ANTIPLAGUE VACCINE AND THE RESULTS OBTAINED FROM ITS USE; WITH AN ANALYSIS OF 158,550 INOCULATIONS.

Thesis for the Degree of M.D.,

by

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ANTIPLAGUE VACCINE AND THE RESULTS OBTAINED FROM ITS USE; WITH AN ANALYSIS OF 158,550 INOCULATIONS.

I. INTRODUCTORY.

Various observers have given at different times varying figures regarding the mortality from plague amongst the uninoculated, and also regarding the incidence and mortality amongst the inoculated.

One of the reasons for the want of uniformity in these figures, probably is that they have been collected from different parts of the countries where plague has prevailed, and the incidence and mortality have been modified by climatic variations.

Another cause, which undoubtedly affects the statistics collected in India, is the great diversity of the races inhabiting the country and the differences in their habits and social customs.

Again, in many cases in which the incidence and mortality amongst inoculated and uninoculated have been compared, the proportion of the former to the total population has been too small for the comparison to be of real value.

For these reasons it is of interest to analyse the results of a large number of cases occurring both amongst /
amongst the inoculated and uninoculated during the course of one epidemic in a single district, where the climatic conditions were uniform and the habits of the people more or less similar however they differed in race, and where a larger than usual proportion of people had been inoculated.

The following embodies the results of the use of antiplague vaccine in 158,550 cases in one district (Hoshiarpur) during the inoculation campaign of 1902-03, carried out while the plague epidemic of that season was at its height.

Hoshiarpur is a submontaine district in the Punjab with a population of some 989,782 according to the census, and had been plague infected since 1897. During the plague season in question one in every six of the inhabitants were inoculated against plague.

In 1902 the Punjab Government, being convinced of the efficacy of antiplague inoculation as a measure diminishing both the incidence and mortality of the disease, conceived the idea of an antiplague inoculation campaign throughout the Punjab, hoping that a large proportion of the people might be induced to accept it. Unfortunately this hope was not realized, as in none of the other districts was there anything like the same proportion of people inoculated as in Hoshiarpur.

As a sufficient number of officers of the Indian Medical/
Medical Service were not available to carry out the operations in question, thirty seven medical men were engaged in England to complete the number requisite. A certain number of these were drafted to each district and each provided with an interpreter. An officer of the Indian Medical Service was also sent to each district to carry out the administrative part of the work in addition to inoculating.

I was sent to the Hoshiarpur district along with five of the men engaged in England and a native assistant surgeon.

The work of inoculation began on the 1st October, 1902, and was carried on until the 10th May, 1903, that is, before, during, and after the plague epidemic of that season.

II. THE VACCINE.

1. The antiplague vaccine used was that prepared after Haffkine's method in the Plague Research Laboratory, Bombay.

Antiplague vaccine was first prepared by Haffkine in 1897, and its composition and effects were enquired into by the Indian Plague Commission, which decided that its employment was free from danger and injurious after/
after effects (Report of the Indian Plague Commission, Chapter IV). It is a culture of the plague bacillus grown on broth for six weeks, the bacilli then being killed, sterilised and 0.5% carabolic acid added.

2. Preparation of the broth.

Up to September 1902 it was prepared as follows:-

Goat's flesh is minced and mixed with pure hydrochloric acid (1 kilogramme of flesh with 80 cubic centimetres of hydrochloric acid). This is kept at 70°C for three days. By this means the native albumins of the meat are hydrolysed to proteoses and peptones. The resulting fluid is now diluted, 9 litres of water being added for each kilogramme of meat used. This is neutralised with caustic potash and sterilised for an hour at 100°C.

The fluid is now filtered and the filtrate heated to precipitate the phosphates and coagulable proteins. It is again filtered, sterilised in flasks plugged with cotton wool, and is now ready for use. The broth contains 0.5% sodium chloride from the action of the hydrochloric acid on the caustic soda.

It is also prepared in a similar way using wheat flour instead of meat on account of caste prejudice against the use of meat.

When prepared the broth is a fluid, clear, and of an/
an amber colour.

Agar-agar has also been used as a medium for the growth of the bacillus.

3. Isolation of the bacillus.

The bacillus is obtained from the bubo or blood of a plague patient and sown on some of the broth to which agar-agar has been added. After it has grown, it is identified, isolated, and cultivated for a fortnight.

4. Incubation.

From this growth flasks containing each a litre of the broth are sown with plague bacilli. These flasks are kept in the dark for six weeks to allow of the growth of the bacilli, and at the end of that time the broth is seen to have become turbid.

5. Testing of purity.

A sample of each flask is taken and sown on agar-agar, and the resulting growth examined for the presence of extraneous organisms.


If the cultures are found to be pure, the flasks are heated to 55°C for 15 minutes to kill the living organisms.

7. Carbolising the vaccine.

Carbolic acid to the amount of .5% is now added to
6. The contents of each flask to render the vaccine an unsuitable medium for the growth of bacteria.

8. Bottling.

The bottles are exhausted of air by means of an air pump and hermetically sealed, then sterilised for three hours at a temperature of 180°C.

A sterilised syphon attached to a sterilised glass bulb with a rubber diaphragm is now introduced into the flask. The drawn out hermetically sealed end of the bottle is introduced through the rubber, after sterilising the former at a Bunsen flame, and broken off against a metal tube inside the bulb. The vaccine now rushes into the bottle which is withdrawn from the bulb and hermetically sealed.


The bottles are set aside for a week and then two of those which have been filled from each flask are examined both aerobically and anaerobically.

10. A little of the vaccine in the bottle is now allowed to run into the long neck of each bottle, and this is separated off hermetically from the bottle by means of a blow-pipe and kept as a sample.

In September 1902 the Director of the Plague Research Laboratory, Bombay, omitted the addition of the carbolic/
carbolic acid.

This was done to ensure greater rapidity of manufacture, as the large demands of the Punjab Government for supply told heavily on the resources of the Laboratory, and the other methods of sterilisation were considered by him to be sufficient to ensure sterility.

However on the 6th November, 1902, at the village of Mulkowal in the Punjab, 19 persons who were inoculated from a single bottle developed tetanus and died.

A commission was appointed to enquire into the occurrence, and came to the conclusion that the tetanus bacillus was introduced into the bottle before opening, and that the omission of the addition of carbolic acid to the vaccine was a mistake. The Lister Institute expressed its agreement with this opinion.

III. METHOD OF INOCULATION.

1. The bottle of vaccine is examined for any abnormal features such as unusual colour of the vaccine, presence of foreign bodies in it, cracks or flaws on the bottle, and if any of these be observed the bottle is to be rejected. The bottle is now shaken to mix the sediment which consists of dead bacteria. The bottle is opened by heating the pointed end in a flame, and jerking/
jerk ing the vaccine on to the heated glass which cracks, and a tap with a pair of forceps is sufficient to knock the neck off the bottle. If the vaccine seems to have an unusual odour it is to be rejected. If not, the needle of the sterilised syringe is introduced into the bottle and the syringe filled.

2. Sterilisation of syringe and needles.

Olive oil or vaseline is heated over a spirit lamp and the needles placed in this. When the temperature of the oil or vaseline reaches 160°C, which may be judged by the fact that at this temperature a crumb of white bread begins to turn brown, the needle is picked out of the oil or vaseline with forceps and adjusted to the nozzle of the syringe. The latter is now filled with hot oil, which is again ejected from the syringe. Before introducing the needle into the bottle of vaccine the needle is again dipped into the heated oil and the syringe allowed to cool.

3. Dosage of Vaccine.

The average dose for an adult male is 4 cubic centimetres, and the fractions of this dose are given for different ages as follows:-

<table>
<thead>
<tr>
<th>Age</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 2 years</td>
<td>$\frac{1}{5}$</td>
</tr>
<tr>
<td>2 to 5 years</td>
<td>$\frac{2}{5}$</td>
</tr>
<tr>
<td>6 to 11 years</td>
<td>$\frac{3}{5}$</td>
</tr>
<tr>
<td>12 to 15 years</td>
<td>$\frac{4}{5}$</td>
</tr>
<tr>
<td>16 to 50 years</td>
<td>full dose</td>
</tr>
</tbody>
</table>
Patients over 50 years of age should be given $\frac{1}{10}$ of the adult dose less for each decade over that age. Females should get $\frac{1}{10}$ less than males of corresponding ages.

4. Operation of Inoculation.

The best site for inoculation is on the left upper arm a little above and behind the insertion of the deltoid muscle. The left arm is chosen so that any subsequent pain or swelling at the seat of inoculation may not interfere with the use of the right arm in the patient's usual vocation.

The skin over the spot chosen is first scrubbed with soap and water and then with 1 in 20 carbolic lotion; the arm is then gripped between the fingers and thumb of the left hand in such a way as to draw the skin tight over the selected spot. The needle is entered in a sloping direction into the subcutaneous tissue and the vaccine injected slowly. The needle is then withdrawn and a pad of lint soaked in 1 in 20 carbolic applied. Between each inoculation the needle of the syringe is sterilised by dipping it into the oil at 160°C.
IV. CONTRAINDICATIONS TO INOCULATION.

These are obvious, namely apparent ill health due to malaria or acute disease of any kind.

However, patients suffering from chronic disease bear inoculation well. Pregnancy unless far advanced is not a contraindication.

V. SYMPTOMS CAUSED BY INOCULATION.

In about 4 hours after the inoculation, in the majority of cases, pain and swelling occur at the site of inoculation; there is also a rise of temperature, sometimes reaching to 103°F., and general malaise.

The general symptoms usually last for 36 hours and then subside. The local symptoms last for about 4 days. In the cases coming under my observation 27 showed an induration at the site of inoculation which persisted for months, but without pain. In none of my cases did abscess formation occur, although this complication has been noted by some.

Indeed considering the primitive ideas which the native of India, as a rule, possesses regarding cleanliness, treatment of wounds etc., the operation of inoculation is attended with exceedingly few untoward results.
VI. EFFECT OF INOCULATION ON PLAGUE INCIDENCE
AND MORTALITY.

The percentage mortality from plague seems to vary
in different epidemics. Thus in the Bombay epidemic
of 1896-97 the average mortality was 61.5%. In the epidemic of 1897-98 it was 78.5% and in that of 1898-99, 78.97% to 81.4%.

The average mortality as given by Chowsky (Treatment of plague with Professor Lustig's Serum, Chowsky, Bombay, 1903) is 80%.

In India the average mortality is from 70 to 85% and seems to be affected by various climatic influences, for example:-
1. Susceptible races may become less susceptible to plague out of their own country.
2. Susceptibility may vary in the same race in different localities (Simpson, Treatise on Plague, 1905).

The figures collected up to the present have not been sufficiently large to enable one to give any uniform expression of the reduction in incidence and mortality amongst the inoculated.

Perhaps the most complete statistics on the subject are/
are those collected by Major Bannerman, Indian Medical Service, Superintendent of the Plague Research Laboratory, Bombay, (British Medical Journal, September 14th 1901), and these only extend to some 924 attacks of plague in the inoculated.

The Indian Plague Commission in its Report Volume V. states that:—

1. "Inoculation sensibly diminishes the incidence of plague attacks on the inoculated population, but the protection which is afforded against attacks is not absolute.

On the one hand plague has attacked persons who have undergone inoculation as many as four times in the course of two years previous to their attack. On the other hand as many as 8% of the inoculated population may suffer from plague, as was the case in Bulsar. Many varying influences have been at work in determining the rate of attack in different places, and it is impossible to give a numerical expression for the measure of protection against attack which inoculation confers."

2. "Inoculation diminishes the death rate among the inoculated population. This is due not only to the fact that the rate of attack is diminished, but also to the/
the fact that the fatality of attacks is diminished. Here again no numerical expression for the amount by which the death rate is diminished can be given."

To endeavour to elucidate these points 710 villages were chosen in which both plague had broken out, and in which inoculations were performed. These villages had a total population of 466,836, and of this number 120,574 were inoculated, this being a proportion of one inoculated to 3.91 uninoculated.

The following table shows the period of the epidemic in which the inoculations were performed.

<table>
<thead>
<tr>
<th>Months</th>
<th>Number of Villages infected.</th>
<th>Number of Villages inoculated for the first time.</th>
<th>Number of inoculations performed.</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>42</td>
<td>11</td>
<td>2,144</td>
</tr>
<tr>
<td>November</td>
<td>47</td>
<td>7</td>
<td>951</td>
</tr>
<tr>
<td>December</td>
<td>111</td>
<td>23</td>
<td>2,660</td>
</tr>
<tr>
<td>January</td>
<td>83</td>
<td>100</td>
<td>10,337</td>
</tr>
<tr>
<td>February</td>
<td>128</td>
<td>231</td>
<td>48,403</td>
</tr>
<tr>
<td>March</td>
<td>136</td>
<td>266</td>
<td>53,340</td>
</tr>
<tr>
<td>April</td>
<td>163</td>
<td>72</td>
<td>2,730</td>
</tr>
<tr>
<td>Total</td>
<td>710</td>
<td>710</td>
<td>120,574</td>
</tr>
</tbody>
</table>
Out of this population of 466,836 the percentage rate of incidence among the uninoculated and inoculated respectively was 8.6 and 1.6. This and other particulars are shown in the following table.

### TABLE II.

<table>
<thead>
<tr>
<th>Classes</th>
<th>Number in each</th>
<th>Cases of Plague</th>
<th>Attack rate %</th>
<th>Deaths</th>
<th>Death rate %</th>
<th>Case mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Uninoculated</td>
<td>346,262</td>
<td>30011</td>
<td>8.6</td>
<td>17288</td>
<td>4.9</td>
<td>57.53</td>
</tr>
<tr>
<td>II. Inoculated</td>
<td>120,574</td>
<td>2063</td>
<td>1.6</td>
<td>313</td>
<td>.25</td>
<td>15.10</td>
</tr>
</tbody>
</table>

Out of the total number inoculated in the district during the epidemic, namely 158,550, 2521 contracted plague. Out of these 461 died giving an attack rate per cent of 1.6, a death rate per cent of .22 and a case mortality per cent of 18.28.

This is shown as follows:

### TABLE III.

<table>
<thead>
<tr>
<th>Number of Plague</th>
<th>Cases of Plague</th>
<th>Attack rate %</th>
<th>Deaths</th>
<th>Death rate %</th>
<th>Case mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>158.550</td>
<td>2521</td>
<td>1.6</td>
<td>461</td>
<td>.28</td>
<td>18.28</td>
</tr>
</tbody>
</table>
The above figures are of value inasmuch as the inoculations were all performed during one plague epidemic and in the same district, under which conditions the different influences determining the rate of attack were as nearly equal as could possibly be obtained.

VII. EFFECT OF INOCULATION DURING THE INCUBATION PERIOD.

In their Report the Indian Plague Commission points out that inoculation does not appear to confer any great degree of protection within the first few days after inoculation has been performed.

Calmette before the International Congress of Hygiene and Demography at Paris in September 1900, and also in the Herben Lecture in London on the 24th November, 1900, (British Medical Journal 27th October, 1900) made the following statement:

"A person in the period of incubation for a slight attack of plague would find the disease considerably aggravated if he submitted during this period to a preventive inoculation of Haffkine's vaccine. The case would almost certainly end fatally."

However/
However the following table, giving cases, deaths, and case mortality per cent in those contracting the disease at periods varying from one to seven days after inoculation, refutes this opinion, as well as showing that the protection afforded by inoculation takes some time to develop, increases as the time between inoculation and attack increases, up to 50 days. It also shows that even in the early days after inoculation a certain protection is afforded.

**TABLE IV.**

Showing time of attack after inoculation in 457 cases with 123 deaths.

<table>
<thead>
<tr>
<th>Time after inoculation</th>
<th>Number of cases</th>
<th>Deaths</th>
<th>Case mortality per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 7 days</td>
<td>82</td>
<td>25</td>
<td>30.4</td>
</tr>
<tr>
<td>7 to 14 &quot;</td>
<td>66</td>
<td>18</td>
<td>27.2</td>
</tr>
<tr>
<td>14 to 21 &quot;</td>
<td>86</td>
<td>23</td>
<td>26.7</td>
</tr>
<tr>
<td>21 to 30 &quot;</td>
<td>223</td>
<td>57</td>
<td>25.5</td>
</tr>
<tr>
<td>Total</td>
<td>457</td>
<td>123</td>
<td></td>
</tr>
</tbody>
</table>
The case mortality here shown in the first seven days, namely, 30.4\% compares very favourably with the percentage case mortality shown amongst the uninoculated in table II. (viz. 57.53\%).

VIII. LENGTH OF TIME PROTECTION LASTS.

The questions now arise, How long does it take for the full protection obtainable to develop? How long does it last? So far as personal observations go I can only present statistics on these points up to a period of eight months or so after inoculation.

The following table brings out the points at issue sufficiently.

**TABLE V.**

Time of attack after inoculation in the 2521 cases and 461 deaths shown in table III.

<table>
<thead>
<tr>
<th>Time of incidence of plague after inoculation</th>
<th>Cases</th>
<th>Percentage of cases to total</th>
<th>Deaths</th>
<th>Death rate %</th>
<th>Case Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 30 days</td>
<td>457</td>
<td>18.12</td>
<td>123</td>
<td>4.87</td>
<td>26.9</td>
</tr>
<tr>
<td>1 to 2 months</td>
<td>396</td>
<td>15.7</td>
<td>50</td>
<td>1.98</td>
<td>12.6</td>
</tr>
<tr>
<td>2 to 3</td>
<td>511</td>
<td>20.2</td>
<td>81</td>
<td>3.21</td>
<td>15.6</td>
</tr>
<tr>
<td>3 to 4</td>
<td>378</td>
<td>14.9</td>
<td>76</td>
<td>3.01</td>
<td>20.1</td>
</tr>
<tr>
<td>4 to 5</td>
<td>337</td>
<td>13.3</td>
<td>38</td>
<td>1.52</td>
<td>11.2</td>
</tr>
<tr>
<td>5 to 6</td>
<td>147</td>
<td>5.8</td>
<td>35</td>
<td>1.38</td>
<td>23.8</td>
</tr>
<tr>
<td>Over 6</td>
<td>295</td>
<td>11.7</td>
<td>58</td>
<td>2.30</td>
<td>19.6</td>
</tr>
<tr>
<td>Total</td>
<td>2521</td>
<td>461</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
IX. CONCLUSIONS.

1. Inoculation though causing a temporary inconvenience is without subsequent immediate or remote ill effects.
2. It lessens the incidence of plague attacks.
3. It lessens the death rate and reduces case mortality.
4. Even when performed during the incubation period of the disease it is distinctly protective, and lessens case mortality.
5. The protection afforded increases up to at least six months after the period of inoculation.