was received by last mail, and they summarise their conclusions as follows :—

The conclusions of the Institute coincide with those of the Commission that in all probability tetanus was at the time of inoculation in the fluid contained in the bottle, but that it is impossible to determine at what stage in its history or in what way the bottle (53 N) became contaminated.

* * * * *

(Signed) W. S. Marris,
Deputy-Secretary to the Government of India.
Home Department (Sanitary),
Calcutta, *December* 13th, 1904."

LETTER FROM THE LISTER INSTITUTE, DATED NOVEMBER 9, 1905.

Vide p. 34.

MR. HAFFKINE'S OBSERVATIONS ON THE REPORTS OF THE INDIAN COMMISSION AND THE LISTER INSTITUTE.

Dated Paris, February 14, 1906.

From W. M. Haffkine, Esq., C.I.E.

To the Under-Secretary of State for India.

Sir,—I have the honour to submit the following remarks on the Mulkowal tetanus accident, and the reports thereon by Sir L. Jenkins's Investigation Commission and the Lister Institute.

The Commission's enquiry brought to light certain facts which pointed to the conclusion that a bottle contaminated with tetanus germs, at, or very shortly before, the time of its use, was the cause of the accident. The details of the operation itself, at the time of opening the bottle, were further found to have been directly conducive to such an accident.

The Investigation Committee subordinated these facts to an *a priori* thesis according to which contamination of the bottle at Mulkowal could not have affected nineteen persons in the way it did. I make some remarks on this thesis in the appendix to this letter under (1*a*) and (1*b*), pages 52 to 54 below.

Surgeons are aware that man is extraordinarily susceptible to tetanus; that to cause an accident an invisible impurity, on the cleanest instrument, may be sufficient; and that to obviate such accidents efficacious processes of sterilisation must be resorted to.

Similarly, a multiple infection with tetanus germs from an insignificant impurity, or derived from one and the same apparently clean instrument, can be realised experimentally.

The Lister Institute has, therefore, rightly reported, in their letter of November last (1905) (p. 34) that the possibility of contamination having effected an entrance into the fluid at Mulkowal could not be excluded.

This proposition sets aside the thesis which the Indian Commission had opposed to the facts above referred to, and upon which they condemned the laboratory.

I recapitulate here the facts in question, and believe it useful to state my remarks with a certain amount of detail; an abbreviated summary of them is, however, given on pp. 51 and 52 below.

On p. 52 are mentioned the circumstances of the Mulkowal operations.

Certain essential points, which I consider by themselves, are referred to, in the form of an Appendix, on pp. 52 to 55.

On pp. 55 to 59 I examine the Indian Commission's Report,

And on pp. 59 to 61 that of the Lister Institute.

I.

THE FACTS OF THE CASE.

A.

The first and the most direct indication regarding the origin of the Mulkowal tetanus germs has been referred to in my letter to you of December 6th last. At the temperature of the Indian climate, when left for a day or two in the plague prophylactic, these germs develop a pungent, disagreeable smell, which becomes the more accentuated as the culture gets more matured. I refer to this subject in the Appendix under (2) (p. 54).

The Mulkowal bottle, which had been prepared forty-one days, and despatched from Bombay twenty-six days, prior to its opening had, when opened, no odour of any description, but acquired the tetanus smell subsequent to that moment. The matter is dealt with in detail in the Appendix under (3) (p. 54). The bottle, therefore, had not cultivated tetanus germs even for a few days prior to its opening.

The above refers to the only, but at the same time precise, method by which the origin of the tetanus germs could be indicated by an examination made at the time of the operations.

The facts elucidated subsequently and mentioned below have all proved the correctness of that examination, as well as the straightforwardness exhibited by the Mulkowal operators in their depositions. To some of these facts I drew the Commission's attention in Bombay; others I was only able to mention hypothetically, as my information at that time was incomplete; others, again, appeared subsequently, in the course of laboratory experiments made by Sir L. Jenkins's Commission, the Lister Institute, and by myself. I quote in the lines below only the testimony derived from the experiments of the Commission and the Institute.

B.

While most of the microbes around us (the so-called saprophytic or harmless germs) require air for their propagation, the activity of the germ of tetanus is, on the contrary, inhibited by the oxygen of the air. The result of this particuliarity is that there exists a fundamental difference between the behaviour of tetanus germs which are corked up in a bottle full of

fluid, and of those that may find themselves in a few drops left in a spacious receptacle full of air.

It has been found that in the "water-agar" plague prophylactic tetanus germs give a particularly abundant growth, and that in a bottle filled in the ordinary way, up to the top, and corked, a rich and very active culture develops in a few days.

Lieutenant-Colonel Semple, of the Investigation Commission, tested the actual specimen of tetanus bacilli obtained from Mulkowal; and contaminated with it ordinary, *i.e.* full bottles of the Bombay water-agar prophylactic as used in the Punjab in 1902.

This contamination he effected sometimes with tetanus bacilli alone, at others, with these bacilli associated with harmless microbes, as always found to be in Nature. He examined his bottles after seven days and found under all circumstances a rich growth of tetanus, which affected animals when inoculated in very minute doses. His experiments show that the amount required for such an effect was many hundreds of times smaller than the doses of an ordinary tetanus culture essayed by him about the same time. The effect on the animals was also very much quicker, as is to be seen in the Appendix to this letter under (4) (p. 54). This represents about the condition in which a tetanus culture would have been found in the Mulkowal bottle No. 53 N, had that bottle been contaminated in Bombay four to seven weeks previously, or even only seven days before its opening at Mulkowal. For it must be mentioned that a culture of tetanus goes on maturing for about a fortnight, and its "toxicity" continues further to increase for another fortnight. A Bombay contaminated bottle would have been, therefore, in these two respects (abundance of germs and of toxic matter in the fluid) richer than the bottles experimented with by Lieutenant-Colonel Semple.

On the other hand, when a bottle of prophylactic, just contaminated with an impurity, is emptied of its contents, and only a few drops of fluid remain in it, the development of tetanus germs in such remnants is inhibited by the air, and either does not take place at all, as was the case in the experiment made on the subject by Lieutenant-Colonel Semple (Appendix B of the Commission's Report, Experiment 7), or else a culture does take place, but only in proportion in which other germs present use up the oxygen of the air (*vide* Lister Institute's Report, Section 3, and Appendix E, Experiments 5 to 8). Such a culture is always reduced—in a degree varying with the circumstances—as regards its abundance and toxicity.

A small sample of fluid remained in the bottle after the Mulkowal operations, and was examined by Lieutenant-Colonel Semple, at Kasauli, fifteen days after the bottle had been used. In this sample he found an association of a common microbe with what, in his view, was "a poor growth of tetanus," in comparison with what should have been there had the culture been growing for only a week in a full bottle (*vide* the Indian Commission's Report; also Appendix to this letter under (5*a*) (p. 54). The effect of that fluid on animals was also several times weaker and slower in action than what he found in cultures grown in full bottles (Appendix to this letter under (5*b*) (p. 54).

The condition of the bottle on its examination at Kasauli corresponded, therefore, to that of a bottle in which tetanus had grown after it had been emptied, and not to that of a bottle in which the germs had resided while it was full; and this finding corroborated the result of the primary examination made at Mulkowal.

C.

The following details show the difference in the conditions under which accidental tetanus cases occur, and those in which the Mulkowal patients would have been infected had bottle 53 N contained a several weeks old tetanus growth.

In the ordinary course of events man can get into his tissues only a mere trace of tetanus virus; for every visible impurity affecting him is immediately removed; a splinter or thorn is pulled out; an injured surface of the skin is washed and left to all appearances clean, &c.

But a tetanus-bearing impurity, when effecting entrance into a cultivation medium, gets multiplied an infinity of times; and, as stated, the plague prophylactic has shown itself to be a particularly rich medium for such a multiplication. An ordinary clinical case can never get infected with anything approaching in amount a measured volume of such a culture; which volume in the Mulkowal cases was that of 1½ cc. Approximately a hazel-nut full of an extremely rich tetanus growth would have been left under the skin of every man inoculated, in place of what can remain after the withdrawal of a thorn, or the washing of an abrasion or wound.

The prophylactic produces, in the case of man, at the seat of injection, an inflammation which develops in a few hours and causes a swelling and induration of the tissue involved. The site so inflamed is comparable to a severe contusion, which, even in a slight form, is known to be an important determining factor in tetanus. (It may be remarked here that the skin of guinea-pigs and mice, on which tetanus experiments are generally made, is not so affected even by large doses of the plague prophylactic. In their case there are other agents by which it is possible to create a state corresponding to the above inflammation). This circumstance should have placed the Mulkowal cases at a great disadvantage.

Lastly, when the tetanus germ gets into animal tissue and the requisite predisposing conditions exist, such as a contusion, or more severe injuries, or the presence of impurities leading to local inflammation, &c., the germ starts manufacturing toxic substances which cause the clinical tetanus symptoms. A certain time is required for the elaboration of these substances; and then another period passes during which the tissues susceptible to this virus, notably the nervous centres, are gradually reached. The two periods make up the "incubation" interval between contamination and the appearance of first symptoms.

But these germs manufacture also toxin in artificial cultivation media. In the case of tetanus the toxin thus produced is of a potency unequalled by any other microbial toxin so far known. Various strains of the same species of microbes, and various media in which they are grown, differ, however, in regard to the

amount of toxin which they elaborate. . Regarding the Mulkowal microbe, it has already been mentioned that a bottle of prophylactic in which it had been growing only for seven days appeared to contain a virus many hundreds of times more deadly than that found by Lieutenant-Colonel Semple in an ordinary tetanus culture. Its action was also several times quicker than that of the latter (Appendix to this letter, under (4) (p. 54)). Had, therefore, the Mulkowal bottle been contaminated in Bombay, the patients would have received into their tissue 1½ cc. of such a potent and rapidly acting toxin ready prepared, instead of, as is the case in accidental tetanus, benefiting by an interval required for the elaboration of toxin in their own tissues.[1]

The large amount of germs that would have been present in a dose of 1½ cc. of a Bombay contaminated prophylactic as compared with the doses in accidental cases; the introduction of that dose right into the tissues; the predisposing inflammation created by the prophlactic; and the presence of 1½ cc. of ready-made and rapidly acting toxin, should have caused the disease to manifest itself with a rapidity and gravity far above those observed in average cases; but, whereas in ordinary Indian practice cases may be met with of an incubation period of less than twenty-four hours, ending fatally within another twenty-four hours, the patients infected at Mulkowal took five and six days to develop the first symptoms, and the length of their illness—which in tetanus is usually fatal—varied from two to five days, and in one case was of one day. (Some remarks on this matter are made in the Appendix, under (6) (p. 55).)

The Commission had before them an important term of comparison in the isolated case of tetanus which they investigated together with the Mulkowal case, and which had occurred at Ferozepore, Punjab, eleven days before the accident at Mulkowal.

On this case the Commission reports as follows: "We are satisfied that the isolated case of tetanus cannot be ascribed to contaminated fluid." The contamination was therefore conveyed through that natural dose of a tetanus impurity, unmultiplied by growth in a culture, as causes ordinary tetanus cases. The patient died two and a half days after the time when she was inoculated, the first symptoms appearing sixteen or eighteen hours after that time, and the illness lasting forty-six hours. The details of the case are reproduced in the Appendix of this letter under (7) (p. 55).

The Mulkowal cases were less affected by the contamination which fell to their lot. The character of their disease, when compared to that of the Ferozepore case, was such as to induce one almost to say that the amount of tetanus impurity which, in Ferozepore, affected a single person, was, in Mulkowal, divided by an accident of procedure into nineteen shares, and weakened in its effect.

D.

Lastly, the following fact refers to the condition of the fluid at the time of using it at Mulkowal.

[1] *Vide* Lister Institute Experiments, 2 and 6, Appendix F (B), p. 77; Indian Commission's Experiment 1, Appendix B., p. 43.

w The syringe with which contents of bottle 53 N. ere injected was dealt with afterwards as follows: It was filled with carbolic lotion 1 in 20, left so for a few (three or four) minutes; then the lotion was squirted out, and the instrument used for inoculating other people. None of the latter suffered. In a culture of four to seven weeks, as it would have been had the bottle been contaminated in Bombay, and even in one of a much shorter duration, tetanus germs develop spores or special forms of resistance, made to withstand heat, desiccation, adverse chemical action, &c. For destroying such spores by carbolic lotion an action of some fifteen hours is required. The procedure applied to the contaminated syringe thus amounted practically to an imperfect cleaning.

If spores of such an abundant culture as would have developed in a bottle of prophylactic had been drawn into the syringe, the above cleaning would have left the instrument very grossly contaminated (*vide* the Lister Institute's Report, Appendix G, Experiment 1; and the Indian Commissioner's Report, Appendix B, Experiment 2 (pp. 48 and 43)).

The fact that the passage through that instrument of one syringeful of lotion was sufficient to render it harmless, points to its having been in contact with only a small amount of impurity, such, for instance, as was experimented with by the Lister Institute in Appendix G, Experiment 2 (p. 48). The force of this argument is the greater that, as has been mentioned already, man is so sensitive to tetanus that an imperceptible trace of virus left on a surgical instrument exposes him to the disease; and in the Mulkowal circumstances the subjects were made particularly susceptible by the inflammation from the prophylactic injection.

Summary of the Above.

The following facts indicate that the germs of tetanus had not effected an entrance into the Mulkowal bottle even only a few days prior to its opening.

The bottle was odourless at the time of using it, whereas in less than three days a tetanus odour becomes unmistakable in a contaminated bottle.

Tetanus germs generally, and the Mulkowal specimen in particular, when growing in a full bottle of prophylactic, give in a few days a rich and extremely active culture; whereas in the sample left in the Mulkowal bottle a poor culture was found of a much lesser activity, such as would develop in the remnants of prophylactic fluid, in a bottle emptied of its contents.

The character of the disease exhibited by the nineteen patients was that observed in average clinical cases in India, of a not very severe description, *i.e.*, in cases due to minute accidental doses of virus, not multiplied by culture; and that character is incompatible with the supposition that the Mulkowal cases were injected with 1½ cc. of a virus of such potency as was found in infected, full, bottles of prophylactic, after even only a few days accumulation of virus in them.

And, lastly, the fact that contamination could be easily removed from the infected syringe and the latter rendered harmless, indicates that it had been in contact with only a small amount of virus; whereas when

brought in contact with a seven days old culture, it has been shown to retain, after rinsing, enough infection to affect seventeen out of nineteen animals, *i.e.*, probably thirteen out of thirteen injected with a first syringeful, and four out of six injected after a further dilution.

The Indian Commission reported to have "*found that the germ of tetanus had been introduced into the fluid before the bottle was opened at Mulkowal*," *and attributed the accident to one of the laboratory operations* (*vide* the Government of India's *communiqué* to the Press of December, 1904 (p. 48)). Not one of the facts of the case contains the slightest indication to this effect; everything brought out by the enquiry points in the opposite direction; and the Commission subordinated all direct indications to a thesis adopted *a priori*, and negatived in the Lister Institute's statement of November last.

THE CIRCUMSTANCES IN WHICH THE MULKOWAL ACCIDENT TOOK PLACE.

Vide pp. 39 and 40, above.

Appendix to Part I. of this Letter.

I now submit remarks on certain points touched upon in the foregoing text and marked therein for reference by bracketed figures.

THE COMMISSION'S PERMEATION THESIS.

(1*a*)

The following is the thesis upon which the Commission reported to have found that the Mulkowal bottle was contaminated prior to its opening, notably in the laboratory:—

"The specific contamination permeated the whole fluid in the bottle, as is shown by the uniform results on the sufferers, and without a series of suppositions opposed to all reasonable probability, these results can only be attributed to a growth of some duration, though not necessarily of great vigour, a view strongly supported by the experiments mentioned above."

The experiments are referred to lower down, under (1*b*) (p. 53). The following concerns the rest of the proposition:—

The Commission do not specify the suppositions involved in their thesis; but the latter embodies obviously the following one: An impurity having fallen into the fluid, the latter would afterwards be drawn into the syringe through the narrow lumen of the hypodermic needle. This must have caused a fair mixing up of the contents. The contamination, though permeating every dose, even fraction of a dose of this fluid, as happened in Lieutenant-Colonel Semple's Experiment 8, Appendix B, and in mine, Appendix C, of the Commission's Report (p. 44), could not, in the Commission's opinion, permeate it so uniformly as to correspond to the degree of uniformity exhibited by the Mulkowal cases (incubation period of five and six days; length of illness varying from one to five days; issue, as is practically usual in tetanus, fatal).[1]

The Lister Institute refers to the above supposition thus: "that the growth was a fairly uniform one, as must be supposed from the similiarity and fatal issue of the disease in the nineteen cases."

Had an impurity fallen into the bottle at the time of using it, variation in the dose of tetanus virus contained in different portions of the fluid should have caused variation of the disease, notably a greater variation than was observed at Mulkowal: this is the supposition involved.

But this supposition is destructive of the one which the Commission adopted a few lines higher up, when they were dealing with a most decisive feature of the case.

Notably, they were confronted with the variation of dose as is exhibited, on the one side, by the immeasurably small amount of virus which "clinical cases" get infected with in Nature; and, on the other, the dose of 1½ cc. of a several weeks' accumulation of microbes and toxine which would have been injected into the Mulkowal cases had tetanus been growing in the bottle since its preparation or its despatch from Bombay. In the presence of this extraordinary variation of dose, the Commission laid it down that the Mulkowal patients might have exhibited the same course of the disease as is exhibited by not very severe average clinical cases in India. (The matter is referred to in the text of this letter under C (p. 50)). In this case the variation of dose was not considered by them as entailing a variation in the resulting disease.

The Commission showed that they were aware of the incompatibility of the two suppositions when they inserted in their thesis the words "though not of great vigour," which are obviously meant to mitigate the contradiction contained in their preceding passage. But this reservation is also inadmissible; for, had the bottle been contaminated in Bombay, the vigour of the growth that would have developed in it is now known, *vide* the text of this letter under B (p. 49). It would have been *great;* and this is in the way of the Commission's conclusion.

The following seems to me the correct conception of the matter:—

(*a*) If the Mulkowal cases exhibited the same course of illness as average clinical cases in India do, this is a decisive indication that the dose of virus which they received substantially corresponded to the natural dose, unmultiplied by culture, that is such as clinical cases get in spontaneous infection.

(*b*) Whatever amount of uniformity the Mulkowal cases exhibited, is intelligible in view of the common origin of the tetanus specimen injected, and of the common way in which the patients were contaminated. For, in nineteen natural cases the infecting microbe would have varied as to its origin, and consequently, its biological and toxic properties, and as to the accidental impurities which would have accompanied it; the

[1] Report dated November 12th, 1902, from Captain C. James, I.M.S., Inspecting Inoculation Officer, to the Chief Plague Medical Officer, Punjab

mode of infection would have varied as to the part of the body involved (trunk, limb, or head), the depth of the wound and the kind of tissue in which the microbe had been deposited; as to the extent of contusion or mortification inflicted on that tissue at the time of infection, and so forth. In the Mulkowal cases all these conditions, which are known to be the direct determining factors in the character which this disease assumes, were the same in each patient, viz., the same strain of microbes, the same accompanying impurities; the prophylactic as cause of the predisposing inflammation; the same part of body—the arm; the same tissue involved, the same closed wound, viz., that from a puncture of a hypodermic needle, &c. With all this, their cases presented enough variety to be put on the account of the variation of dose and the variation of individual idiosyncrasies.

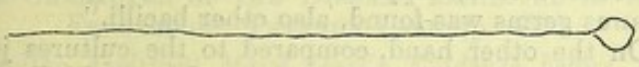
(1*b*)

The following refers to the experiments to which the Commission allude in their above thesis.

The result of Experiment 5, Appendix B (p. 43), is antagonistic to that thesis.

Its bearing would, however, appear somewhat diminished by Lieutenant-Colonel Semple having used for contaminating bottle No. 3 a large platinum loop.

The following is a platinum loop. A wire made of that metal of the thinness of an ordinary thread is bent so as to form a circle (loop) at one of its ends, thus—



By means of such a wire samples are taken from fluids, &c., containing microbes; the bent end of the wire is dipped into the fluid and withdrawn, a thin film then remains stretched over the circle, and by this film another medium is contaminated when the wire is plunged into it. When the loop is small, the film of fluid may attain some thickness and form a droplet.

The larger the loop the thinner generally is the film. Should the diameter exceed that in the above sketch, and generally even in one of that size, when the fluid is a watery one, the film is of an, as it were, inappreciable thinness, and with a further increase of size, one finds on withdrawing the wire from the fluid, that nothing at all remains in the circle—the film breaks. So, after a certain, generally fairly modest, size, the larger the hole of the loop, the poorer a carrier of water it is.

It was such a contaminated loop that Lieutenant-Colonel Semple introduced into bottle No. 3, and he found that every dose taken from the bottle immediately after that gave tetanus to a guinea-pig. The experiment was a fundamental one, for it was undoing the thesis which was adopted by the Commission only *a priori* and upon which the laboratory was condemned. If the objection to the result was that the loop seemed to the Commission to have been large, it was interesting to repeat the experiment with a loop of a somewhat smaller size, and ascertain that in that case the fluid could in no wise be permeated.

To me, however, such a controlling experiment does not appear to have been necessary; for there existed no fluid cultures of tetanus in the environment of the Mulkowal operators; bottle 53 N is likely to have been contaminated with some solid bit of impurity, and it is certain that an agglomeration of microbes grown into what is called a "colony" on a solid substratum, such as a bit of vegetable, or dung, or cultivation soil, and packed together into a particle even of the smallest dimensions, would have contaminated Lieutenant-Colonel Semple's bottle No. 3 to a far greater extent than the largest, or, to say more correctly, any loop dipped into a fluid culture. All those who have made bacteriological analyses of garden earth, or who have examined a microscopic slide made from a fluid culture on the one side, and from a solid on the other, will agree with this.

In Experiments 6 and 8, Appendix B (p. 44), Lieutenant-Colonel Semple (and subsequently also the Lister Institute) (p. 47) followed the plan of my Bombay experiment with a bit of straw.[1] But my experiment was intended to show how easily and uniformly a bottle of fluid gets "permeated" with germs from the smallest amount of impurity dropped into it. Indeed, a fairly even average of fifty colonies appeared in each of the nineteen agar tubes which I inseminated from such a bottle immediately after. My experiment was not intended to show the *amount* of contamination which can easily get into a bottle. By moving a bit of straw in a fluid, as Lieutenant-Colonel Semple has done, only a relatively small number of germs will stick to its surface, as the latter, in the case of a straw, is smooth and slippery, and often not easily moistened by a watery fluid; nevertheless, that operation appeared many times more than sufficient to convey tetanus germs into each of the 1½ cc. dose of prophylactic. But certainly, the number of germs which could adhere to a straw under the above conditions must have been absolutely trivial when compared to what it might have been if even such a bit of straw had been allowed to reside for hours together in the intestinal tract of an animal harbouring tetanus germs, as would have been one of the natural origins of tetanus. Had I wished to give an idea of the amount of contamination which can get into a bottle handled without precaution, I would have taken, not an impervious body, as a straw, but the smallest particle of loose garden soil, such as mentioned above, and of which, one may say, the whole substance is practically composed of one solid agglomeration of microbial germs. In such a particle of matter the number of microbes is so enormously great as to it being almost difficult to express it in figures.

There is, further, ample ground for saying that even the procedure adopted by Lieutenant-Colonel Semple was sufficient to convey to each fraction of a dose in bottle No. 4 enough germs not only to be revealed by

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[1] Appendix C, Indian Commission's report, p. 44.

culture (his Experiment 8) (p. 44) but to give tetanus to an animal or man. For, as I had mentioned to the Commission, Vaillard and Rouget have shown fourteen years ago that two or three tetanus germs conveyed with a particle of earth were sufficient to give tetanus to a guinea-pig. In the Mulkowal cases the inflammation caused by the prophylactic was there to act as a predisposing factor; and in each $1\frac{1}{2}$ cc. dose of Lieutenant-Colonel Semple's bottle No. 4 there were numerous tetanus germs, as a *drop* taken from the $\frac{1}{2}$ cc. of fluid left in the bottle gave a culture in Experiment 8. The drop contained, therefore, at least one bacillus; but most probably there were many more than one to start the culture with; for the latter did not take long to develop; "a typical growth of tetanus was present on the third day."

(2) and (3)

THE ODOUR OF A TETANUS CULTURE.

Vide above, "Mr. Haffkine's observations on Dr. Elliot's evidence," pp. 37 and 38.

(4)

EFFECT ON ANIMALS OF TETANUS CONTAMINATED PROPHYLACTIC.

(*a*) *Vide* Experiment 3, Appendix A, Indian Commission's Report (p. 43): $\frac{1}{20}$ cc. of an ordinary tetanus culture failed to affect a guinea-pig; whereas, in the case of a tetanus culture grown in prophylactic, $\frac{1}{30}$ part of a *loopful* gave tetanus to a guinea-pig on the fourth day (Experiment 5, Appendix B).

(*b*) A numerical comparison is possible from the following (*vide* Experiment 2, Appendix B, of the Commission's Report): According to the Lister Institute's calculation, the dose of $1\frac{1}{2}$ cc. injected, in the experiment just referred to, into the first thirteen animals, corresponded to $\frac{1}{3.600}$ of that amount of original fluid, *i.e.*, to $\frac{1}{2.400}$ cc. (*vide* Lister Institute's Report, Section 3) (p. 45). These may have been the animals that were first and most severely affected. After the injection of these thirteen doses, *i.e.*, of $19\frac{1}{2}$ cc. of fluid, there should have remained in the 20 cc. syringe, $\frac{1}{2}$ cc. of that 3·600 fold diluted virus. This amount was then further diluted to make up a volume of 9 cc. by a second refilling of the syringe, and the dilution so effected brought the dose of original fluid, as given to each of the remaining six animals, down to $\frac{1}{43.200}$ cc. These six animals included probably those that manifested symptoms later, and of a milder type, as well as the two that escaped altogether. Now, in addition to being diluted, the doses of that virus were further reduced by the action of carbolic acid which preceded the dilutions, and which, besides affecting the fluid itself, eliminated all those germs as had not yet formed spores. Of such, in a seven days old culture, there is present still a considerable proportion.

The above specified doses are to be compared with the dose of $\frac{1}{20}$ cc. of the ordinary culture (Indian Commission's Report, Appendix A, Experiment 3), which failed to affect a guinea-pig, and the dose of $\frac{1}{10}$ cc. which gave a guinea-pig tetanus on the third day (same Appendix, Experiment 4). This ordinary culture had not been acted upon by carbolic acid.

(*c*) The above comparisons refer to the activity of the Mulkowal germ grown in prophylactic, as revealed in the doses in which it was capable of taking effect.

The rapidity with which it acted is shown in the following experiments: From a dose of $\frac{1}{20}$ cc. of such a culture a guinea-pig was found dead the next day (Appendix B, Experiment 1) (p. 43); while double the above dose of Lieutenant-Colonel Semple's ordinary culture only rendered a similar animal ill on the third day (Appendix A, Experiment 4). A culture of the Mulkowal microbe, had such been developed in bottle 53 N, should, therefore, have left to the Mulkowal patients a very short incubation period, a matter which is considered in the text of this letter, under C (p. 50).

(5*a*)

THE CULTURE FOUND IN THE REMNANTS OF FLUID IN THE MULKOWAL BOTTLE.

The following was seen by Lieutenant-Colonel Semple in full bottles of prophylactic when they were contaminated with tetanus only seven days before: Experiment 5, Appendix A, Indian Commission's Report (p. 43): "Numerous tetanus bacilli were present in stained specimens."

Experiment 6, same Appendix: "Numerous tetanus bacilli and also micrococci were present in stained specimens."

Bottle No. 1, Appendix B: "A rich growth of tetanus germs was found, also other bacilli."

On the other hand, compared to the cultures just referred to, the sample of fluid found in the Mulkowal bottle showed what, in Lieutenant-Colonel Semple's view, was only "a poor growth of tetanus" (*vide* the Commission's Report) (p. 41).

In his original report on that bottle, he said: "A preparation made direct from the contents and stained showed a few tetanus bacilli (also micrococci)." *Vide* also the description given by Lieutenant-Colonel Semple at the Commission's sitting in Bombay on January 26th, 1903, and incorporated by the Commission in the evidence which I was giving at that moment (the India Office print, communicated to the Lister Institute, p. 19): "Major Semple observed that when he examined the remnants of the fluid microscopically he found a couple of microbes in a field; in another none; in another a few."

(5*b*)

Vide p. 50. Instead of a dose of $\frac{1}{20}$ cc. being fatal to a guinea-pig in twenty-four hours (Appendix B, Experiment 1, Indian Commission's Report),[1] as was the case with a culture from a full bottle, double the above dose taken from the Mulkowal bottle rendered a guinea-pig ill after twenty-four hours, and

[1] Page 43.

caused death a day later, as reported by Lieutenant Colonel Semple upon his examination of the Mulkowal bottle.

(6)

EXPERIMENTAL FACTS WHICH WERE NOT KNOWN WHEN I WAS GIVING EVIDENCE IN BOMBAY.

At the time of giving evidence I referred to the tardy manifestation of symptoms in the Mulkowal patients, as well as to some other subjects, as a matter for the Commission's consideration and enquiry. My own information in connection with the point in question was incomplete; notably, I did not know (1) whether the plague prophylactic in its water-agar variety, which is a vegetable medium, was as suitable for the development of tetanus bacilli as are the special media made for them in laboratories, and in which a virus of great potency is generally developed; and (2) whether the particular bacillus found at Mulkowal came up to the ordinary standard of potency as observed in tetanus bacilli, or did not possibly fall short of this.

Subsequent experiments gave a very emphatic positive reply on these two points.

On the other hand, I was mistaken in admitting that the tetanus bacillus might secrete in that special vegetable medium as much gaseous matter as it secretes in other media containing, *e.g.*, a trace of sugar. Consequently the pressure of gases, which in other instances may cause a stopper to come out with a noise, is not manifested in that way in tetanus contaminated prophylactic as I thought it might be.

(7)

THE CHARACTER OF THE DISEASE EXHIBITED IN THE FEROZEPORE TETANUS CASE (p. 51).

Hospital Assistant Maya Dass's Evidence before the Indian Commission.

I recollect the case of a woman inoculated by Captain Perry, and who afterwards died at 4 a.m. on the 22nd. I saw the woman. I think she died of tetanus. She had occasional cramp, and her teeth were continuously closed; I could not put my fingers in. She had a temperature of 102°. She drank nothing; everything poured inside her lips she rejected.

I saw her at noon on the 21st. I stayed with her, and sent a clerk to get a tub from the bungalow to give her a warm bath; when we got the tub we put her into a warm bath.

I saw her last at 5 p.m. on the 21st.

I heard there had been another case six months before in a neighbouring house; the two houses had a common wall.

I have seen tetanus at the Medical College. I am sure this was the same disease.

There was nothing wrong with the arm, and I could not see the needle puncture.

I made no examination of her person.

I have seen two cases of tetanus.

She was inoculated before two o'clock on the 19th. The relatives said she exhibited symptoms, *i.e.*, cramp, on the morning of the 20th.

I saw her for the first time for an hour. The second time they would not let me examine her.

The spasms were less when she was taken into a darker room.

II.

REMARKS ON THE INDIAN COMMISSION'S REPORT.

I find in the Indian Commission's Report and Proceedings (pp. 40-45) the following points to which, I believe, I am right in taking some exception. I mention first those to which reference has already been made, viz.:—

(1) The part of the Mulkowal operator's evidence, when he examined before the Commission the Mulkowal and other bottles, has been recorded in an abbreviated form, which gives it a bearing I believe to be different from the one it had actually. The matter has been referred to above in the Appendix, under (2) (p. 54).

I find less important omissions, but in an essential part of the evidence, in the deposition of Dr. Elliot's compounder. These have been indicated in footnotes on p. 40.

(2) The Commission, when discussing in their Report the fact that the bottle was free from odour at Mulkowal, refer, in their argument, to *specialisation* of a smell instead of to detection of the existence of a smell. It is specialisation that requires, as the Commission say, an experienced person, *i.e.*, one acquainted with the nature of the smell which he is to specialise; and it is again specialisation that is facilitated by the process (fermentation or culture) producing the smell being pure, *i.e.* unadulterated by the odour of some other concomitant culture. But specialisation of an impurity was not and could not be the object at Mulkowal. Even in the laboratory, though provided with trained bacteriologists and the necessary apparatus, on no occasion is the task of specialising an impurity attempted, as that task, in bacteriology, generally requires days and sometimes weeks of investigation; and in many instances cannot be solved at all.

(3) In the same discussion, the Commission refers to the ordinary ("old" or "standard") carbolised broth prophylactic, in place of the water-agar variety. The first gives out a slight mixed odour of broth and carbolic; the Punjab operators were not dealing with this prophylactic at that time; the other has no odour, and it is with this material that Dr. Elliot was working at Mulkowal.

The importance of this *quid pro quo* is apparent from the circumstance that the Commission have based on it their not taking account of one of the most essential facts brought out by their enquiry. They say: "Our personal observation leads . . . to the conclusion that all bottles of prophylactic have a peculiar and not agreeable smell, so that Dr. Elliot, under the circumstances in which he was working, may well have failed to specialise a tetanus smell."

The difference between the two prophylactics was indicated by Dr. Elliot himself, in his evidence referred to in the Appendix under (2) (p. 54); and he mentioned the absence of any odour in the water-agar prophylactic in further support of his statement that he could not have missed a bottle having an odour of any kind. At the same time he proved before the Commission that he would not have missed

a tetanus contaminated bottle even amongst bottles of ordinary, *i.e.* broth, prophylactic.

(4) In the passage just quoted the Commission refer to the circumstances under which Dr. Elliot was working; and a few lines higher up they say that the smell may no doubt be detected by one on the alert. This may possibly suggest that there was something in the circumstances to prevent Dr. Elliot from detecting the smell, and that he was perhaps not on the alert; or that without being specially on the alert the smell given out by a tetanus culture cannot be felt. But every detail on record indicates an opposite condition of affairs. I beg to refer to the evidence quoted in the Appendix under (2) (p. 54).

I mention now the points to which I have not yet had an occasion of referring, and begin with a passage which attaches to the matter just treated.

(5) The Commission say: "The failure to perceive a smell is undoubtedly a fact that calls for consideration, but we do not regard it as so conclusive in favour of tetanus having been introduced at Mulkowal as to outweigh all other considerations." It must be noted that—

(*a*) A fact, the result of direct examination, would have to be outweighed by considerations: it is, indeed, an *a priori* thesis that the Commission opposes, lower down, to the facts brought out by the investigation.

(*b*) The result of the examination in question, *i.e.*, freedom of the bottle from tetanus odour at the time of opening it, has been confirmed by every one of the facts observed and found out subsequently, all of which will have to be rejected before an opposite conclusion is possible; and

(*c*) The Commission speak of "all other considerations" to be outweighed; whereas by this they refer to the only consideration adopted *a priori*, and which has been negatived in the Lister Institute's declaration of November last.

(6) The Commission broke up the facts opposing that consideration into two parts, and dealt with one part of them as being the whole.

I beg to refer to the two broad alternatives between which the Commission divided their discussion on the origin of the Mulkowal tetanus. What they had determined to be the argument on the first alternative (*vide* their text) should, I believe, have ended before the commencement of the following sentence: "There is also the important circumstance that on the examination of the bottle at Kasauli it was found to contain tetanus bacilli; and though not absolutely conclusive on the point, we have always regarded this fact as strongly suggestive of the inference that the contamination was in the fluid."

My contention will be clear from the following. The above sentence, though containing a very definite and clearly expressed idea, obviously lacks verbal completeness; for as it stands it would seem to affirm that the circumstance that the bacilli were found in the fluid was not absolutely conclusive as to their being in the fluid; but that the Commission considered this circumstance as suggestive that the bacilli were in the fluid.

I believe I am right in saying that the Commission only omitted at the end of their sentence the words, "before it had been made use of," or some similar ones, and that they meant to say the following: The fact that the bacilli were found in the bottle was not absolutely conclusive on the point as to when they had entered it; but the Commission have always regarded this fact as strongly suggestive of the inference that they had entered it before the fluid was made use of, *i.e.*, before it was drawn into the syringe.

By adding the missing words the Commission would have found themselves in the necessity of attaching the subject in question to the second portion of their argument; for that subject refers to the *time* when the bottle was contaminated: before its handling at Mulkowal, or during that handling.

I need, I think, hardly add that the passage quoted contains no explanation as to the reason why the Commission have considered the mere presence of the bacilli in the fluid as suggestive of the time when they had entered it.

Further, the sentence next to the above passage begins thus: "We are now confirmed in this view. . . ." Obviously, not in the view that the bacilli were in the fluid; for these had been seen in it by direct examination, and the matter could not be treated as a view; but—as will be seen directly—the Commission were confirmed in the view that the bacilli were in the bottle before any fluid was drawn from it.

"We are now confirmed in this view by the experiments set out in Appendix B (bottle No. 4, Experiments 7 and 8) which establish[1] that, though there were tetanus germs in the ½ cc. of the fluid left in the bottle No 4, the conditions in it, when thus emptied, did not permit of their growth, and this points to the conclusion that the bacilli found by Lieutenant-Colonel Semple in the Mulkowal bottle were the result of a growth that had taken place in the fluid."

This sentence, as it stands, would seem again to affirm that the conditions in the bottle, when almost emptied, did not permit of the bacilli's growth, and that this pointed to the conclusion that they were the result of a growth. The sentence appears to lack at the end of it the words: "before the bottle was emptied." The reference to the *time* at which tetanus growth had taken place in the fluid, transfers again the matter to the second portion of the Commission's argument, *i.e.*, the one dealing with the time of the contamination of the bottle.

Similarly, the whole of the remaining text of that first, the ampler, half of the Commission's argument belongs to the second, the shorter, portion. The phraseology appears to me to betray this in the following sentence: "We therefore are unable to accept the argument that the freedom from tetanus of those persons subsequently inoculated shows that the specific contamination could not have entered the syringe, and after full consideration we think that the contamination was in the fluid." The connection between the two parts of the sentence, bound together by the conjunction "and," is not established; and the words "before the latter was drawn into the syringe," must

[1] As is to be seen from the Lister Institute's Report, Section 4, Lieutenant-Colonel Semple and the Commission were misled by the result of the experiments in question.

be put at the end of the passage to make at once clear the meaning of the whole. Moreover, the statement, "after full consideration we think that the contamination was in the fluid," when a little while before it was stated that the bacilli had been seen in the fluid, is inexplicable without the omitted words. As the sentence meant not to affirm that the contamination was in the fluid, but that it was there before the fluid was drawn into the syringe, the matter belongs to the second half of the Commission's argument.

It will be seen from the Lister Institute's Report that, whereas the Indian Commission's plan of discussion was, not to refer to the time of contamination only in the first half of their argument, the Institute adopted that plan for the whole of their Report.

The first half of the Commission's argument calls further for the following, mostly technical, remarks:—

(7) They say: ". . . To deal first with the theory that the needle may have been the seat of the mischief, it is a remarkable circumstance that the contamination should have survived only so long as bottle 53 N was in use, and then have ceased to manifest itself."

The possibility of contamination by a needle has been verified in the Lister Institute's Experiment 2, Appendix G to their Report (p. 48): a contaminated needle carried tetanus germs down to the seventeenth consecutive injection (an eighteenth and nineteenth, to correspond to the Mulkowal numbers, have not been tried). After this a syringeful of carbolic was drawn into the syringe, as was done by Dr. Elliot at Mulkowal, and no more tetanus germs were got out of that needle. A susceptible animal having the seat of injection bruised or inflamed by a concomitant impurity, or prepared by a chemical reagent, would have taken tetanus after each of the above seventeen injections. (*Vide* indeed Vaillard and Rouget's experiments in the *Annales de l'Institut Pasteur*, 1902, referred to above (p. 54) in the Appendix, under (1*b*).)

(8) The Commission say: "Then Lieutenant-Colonel Semple has made an experiment in which a similar needle dipped in a tetanus culture, and used immediately afterwards on a guinea-pig, failed to impart tetanus."

It is not stated, in the description of this experiment, whether the needle was not one sterilised by the hot oil method; nothing would adhere to such an oiled needle momentarily dipped into a fluid culture. The matter is not essential for the same reason as mentioned on another occasion: there were no fluid cultures of tetanus in the operator's environment at the moment of fixing the needle on the syringe, to contaminate it in the manner under consideration.

If, in place of that procedure, Lieutenant-Colonel Semple had taken a tetanus contaminated grain of earth and dropped such into the nozzle of the needle at the time of fixing the latter on to the body of the syringe, as might occur with a needle picked up from the ground; or even if he had proceeded as the Lister Institute did in the experiment quoted above, in which tetanus germs were allowed time to adhere to the needle (Experiment 2, Appendix G of their Report), the result would have been greatly different. It must also be pointed out that both Lieutenant-Colonel Semple and the Lister Institute used "pure" cultures of tetanus. Such are never met with in Nature. In bottle 53 N also, Lieutenant-Colonel Semple found a concomitant impurity, as was inevitable; and it is impurities, of which a number have now been pointed out by tetanus investigators, that help this bacillus to take effect. Moreover, as mentioned before, the prophylactic fluid causes in man a very considerable inflammation, and thus creates another "tetanus favouring" circumstance; whereas the animals experimented upon by Lieutenant-Colonel Semple and the Lister Institute are hardly affected by the prophylactic at all.

(9) The Commission say: "On this fact has been founded the argument that the freedom from tetanus of those subsequently inoculated is inconsistent with the theory that the specific contamination could ever have entered the syringe." For adequately treating this matter, the sentence should have been completed by the addition of the words: "in such an amount as would be represented by a tetanus culture in a bottle of prophylactic." In my letter to the Commission's Secretary (Lieutenant-Colonel Semple), dated Bombay, February 4th, 1903 (p. 35), I explained as follows: "The objection I mentioned against a tetanus culture having been drawn into the syringe, is maintainable in regard to such a culture as found by Major Semple. If, on the other hand, contamination was introduced just before the syringe was filled, the amount of it may have been such as to admit of the rinsing of the syringe rendering it innocuous." I stated this in the above letter, as I thought that, in my verbal evidence, I had not presented that consideration with the fulness pertaining to it; and further took some pains to render the matter clear by a specially made experiment, and wrote about it to the Commission's Secretary, on the 8th of the same month (*vide* Appendix C of the Commission's Report), (p. 44.)

At the time of my giving evidence and writing those letters in Bombay, I did not know the real abundance of a tetanus culture which develops in a full bottle of prophylactic. At present it is known that had tetanus been in such a bottle for a few days, the abundance of organisms would have been far above that which Lieutenant-Colonel Semple found in the sample of 53 N when it reached him at Kasauli.

(10) The Commission say: "The experiments that have been made by Lieutenant-Colonel Semple, appended to this Report, show, we think, that the argument is so far well founded that where a fluid containing a culture of tetanus is used, the syringe, even after it has been washed out with a 1 in 20 solution of carbolic acid, may retain enough contamination to affect fluid with which it is subsequently filled. The extent, however, of its influence varies according to the richness of the growth between fatal results and absence of all manifestations."

The culture which in Lieutenant-Colonel Semple's experiments gave fatal results (Experiment 2, Appendix B), (p. 43) had been made just in the conditions in which tetanus would have developed in bottle 53 N, had the latter been contaminated in Bombay, or even only a few days before the Mulkowal operations. It is such a culture that interests us in the present instance, and

of which it is desirable to know whether it existed in the bottle at the time of inoculation.

That it did not so exist is indicated by two concrete results which mutually agree and explain each other, viz. (*a*) on examining the Mulkowal bottle Lieutenant-Colonel Semple found in it no such culture; and (*b*) after a mere rinsing of the syringe there was, at Mulkowal, absence of all tetanus manifestations, which would have been paradoxical had Lieutenant-Colonel Semple been wrong in his above finding, and had bottle 53 N contained a Bombay infected fluid.

The Commission do not draw these conclusions, and explain as follows: "There are no means of forming an opinion as to the precise richness of the growth in the bottle 53 N beyond Lieutenant-Colonel Semple's view that it was a poor growth in comparison with that which he used in his Experiments 1 and 2, Appendix B," and they conclude, "after full consideration we think that the contamination was in the fluid."

In this explanation the Commission assume that the condition of the bottle as to tetanus development was the same when Lieutenant-Colonel Semple saw it as when it was first opened at Mulkowal; for, if not the same, an opinion on the precise richness of the growth found by Lieutenant-Colonel Semple would not advance us, in the present argument, in regard to the Mulkowal events. The argument then (even after being completed with the words mentioned on p. 56 above) appears affected by a double *petitio principii*. For the assumption that the growth seen by Lieutenant-Colonel Semple had already developed when the fluid was drawn into the Mulkowal syringe cannot figure in an argument enquiring whether that growth ever entered the syringe, or ever was in the fluid; and if it entered the syringe, it is impossible to consider as an unknown, and useless to examine by experiment, the question as to whether it was of a richness to be followed by fatal results or by an absence of all manifestations; for it is known that none of those inoculated at Mulkowal, after the rinsing of the syringe, suffered.

The assumption which figures in the above argument as a premise is itself founded upon an experiment by Lieutenant-Colonel Semple, as referred to by the Commission higher up. This experiment had not given a full reply on the point, as the Lister Institute has correctly ascertained (p. 45), and had led the Commission to a premise which, even when taken outside the above argument, is unwarranted.

Regarding the whole point, on which I have dwelt perhaps longer than I should have done, the Commission say: "We therefore are unable to accept the argument that the freedom from tetanus of those persons subsequently inoculated shows that the specific contamination could not have entered the syringe."

If the wording of the argument they refer to is completed, as it should have been, by the addition of the words, "in such an amount as would be represented by a tetanus culture in a bottle of prophylactic," that argument is to be maintained.

(11) In the above reasoning the Commission refer to Lieutenant-Colonel Semple's Experiments 2, Appendix A, and 2, Appendix B, in the first of which the result was absence of all manifestations, and in the second, fatal incidents.

Regarding these experiments, it must be remarked that Experiment 2, Appendix A (p. 43), was evidently only a preliminary one and very much different, as to its circumstances, from the Mulkowal case and from Experiment 2, Appendix B (p. 43)—I refer to the size of the syringe; the time during which the contaminated fluid was left in contact with that instrument; the number of animals on which the syringe was tried; the fluid—sterile broth—which was used instead of a fluid causing inflammation. Then the syringe was "washed out" with carbolic; this procedure possibly differed from the one used in Experiment 2, Appendix B, and which corresponded to the Mulkowal procedure; and, lastly, it is not certain whether the syringe used was not one usually sterilised by the hot oil method, as is practised in the Kasauli Laboratory, or had not its plunger oiled with vaseline; neither of which conditions obtained in the Mulkowal case. Contaminated fluid would not moisten and adhere to an oiled barrel.

Under the conditions in which that experiment was done it is impossible to draw from it conclusions regarding the Mulkowal events, or to compare its result with that of Experiment 2, Appendix B, in which the Mulkowal conditions were very much more approximated. In other words, the difference in result between these two experiments was prepared by a greater deviation of conditions than merely a difference in the richness of growth.

It remains thus admissible that even with the poorer growth used in Experiment 2, Appendix A, the result might have been a positive one had the Mulkowal conditions been more closely realised. This experiment, however, lost its importance on account of Experiment 2, Appendix B, having given positive results; and it may only be pointed out again that even in the latter experiment the animals experimented upon differed from man by not getting the inflammation which he gets from the plague prophylactic. Further, for gathering an accurate idea of the state of infection of the syringe, information is wanted as to whether the two animals which escaped, out of the nineteen, as well as those that developed milder and more tardy symptoms, were not injected after the second refilling of the instrument. A detail of lesser importance is that the syringe had not been filled with contaminated fluid twice, as was the case in Mulkowal. It was, however, left in contact with that fluid for two minutes.

In the second half of the Commission's argument, in addition to the remarks made already, the following is essential:—

(12) The Commission mentions as an admissible premise—which they afterwards conclude to be inapplicable to the Mulkowal case—that the contamination might have effected an entrance at Mulkowal, and say: "Thus, by way of illustration, the stopper or the forceps might have been dropped on the ground and applied to the mouth of the bottle with contamination adhering, or spores settled between the rubber stopper and the rim of the bottle, might have dropped in as the stopper was pulled out."

It appears to me gravely prejudicial to the

elucidation of the case that the Commission quote the above by way of illustration, as a possibility, and omit bringing into line the following actual circumstances, viz., that it came out on evidence that the forceps was dropped in opening the Mulkowal bottle, notably when that opening was partly done, the operation being then completed with that forceps picked up from the ground; and that such an occurrence, as well as the dropping of the cork, was a common one in the practice of the compounder who was dealing with the Mulkowal fluid; that the procedure by which danger should have been obviated, viz., sterilising the instrument in the spirit lamp, was abolished by the rules prescribed to and practised by the operators; that the contents of the box in which bottle 53 N was carried was exposed to dust, in the open, on successive occasions since at least the week before the opening of that bottle; that the latter itself was so exposed during the operation, at the time when a gathering of peasants was crowding around; and that the procedure of burning the dust on the cork and rim was equally abolished in the Punjab technique and substituted for by a manipulation which could not prevent danger.

(13) The Commission's reasoning contained in the passage beginning with the sentence: "It must be conceded that . . . ." and ending with the sentence: "It is therefore clear, accepting all that this train of thought implies . . . " will, I think I may venture to say, meet with a chorus of dissension from every bacteriological institution in the world.

Besides what I am bound to designate as its intrinsic arbitrariness, it presents this particularity, which distinguishes it from all the Commission's preceding mode of reasoning, that, while up to that point they were taking for guidance the result of animal experiment, and this not only in principle, but even in degree, in the present instance they for the first time refuse to apply to man a rule which has no exception, whatever susceptible animal or whatever disease be taken, viz., that a dose of ready manufactured toxin hastens the incubation of a disease, and the development of a fatal issue.[1] On this subject other remarks are to be found in the text of this letter, under C (p. 50.)

(14) While in the passage just referred to the Commission refuse to admit the influence of the dose of virus upon the symptoms of the disease, in the *a priori* thesis on which they condemn the laboratory, they base themselves on a postulate requiring rigorous equality of dose to correspond to a *certain* similarity of symptoms. On this point further remarks are contained in the Appendix to this letter, under (1*a*) and (1*b*) (pp. 52 to 54).

In concluding the above remarks I do not know whether I need mention again that when I admitted in Bombay, before I could make actual experiments, that the stopper of a bottle of prophylactic contaminated with tetanus might come out with a noise, I was mistaken, as I have already explained in the Appendix to this letter under (6) (p. 55). The Commission are therefore right in saying: "To the absence of explosive noise we attribute no importance."

[1] *Vide* Commission's Exp. 1, App. B., p. 43; Lister Institute's Exper. 2 and 6, App. F.(*b*), p. 47.

III.

REMARKS ON THE REPORT OF THE LISTER INSTITUTE.

It has been mentioned above that the Indian Commission opposed to the facts brought out by the enquiry an *a priori* thesis to the effect that contamination entering the fluid at Mulkowal could not have affected nineteen people in the way it did.

The position of the Lister Institute regarding this premise has been determined by experiments on the following two points:—

(1) The first related to the question as to whether it was necessary, in the nature of things, for contamination to have entered the fluid at all; whether it could not have happened, as it used to happen in surgery, that tetanus was conveyed to the patients (and fluid) by a septic instrument, notably the syringe needle.

In my Bombay evidence I dealt with this eventuality as a possible one. Lieutenant-Colonel Semple's Experiment 1, Appendix A (p. 43), led the Commission to the opposite conclusion. By a slight approximation to probable real circumstances, notably by using, not a wet needle, fresh from a momentary dipping into a contaminated fluid and which the operators were not likely to have had at hand, but one on which contaminated matter had been allowed time to dry, the Lister Institute obtained different results. They ejected with such a needle a syringeful of harmless fluid, in twenty doses of 1 cc. each, and ascertained that tetanus germs were conveyed down to seventeen consecutive ejections, the eighteenth and nineteenth not having been tried. After filling the syringe with carbolic lotion and emptying it in the way it was done at Mulkowal, at the end of bottle 53 N, there occurred what the Indian Commission had considered as a remarkable circumstance, notably, no tetanus germs were further obtained from that needle (Appendix G to the Lister Institute's Report, Experiment 2) (p. 48). It is true that the writer of the report, in summing up the above result, used the following terms: "This experiment shows that a seriously contaminated needle (dried), under the test conditions, does not give uniform infective results, either before or after the use of the carbolic solution. The infective material tends to disappear at the end of the ejection of the first syringeful and has disappeared after carbolic washing."

But the actual result was there, and it was obvious that the needle used was far from presenting the utmost limits of contamination. Also in the Mulkowal circumstances there were conditions aggravating the case of the patients such as were not present in the Laboratory Experiment (*vide* notes on p. 57, under (7) and (8)).

Consequently, in accordance with their experiments, *the Indian Commission had pronounced it as established that the tetanus impurity was primarily in the fluid*; whereas the Lister Institute was able to follow them only to the extent of accepting this as a probability.

I must add that when discussing the above matter in Bombay I did not know yet how Dr. Elliot had actually dealt with the needle, nor were the facts subsequently described by the compounder as to his procedure at Mulkowal known to me.

(2) The other point was the following: Contamination, whether it came from the needle, forceps, cork, or other source, was brought in contact with the fluid and contaminated the latter. When examining the sample left in the bottle, fifteen days after Mulkowal, Lieutenant-Colonel Semple saw in it a poor growth of tetanus. That growth, when so developed, was sufficient to give the bottle an extremely marked odour.

The facts brought out by the enquiry had indicated that, at the time of using the bottle, there was no such growth. Could it have developed since?

In my evidence in Bombay I stated that it could.

Lieutenant-Colonel Semple tested this in his Experiment 7, Appendix B (p. 44), and concluded that it could not.

The experiments made by the officers of the Lister Institute have amended this result. They saw that the conditions described by Lieutenant-Colonel Semple "did not permit of the growth of a pure culture of tetanus," *i.e.*, of a culture which is never met with in Nature; but that "in those cases where it was sown in association with aerobic saprophytic organisms, growth occurred." The Institute accordingly concluded: "We think, therefore, that Lieutenant-Colonel Semple's experiments cannot be held to exclude the possibility of contamination at the time of the opening of the bottle" (p. 45).

The above may be summed up as follows: The Indian Commission had divided their discussion on the origin of the Mulkowal tetanus into two portions, notably under two alternative heads:—

(*a*) In the first they considered whether contamination had primarily penetrated into the fluid. They concluded that it did, the Lister Institute accepted this only as a probability.

(*b*) In the second half the Commission considered the *time* when contamination penetrated into the fluid, and concluded, on the ground of their *a priori* thesis, that this occurred before the opening at Mulkowal. In this the Lister Institute did not follow them; they negatived the Commission's thesis and abstained from saying anything else.

The Institute's view was conveyed to you by the writer of their Report in the following words:—

First wording: "Although of opinion that the evidence points strongly to the infection being in the bottle at the time of the inoculation, we agree with the Commission that it is quite impossible to determine at what stage in its history, and in what way bottle 53 N became contaminated."

Second wording: "The conclusions of the Institute coincide with those of the Commission that in all probability the tetanus was at the time of the inoculation in the fluid contained in the bottle, but that it is impossible to determine at what stage in its history or in what way bottle 53 N became contaminated."

In the first passage, the initial portion, that beginning with the concessive conjunction "although," expresses what was the Institute's limit of agreement with the Commission; and seems to prepare the reader to find in the next portion the dissentient part.

There it actually is. The Commission had reported that the bottle was contaminated prior to its Mulkowal opening, and indicated three *laboratory* stages between which they thought it impossible to chose, but one of which was, in their opinion, the origin of the mischief.

The writer of the Institute's Report, without drawing undue attention to the matter, included the Mulkowal handling among the bottle's "stages of history"; and to render the pronouncement less obtrusive prefaced it with the words "we agree."

In the second passage, which was to go forth as the Institute's conclusion, he transferred the words of agreement from the middle to the top, and embraced both statements, the one in which the Institute disagreed with the Commission in considering the subject matter as a fact, and admitted it only as a probability; and the other, in which they disagreed with the Commission fundamentally.

When the above was published to the world, every one understood that two scientific bodies, the Indian Commission and the Lister Institute, had found that tetanus contamination which caused the loss of nineteen lives occurred in the laboratory.

In November last, when the Director of the Institute was asked by you to explain more clearly the Institute's position, he went a step further than the Report warranted him. For, on finding, as mentioned above, that the Mulkowal accident was possible without contamination having ever reached the fluid, the Institute had abstained from dealing with the question as to when that contamination had probably reached it. Had it wished to do so, I presume that it would have proceeded not by speculation, but by examining in detail, and making a clear pronouncement on, the facts which bear on that question.

The Mulkowal bottle had been in the Punjab for twenty-six days, and in possession of the Mulkowal operators since at least the week before the accident (*vide* Dr. Elliot's evidence); and as the Director of the Lister Institute has now ventured the surmise that that bottle had probably been contaminated prior to its opening for use, I examine the Institute's Report in order to see whether there are indicated in it any circumstances, occurring within a day or two of the accident, and which were more directly conducive to contamination than were the circumstances at the time of using the bottle (*vide* pp. 39 and 40); or else to see whether the Institute negatives the facts indicating that the bottle had been free from tetanus up to a maximum of three days of the accident.

As was indeed likely, nothing is mentioned in the Report on the first alternative.

Regarding the second, I find there the following references to the facts in question.

(1) and (2) Those detailed in the text of this letter under B and C on pp. 49 to 51 are not mentioned.

(3) Regarding the condition of the bottle as examined at Mulkowal (Section A, p. 49 and Appendix 2, p. 54, of this letter) the Institute says: "From consideration of the evidence, and in the light of experiments (*vide* Appendix F), the Institute agrees with the Commission that in all probability the tetanus was at the time of the inoculation in the fluid contained in the bottle, but the fact that a bottle presumably tightly corked (*vide* Dr. Elliot's evidence)

should contain enough tetanus growth to destroy nineteen people, and yet not be accompanied by sufficient smell to arouse the suspicion of Dr. Elliot, who, according to his evidence, remembers smelling this particular bottle, is difficult to comprehend."

From the Institute's declarations it is patent that in this passage the word "fact" is put in in place of "suggestion." For the whole matter of enquiry, the question asked of the Institute was as to whether it was a fact that bottle 53 N contained a "growth" (a culture) at the time of using it; and the Institute declared that it could not be considered as a fact. The first passage of the Report contains, therefore a refutation of the Indian Commission's pronouncement, but the writer of it mixed up the issues. The corresponding portion of the passage should have been worded approximately as follows : "But the suggestion that it had entered the fluid previously and that there was a four to seven weeks' old growth in the bottle, and yet not to be accompanied by a sufficient smell," &c. . . .

Further, whether a bottle may contain enough germs to cause nineteen tetanus cases and yet not be accompanied by a smell, is evident from the Indian Commission's Experiment 5, Appendix B (p. 43), one contaminated "loop," brought in contact with a bottle of prophylactic, gave it enough tetanus for affecting thirty animals; but that bottle most assuredly had not the slightest perceptible vestige of a smell. If "growth" had been allowed to take place in that bottle, a smell would have rapidly developed, as it developed in bottle 53 N after its handling at Mulkowal. At the latter moment bottle 53 N was in an analogous position to that of Lieutenant-Colonel Semple's bottle No. 3 (p. 43) in that there was no smell in it; in accordance with this, no growth had taken place in it; yet it contained enough tetanus to affect nineteen (in Lieutenant-Colonel Semple's case thirty) subjects.

(4) In connection with the matter considered above under D (p. 51) the Report does not defer nor object to the Commission's decisive Experiment 2, Appendix B (p. 43). Instead, it makes certain remarks simulating some controversy with me. The remarks are irrelevant. In the experiments regarding the number of germs required to cause tetanus to a guinea-pig (p. 47), the Report deals with a culture artificially weakened in its effect on animals, instead of with a microbe of such activity as was found at Mulkowal. Then the culture is taken in a pure condition which is never encountered in Nature, and the animals are not prepared as the men were prepared at Mulkowal by the prophylactic inflammation. It is known, since the first discovery of this germ, that tetanus bacilli obtained in an aerobic or imperfect anaerobic culture do not affect animals, or affect them only feebly. In a number of cases an animal appears as an agent more susceptible to an impurity containing a trace of virus than cultivation media are. A guinea-pig injected with a suspicious sputum reveals in it tubercle bacilli when a culture fails to do so; a rat does the same in regard to mere traces of plague bacilli present in dust, and a guinea-pig does the same in regard to the germ of tetanus in a minute grain of earth in which a culture fails to show the presence of a single bacillus, as evident from Vaillard and Rouget's experiments quoted above.

I abstain from other criticisms to which the text of the Institute's Report and of its Appendices is open."

Letter from Lister Institute, dated December 5th, 1906, *vide* p. 34.