STUDIES ON CERTAIN PROPHYLACTIC MEASURES

AGAINST THE SPREAD OF

BUBONIC PLAGUE

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NOTE.

Much of the original work incorporated in this Thesis has already been published by me in the following papers:-

1. A report on the use of Leybold's portable apparatus for the destruction of rats by the use of carbon monoxide gas submitted to and printed by the Government of India in 1909.

2. "A preliminary report on the killing of rats and rat fleas" Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India No.38, 1910.

3. "A note on the action of hydrocyanic acid gas on grain and especially on rice", Proceedings of the All-India Sanitary Conference of 1911.

4. "The use and advantages of hydrocyanic acid gas as a disinfectant for plague infected houses and ships." (In conjunction with Major W. Glen Liston, C.I.E.,M.D., D.P.H.,I.M.S. and Captain J. Taylor M.D.,D.P.H.,I.M.S.)

5. Some of the experiments upon the factors which influence the immunising value of Haffkine's anti-plague vaccine were published in the annual report of the Bombay Bacteriological Laboratory for the year 1911.

6. "The absence of a "negative phase" after inoculation with plague prophylactic (anti-plague vaccine)" Proceedings of the All-India Sanitary Conference 1912.

I N D E X

INTRODUCTION.

CERTAIN PROPHYLACTIC MEASURES AGAINST THE SPREAD OF BUBONIC PLAGUE.

It is not the object of this Thesis to consider all the anti-plague measures which might be adopted during an epidemic as this subject is too wide to be dealt with at all fully in one paper. I have personally had little experience of the practical outcome of these measures in field work, and I could not pretend to judge between the practicability of the various methods adopted. This depends not only upon the inherent value of the method but upon its advisability in view of the social and religious prejudices of natives in various parts of India. I desire to present here the outcome of various experiments and observations made under laboratory conditions, some of which have been published already. I have started with the assumption which now has been proved by the labours of the present Indian Plague Commission (published in the plague numbers of the Journal of Hygiene) that bubonic plague is a rat disease spread from rat to rat by fleas (chiefly in 99% of cases in India by the agency of/Xenopsylla Cheopis formerly termed the pulex cheopis) and that it is generallyby the agency of rat fleas that the disease is communicated to man. I am aware that this assumption is in some quarters still held as not proven, but in my view there is no reasonable ground for such doubt and I do not intend to recapitulate the proofs given.

Prophylactic measures against bubonic plague would therefore aim at the following results (1) rat and flea destruction in infected houses, clothing, and grain.

The destruction of rats aims at the root of the evil and various methods have been proposed, as by the use of chemical poisons such as:-

"Common sense rat exterminator", Swiz etc. The chief ingredient in most of these preparations is phosphorus. Other methods suggested to reduce numbers of rats have been the propagation of bacterial diseases among them (by Danysz Virus), trapping and killing rats when caught, and lastly the fumigation of infected houses by various gases. The destruction of fleas in plague-infected houses has been obtained by spraying the walls of houses by emulsions of petroleum and other oily liquids, and by the use of various gases as sulphur dioxide gas. An important subject, which I will touch on again in considering the use of hydrocyanic acid gas as a fumigating agent, is the question of the disinfection of clothing of persons coming into a healthy area from an infected one so as to kill infected fleas.

(2) A permanent safeguard against many of the dangers of plague infection would be the erection of well-built houses in which special attention would be paid to the roofs, walls, and floors, so that they were constructed of such materials that rats would not harbour in them. The importance of such houses in the prevention of the spread of plague in large districts was brought out in the Commission report on the absence of any epidemic of plague in Assam owing to the nature of the housing conditions of the people. This may be taken also as one of the chief causes of the immunity to plague shown by Europeans in India.

It should be the aim in the construction of such houses to keep them separate from grain or food shops or stores. Unfortunately, as is brought out in many of the commission reports, it is a common thing in the Punjab and other parts of India to have as a part of the house a grain store or godown which is always infested with rats. When it is necessary to build such godowns they ought to be constructed of concrete and made rat-proof. The system of scavenging ought to be paid special attention to, and cattle, goats etc. ought not to be harboured inside houses. According to Liston[1], health officers in some of the large towns of India are trying to obtain legal powers to amend the present bad housing conditions of the people.

(3) The evacuation of infected houses.

The utility of this measure has been much discussed; in some parts of India it is the means most adopted to stamp out plague. It has been shown by the plague research commission that in the bodies of infected rats the increase of saprophytic bacteria causes a disappearance of the plague bacilli in about 48 hours after death. Further in a climate like Bombay highly susceptible animals allowed to run about on floors contaminated by plague cultures, 24 hours previously, did not become infected. Rat fleas, however infected with plague, remain infected for a considerable period. There are two methods then of insuring the safety of houses evacuated after infection. (a) By allowing a sufficient length of time to elapse so that infected fleas may die a natural death. The safety of reentrance into these houses could then be gauged by first introducing susceptible animals and noting the effect on them.

[1] Plague preventive measures
The proceedings of the All-India Sanitary Conference, Madras 1912.

In connection with this it is important to know the average length of life of fleas apart from their hosts. This subject is considered in the Journal of Hygiene by the Plague Commission[1]. The length of life depends both upon the humidity and the temperature of the atmosphere, especially upon the former. The longest life in some experiments performed in Poona was during August when the humidity was over 80%. The average life of an unfed flea was then 4.3 to 4.6. days. The shortest life was in April when the humidity fell to 45%, 0.7 to 0.9 days. As regards temperature, extremes of heat and cold were unfavourable to life; a moderately cool temperature favours life most. It is obvious that there are two disadvantages in this procedure _____ the length of time nature would take to disinfect the rooms and the difficulty of applying animal tests safely in the out-districts. (b) by the use of artificial methods of disinfection aimed against both flea and rat

(4) Lastly as a measure of personal prophylaxis there is the use of protective vaccines.

In this thesis I have considered two lines of protective work, (1) the use of certain gases for the fumigation of infected houses, godowns, and ships. (2) The various methods of preparing an anti-plague vaccine with special reference to Haffkine's method. In the second division I desire to quote original work on factors which increase the immunising value of the vaccine as shown (a) by animal experiments and (b) by statistics of actual anti-plague campaigns. Further, I have added a paper on the question of existence or non-existence of a negative phase or period of increased susceptibility to infection immediately after immunisation.

[1]Journal of Hygiene Plague Supplement II, January 1913.

This last question has an important bearing on the value of inoculation during an epidemic.

The use of various gases for the fumigation m of houses, godowns, and ships to get rid of plague-infected fleas and rats.

SULPHUR DIOXIDE.

So far gases have been utilised by Port Health Officers for the disinfection of plague-infected ships. In Bombay, as in many other ports both in England and France, sulphur dioxide has been the gas used, and by this time the limits of its action upon rats and fleas as well as on the cargoes and materials of ships have been well defined. I do not intend to go minutely into the work done with this gas as I have had no personal experience of its use. Dr. Wade[1] of the Local Govermment Board came to certain conclusions as to its use which I will briefly recapitulate. (1) Rats, insects, and bacteria like B. Typhosus are killed by sulphur dioxide gas in a dilution of 0.4% if exposed to it for one hour. (2) Exposure to a dilute sulphur dioxide gas for a lengthy time is more efficacious than exposure to a more concentrated form of the gas for a shorter time. (3) As much of the gas is absorbed by the various cargoes, a 3% concentration at the commencement is necessary. (4) Penetration by sulphur dioxide into the interior of bales (except bales of cotton) cannot be affected by sulphur dioxide in any reasonable time, and the same is probably true of the interior of grain bags and grain in bulk. Wade writes: "It follows that disinfection of the interior of absorbent bales and of grain is at present impracticable". He evidently considers this disability to be of little importance, as rats can only penetrate cargo through "Crevices of appreciable dimensions", and their fleas must starve if they leave their hosts. As it seems to me that the problem of thorough disinfection of ship-holds largely hinged on

[1] "Report on further experiments on sulphur dioxide as applied to the destruction of rats and in disinfection on shipboard" by J. Wade D.Sc. L.G.B. Reports 1905-6.

this question of the habits of rats with respect to burrowing into grain bags etc., and on the depth to which fleas may burrow into grain in bulk, I made some observations which I will detail later (p.55) I showed that rats do not burrow during fumigation by hydrocyanic acid gas into the interior of bags of rice, and that fleas do not escape the effects of fumigation by hydrocyanic acid gas by burrowing into grain in bulk.

The administrative problem therefore, as Wade correctly surmised, will be solved if we get a gas which penetrates into "Crevices of appreciable dimensions", and there is no object in searching for a gas which will penetrate into the depth of grain bags or grain in bulk. Wade points out that sulphur dioxide in dilutions even of 8% does not penetrate grain in bulk to more than a few inches from the surface after exposure of 24 hours. Wade finally concluded that an average-loaded hold would be thoroughly disinfected by fumigation with 3% sulphur dioxide for 8 to 10 hours' exposure provided that the hold be left closed till the next day.

The disadvantages of the gas must now be noted. Wade acknowledges that "the problem of fumigating a ship in order to destroy rats etc., without damaging the cargo is as yet unsolved" Those strengths of the gas which are efficacious viz. 3% for 8 to 12 hours, are injurious to wheat, but leave barley and maize unaffected. Nocht and Giemsa[1] Consider the practicability of the various means of exhibiting sulphur dioxide, (1) by burning sulphur 20 lbs in conjunction with charcoal 40 lbs. for every thousand cubic metres of space.

There is danger of fire.

[1] On the destruction of rats on board ship as a preventive of plague infection by Dr. Nocht and Dr. G. Giemsa from the Journal of the Imperial Board of Health vol.20 part I. 1903. (Arbeiten aus dem Kaiserlichen Gesundheitsamte Band XX, Heft 2, 1903).

(2) Piktolin (invented by R. Pictet)

The combination of sulphurous acid gas and carbonic acid gas can be obtained in liquid form, but during disinfection with it holds should be emptied of cargo as the gas does not penetrate. The cost is double that of smoking out by burning sulphur, as in winter 4 lbs and in summer 2 lbs are necessary for every hundred cubic metres of cargo space.

(3) Clayton gas (a mixture of sulphurous and sulphuric acid gas obtained by burning sulphur at a high temperature in special machines) is the method used mostly in England. While the gas is innocuous to dry goods and polished metal (except steel) it damages fresh fruit, flour, and meat. Repeated fumigations also do damage to steel walls of ships. The authors decide that it is impossible to use a gas, as one would be liable to claims for damages. As compared with fumigation with hydrocyanic acid gas and carbon monoxide gas it is an expensive method. Sulphur dioxide as generated by a small Clayton machine was tried on Indian huts in Bombay by Gloster in 1908, but he failed to obtain good results, owing to the structure of the average Indian house with its innumerable apertures prohibiting a proper concentration of the gas; "The gas requires to be rapidly generated in large amount and be continuously pumped into the room for a considerable period to be effective". The cost of these operations both in the necessary plant and in working expenses was prohibitive[1].

[1] Report of the Bombay Bacteriological Laboratory 1908.

CARBON DIOXIDE GAS.

Carbon dioxide has been used occasionally for the fumigation of ship-holds. It was used successfully in Marseilles[1]. The carbon dioxide is stored in liquid form in cylinders. According to Nocht and Giemsa, rats can maintain life in an atmosphere containing 30% carbon dioxide, although lights are extinguished in an atmosphere of 12% carbon dioxide, so that a great concentration of gas would be necessary to fumigate successfully; 20% of the cargo space would require to be filled with gas. The authors calculate that to treat a steamer of medium size, say of 3000 cubic metres cargo space, an expenditure of £20 to £25 sterling would be necessary exclusive of the cost of labour, piping, transport, etc. In addition, great difficulties are met with during the actual operations from the frozen barbonic acid plugging up the nozzles of the cylinders.

FORMALDEHYDE.

Formaldehyde vapour has been suggested as a fumigating agent, but it is said in moderate concentrations to be non-toxic to rats and insects.

CARBON MONOXIDE.

The effects of carbon monoxide gas have been tried in Germany by Nocht and Giemsa[1] in 1900 and in England by Haldane[2] in 1902.

[1]Nocht and Giemsa-----Supra.

[2]Haldane - 30th annual report L.G.B. 1902.

I should first of all state that while the rôle of the rat in disseminating plague was well known then, the transmitting action of the flea was unknown. At that time allthat was demanded was a gas lethal to rats. Giemsa and Leybold pointed out first that the so-called "Generator Gas" is rich is carbon monoxide, and can be produced by the imperfect combustion of coke. The Germany Government placed at the disposal of the Hamburg Authorities, at the instigation of the plague committee of the Board of Health, a sum of money to make extensive experiments on sea-going vessels. Most Generator Gases (produced by the action of compressed air with or without steam on burning coke, anthracite or charcoal) are rich in hydrogen as well as in carbon monoxide&carbon dioxide and are highly inflammable. It was the object of the Hamburg Authorities to produce a gas free of hydrogen and composed of such relative proportions of carbon monoxide and carbon dioxide, that it would be non-inflammable. It was shown that when the volume of carbon dioxide was at least double that of carbon monoxide the mixture was non-inflammable. They found that in a generator the carbon monoxide content falls and that of carbon dioxide rises, given a constant blast of air, in proportion to the lessening of the thickness of the layer of coke. By reducing the coke layer to 22 centimetres, using finely broken dry coke, they produced a gas rich in carbon monoxide (5%) with more than 10% carbon dioxide, highly poisonous but non-inflammable. The gas contained on an average carbon monoxide 4.95%; carbon dioxide 18%; nitrogen 77.05%. No oxygen was present, and the specific gravity of the gas as compared with air was 1.08 to 1. The heat of the generator was also used to work pumps and fans to propel the gas into holds or to withdraw it after the end of fumigation. From the large generators 405 cubic metres could be produced in an hour. After fumigation, air free of

carbon monoxide can be restored to holds in six hours. Cabins were opened by operators wearing smoke helmets. Finally as 0.5 parts per mille of carbon monoxide will produce poisoning, and as chemical detection of such amounts is in practice impossible, before entering spaces after fumigation the effects of the air are tested on small animals in cages.

In Haldane's experiments the formation of an explosive compound was avoided by mixing air with the generator gas prior to its introduction into spaces. Wade[1] points out that certain plague-infected ships fumigated with this gas at Hamburg were re-infected. This is no doubt due to the fact that fleas are not killed by the amount of carbon monoxide present in this generator gas. I will detail experiments on this point later (p.24) Wade also mentions the non-lethal action of carbon monoxide on bacteria in insects. He states that "The Hamburg Sanitary Authorities now appear to disinfect ships with sulphur dioxide after killing the rats with carbon monoxide and removing the cargo". In this connection also I would call attention to a paper published by M. Edmond Bongean published in the Bulletin des Sciences Pharmacologiques for January 1908 and quoted in the Lancet of March 21st 1908. He says of this Hamburg process that it is dangerous to man while it does not destroy insects.

A hand machine, a small model of the Leybold's machine for generating carbon monoxide gas and consisting of a generator, a scrubber and fan to draw the gases produced in the generator through the scrubber and to propel the gases through piping, was sent to India in 1908. I made experiments[2] with this machine to test the lethal power of the evolved gases on rats and fleas in Indian houses. With regard to the working arrangements of the machine no instructions

[1] Report on further experiments on Sulphur Dioxide as applied to the destruction of rats and in disinfection on board ship by John Wade D.Sc. Local Govt. Report 1905.6.

[2] I had throughout these experiments the advice of Captain [illegible]

were given to us by the makers as to the rapidity with which the fan should be revolved. We found that the composition of the gases produced, varied as the air was drawn more or less rapidly over the burning coke, in the former case less carbon monoxide being produced. It was found in practice that the fan must be revolved about 90 revolutions per minute at least, to ensure enough pressure to bring the gas through the discharge pipe.

With regard to the fuel to be used in the generator, instructions were sent to use either coke or anthracite coal. In one of our experiments we used the latter but abandoned it in favour of coke, as the gas generated was smoky, whereas by the use of coke a clear, tasteless gas was produced - an advantage in two points ; (1) rats would not be able to know in what direction to flee from it, and (2) the machine is not so liable to get fouled.

With regard to the first point, I might mention that in one of our experiments one rat escaped from its cage. It went through a hole in a door into a neighbouring room, the paper pasted over the hole being eaten through; but it came back into the room into which the gas was being pumped and was found dead there.

The coke we used was a mixture of Australian and Indian coke. It was very porous and was specially picked. The gases were examined for us by the Chemical Analyser to Government.

He stated that the average composition of six samples of gas generated by the Leybold's hand apparatus was:-

Oxygen	... 9.5
Carbon dioxide ...	... 4.35
Carbon monoxide ...	... 4.35
Nitrogen	.:. 81.8

We, therefore, failed to produce an oxygen-free gas, but produced a very poisonous gas with about the average strength of 5 per cent carbon monoxide. My experiments were carried out in two types of rooms.

I. The first experiments detailed were carried out in a comparatively air-tight room, which was chosen in order that the minimum amount of gas, per cubic space, necessary to kill, might be estimated.

It is stated for holds of ships, which can be made comparatively air-tight, that "experiments have shewn that rats under the most favourable conditions in a ship's hold, whether it be loaded or empty, are certainly killed if the amount of introduced gas be half the cubic space and the gas be left in the hold at least 2 hours after stopping up the ventilators.

"If gas of the volume of $\frac{3}{4}$ the cubic space be blown in, the desired result will follow even when ventilation follows shortly after the completed introduction".

The room was of the following dimensions : Length 12 feet 6 inches; breadth 7 feet 8 inches. The roof sloped upwards from a wall 5 feet 8 inches to one of 11 feet 8 inches. The cubic capacity was about 830 cubic feet. The walls were of cement, and the floor was of stamped earth. The roof was of corrugated iron made rat-proof by being firmly fastened to the walls. There was a -

large window 2 fe t 7 inches x 3 feet 6 inches and opposite it a large door 3 fe t 2 inches x 5 feet 6 inches. The window was capable of being tightly closed by shutters and the whole was sealed over with paper; the door leading into another room was also closed and any cracks or obvious holes were sealed over, so that a comparatively air-tight chamber was produced. The results were as follows:-

(1) If the gas to the amount of more than half the cubic space was allowed to remain in contact only two hours and then the polluted air exhausted, only a small percentage of rats were killed.

(2) If the same amount of gas was blown in but allowed to remain in contact over night, all the rats on the floor level were killed.

(3) In these experiments the gas was blown in 1 foot from the ground level. Little diffusion took place into the upper levels of the room, so that rats on the roof would have probably escaped. In later experiments in another godown more airy, presenting conditions of ventilation similar to those obtaining in an ordinary Indian house, the gas was introduced from the roof. The results were not so successful.

(4) Fleas cannot be said to be affected. In ordinary conditions fleas die rapidly. As a control I put 30 fleas in 3 test-tubes in a room overnight and of these 9 died.

This point, that carbon monoxide is non-poisonous to fleas, was absolutely proved later and will be referred to again. It will be seen that in one of the experiments now detailed after the introduction of 1,000 cubic feet of the generated gas into the room of 830 cubic feet, no fleas were killed.

The following are the detailed results:-

Experiment I.

Coke was used as the fuel, 500 cubic feet (over half the cubic capacity of the room) being pumped in through a hole in the top of the window, the delivery tube falling to one foot from the ground. The delivery took one hour five minutes.

The gas was left to diffuse 2½ hours after the delivery had stopped and then by the tube connected with an exhauster of of the Clayton sulphur dioxide machine the air of the room was exhausted.

The room was entered next morning.

Result:-

Of 15 rats put on the floor :

2 were dead.

1 was killed by its neighbours. (In subsequent experiments the rats were put in separate cages).

7 were sick.

Of 30 fleas in test-tubes, open at both ends, and only guarded by some coarse cloth, 11 were dead.[1] The test-tubes were placed in racks on the floor.

As these results were not satisfactory, either it became necessary to introduce more gas or to let the gas remain longer in contact with the animals - that is, use no exhauster at all. For the introduction of fresh air evidently revives the animals, even though in the air of the room immediately after the

[1] It should be noted that fleas in test-tubes may die in the absence of fumigation. A control experiment should have been performed. But the large number of living fleas shows the non-lethal action of the gas for fleas.

introduction of more than the cubic space amount of gas only a trace of carbon monoxide is present - not enough to be measured quantitively. This point is referred to again.

I resolved, therefore, not to use an exhauster at all, and to introduce the delivery pipe with as few curves as possible, as the pressure exerted by the fan being little, any, even small, curves might prevent gas passing. The pipe was let through a hole in the foot of the window.

2. Five hundred cubic feet of gas generated by burning coke were introduced and left in contact all night.

Of 15 rats placed on the floor all died.

Of 30 fleas placed on the floor 12 died.

In addition, to see whether there was much diffusion of gas into the upper levels of the room, four rats were hung on cages at a distance of about 5 feet from the ground. Of these one died and one was very ill in the morning.

To show that there is some diffusion of the gas into neighbouring rooms I would mention the following circumstances:

Some rats were on the floor of the room next to the one experimented on, separated by a door. Two of these rats on the floor were found dead and on section one had lungs of a light salmon colour and both had slight diffuse congestion. But the blood did not show the carbon monoxide spectrum, being reduced to ordinary haemoglobin on adding Stokes Re-agent, so that if they died of carbon monoxide poisoning, very slight amount must have entered into combination with the haemoglobin.

Again at 10-30 a.m., on the experimental room being opened, all the rats - dead and alive - in the room were taken out, and 15 more rats were placed on the floor and were hung at 5 feet

from the ground. Half an hour later one of these at 5 feet from the ground was found to be dead. It also presented a congested and salmon colour appearance of the lungs, but its blood -- spectrum was reduced by Stokes Re-Agent.

There may have been a slight trace of carbon monoxide in the air at the upper levels of the room and if the rats were at all sickly, the small trace might be enough to kill ~~them~~. But I cannot explain why the blood should not show the carbon monoxide spectrum. The blood of the rats placed in the room overnight all showed this spectrum.

3. To ascertain whether by introducing a larger amount of gas the rats on the upper level could be killed, 830 cubic feet were pumped into the room. Out of 5 rats placed at levels of 4 to 5 feet from the ground, only 1 was found dead. 15 fleas out of 30 were found dead and all the 15 rats on the floor as usual died. It is evident, therefore, that there is little diffusion into the upper parts of the room.

4. To show that even 1,000 cubic feet of gas produced by burning coke introduced into this room of 830 cubic feet capacity (the room not being opened till the next morning after the experiment) did not have any effect on fleas; it may be mentioned that out of 30 fleas put in, none were found dead next morning. They were in the open ordinary glass-tubes with not too fine meshing at both ends so that diffusion must have taken place. Fleas loose on the floor could, therefore, much more easily escape as they could bury themselves in the dust. The delivery pipe hung in this experiment 4 inches from the floor and the glass-tubes were in racks placed on the floor. The lower end of the glass-tube did not rest on anything as the tube was suspended

in the middle.

Of 15 rats in cages on the floor all died.

5. When anthracite coal was used instead of coke, 1,000 cubic feet being introduced, out of 30 fleas 20 were found dead in the morning. Possibly the smoke killed them - certainly not the carbon monoxide. The room was not opened till the next morning after the experiment, 22 hours after the delivery of the gas had been completed. Here also out of 15 rats placed on the floor all died.

With regard to the post mortem changes in the rats found dead, it was noted that the extremities of the limbs, the nose and the mouth were of a brighter pink colour than normal.

In all the rats the muscles of the thorax were of a bright pink appearance somewhat suggestive of that found in plague rats. In nearly all cases examined there was a general lung, peritoneal, and intestinal congestion.

In many cases the blood was examined and the carbon monoxide spectrum was obtained, the reducing fluid used being Stokes Reagent. But in certain cases the carbon monoxide spectrum was not decidedly obtained.

II. Experiments in the larger room:-

This room presented much more the conditions which hold in a native hut. Its length was 12 feet 6 inches; breadth 15 feet 7 inches; height at one end 5 feet 6 inches, at the other end 11 feet 4 inches. The cubic capacity was about 1,640 cubic feet. The floor was of stamped earth and the roof of country tiles.

Between the edge of the roof and the smaller end wall a small space communicated with the outside. There was also very free ventilation between the edges of the tiles. There was a large window which could be well closed with shutters and two doors, one of which communicated with the outside and one with the room described above.

In the following experiments as many of the larger ventilating holes as could be filled up were closed with tightly-packed paper and the room was made as air-tight as most native huts can be made.

In the floor of the room four types of rat burrows were -- laid out. The burrows consisted of a small wooden box to contain rats and fleas and a channel leading from it to the floor of the room. The dimensions of the boxes were; 21 inches by 10¾ inches by 12 inches. They were fitted with hinged lid and sunk in the grouhd. Inside the box was a rack to hold glass-tubes containing fleas. The cage for the rats could be placed inside the box easily.

The channels were made of a series of short sections of earthen pipes sunk in a trench. Each section was made up by concave country tiles placed together to form a somewhat tapering pipe, the narrow end of one section fitting into the broad end of the other. The narrow end of the lowest of these sections projected slightly into the box.

Four types of burrows were made. There were:-

Type I.- A blind burrow with a single opening.

Type II.- Onehole with two burrows leading to it with S-shaped curves on the horizontal.

Type III.- Same as II but with the curves vertical.

Type IV.- A single burrow with the cage outside the room

for observation.

Over the tiles 6 inches of earth were placed, and over the boxes, after the rats and fleas had been put in, about 3 inches of earth.

Experiment VI.

The delivery pipe lay on the floor. Four rats were put on the floors.

Four rats were hung up about 4 to 5 feet from the ground.

One rat was put in each burrow box.

10 fleas were put in glass-tubes in each burrow box.

870 cubic feet of gas were pumped in - a little over half the cubic capacity of the room.

After 650 cubic feet had been put in, the box outside the room was opened (Type IV) and the rat was found to be dead. The room was opened next morning and the following results found:-

All the rats on the floor were dead. Of the 4 rats hung up <u>none</u> were dead, but 3 died next day.

In burrow I the rat was dead and 5 fleas out of 10 were found dead.

In burrow II rat living; 2 fleas out of 10 dead.

In burrow III rat dead; 6 fleas out of 10 dead.

In burrow IV rat dead after 650 cubic feet were put in.

The flea count was a failure as the test-tube was broken.

Experiment VII -

In this experiment the conditions of rooms and burrows were the same as in VI, but the delivery tube instead of lying on the floor as in VI was made to discharge into the room 5 feet from the ground. One thousand cubic feet of gas were introduced. It was also decided to allow free rats and fleas into the room.

Five guinea pigs were first of all put into the room to clear it of fleas[1] and later 45 fleas were let loose.

Five loose rats were put in.

A rat was placed in each burrow box and also a glass-tube with 10 fleas.

(Unfortunately the flea count in the glass-tubes in this experiment cannot be relied on as by an oversight instead of being open at both ends ordinary test-tubes were used).

Four rats were put on the floor in cages.

One test-tube containing 20 fleas was placed on the floor.

Four rats were hung 4 to 5 feet from the ground.

Results:-

On the floor.

4 rats in cages dead.

5 rats loose in room dead.

Of 4 rats hanging 4 to 5 feet from the ground none dead.

In burrow I rat dead and 4 fleas dead out of 10.

In burrow II rat dead and 4 fleas dead out of 10.

In burrow III rat dead and 3 fleas dead out of 10.

In burrow IV the rat was found dead after 200 cubic feet had been blown into the room. A second then put in was found dead in the morning, when also 5 fleas were discovered to be dead out of 10.

The characteristic carbon monoxide spectrum was found in the rats examined, specimens being chosen from those dead on the floor in burrows and from those in cages hung up.

[1]This was a method introduced by the Plague Research Commission to clear room of fleas. Further, flea "counts " could be made on guineapigs.

In the morning 5 guinea pigs were let loose to collect the living loose fleas of which 45 had been put in. The count obtained was 15.

As a control to Experiments VI and VII, rats were left one night in the burrows and were found alive next morning, so that free ventilation had taken place in the burrows. As a control to the collection of fleas on guinea pigs, the room was again cleared of the fleas by the guinea pigs, 45 fresh fleas were put in overnight and next morning the five guinea pigs were introduced and the flea count was found to be 20.

Some further experiments were carried out to see whether if the gas were introduced through the roof, the outlet being into the upper levels of the room, the results obtained were better or not. But it was found that, owing possibly to the nature of the roof and the many holes through which the gas could escape, the results were bad both on rats hung up 4 to 5 feet from the ground and also on the rats on the floor and in burrows. When the outlet of the pipe was one foot from the roof, although an amount of gas equal to the full cubic capacity of the room was blown in, no rats either hanging up in cages 4 to 5 feet from the ground nor those on the floor or burrows were killed.

The pipe was then introduced through the roof and the outlet of the delivery pipe was placed at 5 feet 5 inches from the ground. The following were the results after 1,500 cubic feet had been blown into the room of 1,640 cubic feet <u>capacity</u>:-

<u>Rats on the floor</u> : 2 dead; 3 very sick.

Rats hanging up 5 to 6 feet from the ground : 4 looking very ill; 1 escaped.

Rats in the burrows : all alive.

The flea results in this experiment were contrary to the results obtained in the other experiments.

Two glass-tubes hung up 5 to 6 feet from the ground had 10 fleas in each.

After the experiment 8 were found dead in one, 1 alive, 1 escaped.

In the other, 7 dead and 3 alive.

On the floor, in one tube all 10 were dead; in the other tube 9 out of 10 dead.

In burrow 1 : 5 fleas were found dead.
3 alive
1 had escaped.

In burrow II : 4 fleas were alive.
1 was dead.
5 had escaped.

In burrow III: 9 fleas were dead.
1 alive.

In burrow IV : 4 dead.
6 alive.

Some more experiments were carried out, but need not be detailed as they proved that gas introduced high up was not so effectual in the type of room experimented on as gas delivered low down.

As the results detailed above were not conclusive as to the effect of the gas on fleas, the following experiments were carried out. Three of the jars with analysed gas had been returned to us by the Chemical Analyser. As the gas was heavier than air, the mere taking out of the cork from the jar and introducing a small body into the jar would not disturb the gas in the jar, nor cause it to diffuse out into the air very much.

About one inch of test-tube was taken, fleas were put in and the end of the tube covered with a small meshed cloth. The test-tube was then lowered into the jar.

I. Six fleas were put into the jar A, whose gas analysed gave:-

Oxygen	6.6
Carbon monoxide ...	6.4
Carbon dioxide ...	5.8
Nitrogen	81.2

The jar was then corked up.

In 1½ hours the fleas were still living. A small rat was then put in and died in 10 minutes.

The taking of its body out would disturb the air of the jar somewhat no doubt, but still there would be a considerable percentage of carbon monoxide in the jar into which the fleas were again lowered. But 17 hours afterwards 2 were alive, 4 dead.

II. Seven fleas were lowered into the contents of jar C, with a percentage of 5.2 of carbon monoxide. They were alive one hour afterwards. A rat was then put in and died in 20 minutes.

III. Seven fleas were put into jar B, with a perdentage of 4.7 carbon monoxide. Six were alive and one was dead 21 hours afterwards.

These results prove conclusively that carbon monoxide is not poisonous to fleas[1].

[1] This point was also made by Dr. Wade "Report on further experiments on sulphur dioxide as applied to the destruction of rats and in disinfection on board ship", Local Govt. Report 1905-6. Also by Mons. E. Bongean in the Bulletin des Sciences Pharmacologiques, January 1908 referred to in Lancet 21st March, 1908.

Conclusions:-

By the experiments in the smaller comparatively air-tight room it was proved that if the gas was delivered at a low level in the room, 500 cubic feet was as efficacious as 830 cubic feet, i.e. the cubic space of the room - that neither had effect on rats on the upper levels. In the larger room it was proved that 1,500 cubic feet delivered high up near the roof was of no use. The best results were obtained at low levels, the delivery pipe being introduced through the door, not through the roof, and the outlet being about 5 feet from the ground and 1,000 cubic feet being introduced into a room of 1,640 cubic capacity. It would take about 2⅔ hours to introduce this amount. The delivery pipe might just as well lie on the floor, as rats high up on the roof would not be affected at all.

The gases penetrated well into these artificial burrows and probably would penetrate well into natural burrows as, owing to the multiplicity of their opening, free circulation of air must occur. Burrows running up into the walls would probably not be reached by the gas. Possibly if larger volumes of gas were delivered in shorter time, the upper layers of the room would be reached. But with the present fan arrangement, I do not think that with larger amounts of gas any great concentration of carbon monoxide is obtained at any time in the room. As a proof of this, I would refer to two samples of air of the room tested by the Chemical Analyser.

Into the small room (cubic capacity 830 feet) 500 cubic feet was delivered and then a sample of the air of the room taken from the floor. The delivery outlet was close to the floor.

The Analyser gave:-

Oxygen	19%
Carbon dioxide ...	0.8
Remainder	80.2
Carbon monoxide ...	a trace

The delivery was continued till 1,000 cubic feet was passed in and a sample was then taken by syphoning.

Analyses:-

Oxygen	18.6
Carbon dioxide ...	1.2
Remainder	80.2
Carbon monoxide ...	a trace

These results prove that however much gas may be put into a room, more air-tight than any native hut can be, the delivery of gas is so slow that much concentration of carbon monoxide cannot occur.

The advantages of the use of this gas then are (1) that with an amount of gas over $\frac{1}{8}$ the cubic capacity of the room, if delivered low down, rats on the floor and in burrows at the floor level are killed; (2) that as the gas is tasteless and odourless, rats would not flee from it, as they do from sulphur dioxide. In our experiments rats were found lying dead on the floor of the room and had evidently made no effort to escape by the roof.

The disadvantages are (1) that the gas is not a flea killer and (2) that with the present fan arrangement any measurable concentration of gas cannot be obtained in the room. Rats on the upper level and in burrows running up the wall would escape if the gas were delivered low down. Gas delivered near the roof was not effective owing to its escape through the roof. (3) The gas is very

poisonous and owing to its being tasteless and odourless is obviously dangerous in working.

This is specially the case in tenements where one room communicates through so many holes and rat burrows with another. The gas would diffuse into other rooms than that being disinfected, and its presence would not be suspected till dangerous symptoms appeared.

The cost of working the apparatus would be large as regards the labour required and small as regards the coke necessary. The labour required to introduce 1,000 cubic feet would be at least 3 men as the handle revolving the fan has to be turned quickly and continuously for 2½ hours, and, moreover, water has continually to be poured into the reservoir on top of the scrubber.

The amount of coke necessary to produce 1,000 cubic feet is roughly from 16 to 18 lbs. The coke in the scrubber would require to be renewed very seldom, so that its cost is negligible. The price of the Indian coke in Bombay is Rs. 28 per ton.

HYDROCYANIC ACID GAS[1].

At the suggestion of Captain W. Glen Liston, I.M.S., experiments with hydrocyanic acid gas, as a measure to exterminate rats and fleas, were commenced in May 1909. The first experiments were made under his supervision, and on his departure to England I had the advice of Colonel Bannerman I.M.S. Dte. Ind., the Director of the Bombay Bacteriological Laboratory.

[1] This paper was published by me as "A preliminary report on the killing of rats and rat fleas" Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India No.38, 1910

We were later informed by Mr. Lefroy, the Imperial Entomologist, that large use had been made of this gas by the Agricultural Departments in the United States of America and in South Africa. He kindly sent us some of the literature on the subject along with an account of his own experiments conducted at Pusa, all of which have proved of great help to us.

It will be useful to consider briefly some of the previous work done on this subject. Hydrocyanic acid gas was first used in 1886 by Coquillet in California to destroy the "Cottony Cushion Scale" insect on plants; and later, fumigation of nursery stock with this gas has become compulsory throughout certain of the States. It is now used throughout Canada, the United States of America, South Africa, and Australia, not only for the disinfection of nursery stock, but also as a means of ridding orchard trees of their pests. More important for our purpose is the use of the gas for the disinfection of houses, jails, and railway coaches. The application has naturally been very limited on account of the deadly nature of the gas. Most of the information on this point we have obtained from the Reports of the Government Entomologist to the Cape of Good Hope for the years 1898 to 1901. In 1898 he considered the gas too dangerous for house disinfection; but in 1900, at the request of the Colonial Office, he rid the Worcester jail of the bed bug - "Acanthia lectularia" - by fumigation with hydrocyanic acid gas. A year afterwards the buildings were still free from the pest. In 1901 he further treated two more jails - Tokai and Kimberley - without any accident, although in the latter jail so great a quantity of potassium cyanide was used as 271 lbs. At the time the experiment was completely successful, although a year afterwards the buildings were re-infested. He

He also notes in 1901 the"use of the gas for the destruction of vermin in sleeping carriages of the Cape Government railways has now be n practised for three years with perfectly satisfactory results and without any accident".

There is also an interesting note to the effect that Professor W. J. Simpson thought this gas might be utilised to cleanse premises in which cases of plague has occurred, and experiments were actually undertaken to determine what minimum strength of the gas might be necessary to destroy bed bugs, which at that time were considered to be possibly the carriers of the plague germ. The experiments were discontinued owing to the decline of the epidemic and the departure of Professor Simpson.

In America also, fumigation by this gas has been occasionally used to rid buildings of pests. For example, in the "Proceedings of the 17th Annual Meeting of the Association of Economic Entomologists of America", a paper was read on the fumigation of a four-storey building to get rid of the "Clinocoris lectularia". The building was of about 150,000 cubic feet capacity; 80 lbs of potassium cyanide were used; the building was kept shut up for two days. All the insects and eggs were killed. "No eggs appeared to hatch afterwards though examination was made frequently for a period of several weeks".

The Production of the Gas.

The chemicals used - In all the work done the gas has been produced (1) KCN by the action of sulphuric acid on potassium cyanide. These chemicals must be of a certain standard of purity to get satisfactory results. With regard to the Potassium cyanide, elaborate investigations were carried out, chiefly by Everhart of the Southern College of Pharmacy in the United States of America. His results

are detailed by Wilmon Newell in Bulletin No.15 of the Georgia State Board of Entomology. They generally are that failure to obtain satisfactory results in fumigation has in many bases been due to the use of impure and adulterated cyanide which is often represented and sold as being pure. Of the adulterants sodium chloride is the most important, as it produces loss and decomposition of the hydrocyanic acid gas evolved.

Nitrates are another harmful adulterant. The most common adulterant in low grade cyanides being sodium chloride, increased amounts of low-grade cyanides cannot be safely substituted for pure cyanide, though they are much cheaper.

A high percentage of potassium cyanide in a sample is not sufficient unless analysis shows the absence of chlorides and nitrates.

Of several different cyanides examined by Everhart, only two, those of Merck & Co. (98 - 100 per cent) and of the Baker and Adamson Chemical Co. (99 per cent) were found to meet all requirements.

In almost all of the experiments detailed in our tables, carried out before we had read Newell's paper, we used 98 per cent potassium cyanide or Burgoyne Burbidges' potassium cyanide 100 per cent (double salt). Captain Higham, I.M.S., Assistant to the Chemical Analyser for the Government of Bombay, kindly gave us an analysis of Burgoyne Burbidges' salt. It proved to be a mixture of both potassium and sodium cyanides, yielding 33.9 per cent hydrocyanic acid gas/equivalent to 81.6725 potassium cyanide.

One hundred per cent potassium cyanide would yield 41.5 per cent of its weight of hydrocyanic acid and 100 per cent sodium cyanide 55.1 per cent. There were no nitrates; but chlorides were present to the amount 3.778 per cent calculated as

hydrochloric acid.

(2) Sulphuric Acid.

The sulphuric acid ought to be commercial concentrated sulphuric acid, (specific gravity 1.83).

The proportional amounts of potassium cyanide, sulphuric acid, and water are important. Coquillet[1] in 1889 found that the best results were obtained by using 1 part potassium cyanide, 1 part of sulphuric acid, and 2 of water. But the usage of different workers has varied considerably on this point. However, the majority advocate the 1-2-4 formula, i.e., 1 oz. of 98 per cent potassium cyanide, 2 fluid ozs. of sulphuric acid, and 4 fluid ozs. of water. The advantage is twofold: (1) There is enough water to dissolve the potassium bisulphate formed and so prevent the deposit of this salt round the potassium cyanide which would reduce the formation of hydrocyanic acid gas. (2) When 2 ozs. of sulphuric acid at 20°C. were mixed with 4 ozs. of water at 20°C. the initial temperature became 110°C.; one oz of potassium cyanide was added and the whole mixture exposed to the outside air having a temperature of 14°C. for 40 minutes. At the end of that period the mixture had a temperature of 32°C. The boiling point of prussic acid is 26½°C., so that throughout the whole period the temperature was sufficient to volatilize the prussic acid.

If more water is added the initial temperature is less; if less water is added than 4ozs. the addition of 1 oz. of potassium cyanide would also diminish the initial temperature.

These conclusions were arrived at by Wilmon Newell, as noted in the above-mentioned paper. Some experiments were performed on this point by us.

[1] Coquillet's papers have not been consulted by me.

The room temperature was 28°C.

The initial temperature of both the sulphuric acid and the water was 28°C., quite sufficient for the volatilization of the gas.

I. Three parts of water were added to one part of sulphuric acid.

The initial temperature became 88°C.; after forty minutes the temperature was 43°C.

II. Two parts of water were added to one part of sulphuric acid.

The initial temperature became 105°C.; forty minutes later the temperature was 39°C.

III. One part of water to one part acid.

The initial temperature was 115°C.; forty minutes later the temperature was 41°C.

The end results vary then, not only with the initial temperature developed but with the bulk of fluid used. In cold climates, as in the Punjab in the cold weather, it would be advisable to mix the various chemicals in one large vessel rather than distribute them in small charges about a room, as cooling of the mixture in the latter case would occur so much more quickly.

Considering every point the use of the 1-2-4 formula is probably the best.

A few minor points advocated by various authorities are:-
(1) The use of fairly large lumps of potassium cyanide instead of powdering it. The generation of the gas is too violent with powdered potassium cyanide. Further the powdered salt is apt to deteriorate on standing by exposure to air; and, lastly, there is the increased cost of powdering the salt. If there is a sufficient excess of sulphuric acid and water the inter-action of the chemicals is rapid - almost as rapid with large pieces of

potassium cyanide as with small. (F.A.Sirrine, New York Agricultural Department, 1909).

The Entomologist of the Cape of Good Hope advocates the use of lumps of potassium cyanide, the size of an egg for one lb. charges and lumps the size of an acorn for small charges.

(2) The acid ought to be poured into the water if splattering of the acid is to be avoided. The potassium cyanide ought to be added last. The maximum effect of the gas is then produced.

(3) The storage of the potassium cyanide is of importance as under exposure to air and moisture it deteriorates rapidly. After a case containing potassium cyanide has been opened means ought to be taken to prevent ingress of air and moisture. It ought to be kept in a cool dry place. The same precautions are necessary to keep the sulphuric acid from absorbing moisture.

METHODS OF CARRYING OUT FUMIGATION RECOMMENDED BY PREVIOUS WORKERS.

(1) The quantity of potassium cyanide to be used per cubic space to be fumigated.

There is some difference of opinion with respect to this point.

Burgess of Ohio in a paper on "Fumigation of Nursery Stock" states: "A series of experiments in treating peach buds made in August 1902 showed that ½ oz. of cyanide to each 100 cubic feet of space for 40 minutes' exposure killed all San Jose scales and no injury resulted to buds".

Newell of the Georgia State recommends 1 oz. of 98 per cent potassium cyanide to 100 cubic feet. With this dosage he states that "trees heavily infested with the San Jose scales and protected with a heavy layer of earth failed to show any live scale insects within a year of being fumigated".

Sirrine advises 25 grammes of potassium cyanide per 100 cubic feet; or for orchard trees, owing to the moisture and absorption of the evolved hydrocyanic acid gas, 30 grammes, i.e., about 1 oz. to 100 cubic feet.

The Government Entomologist for the Cape of Good Hope in his Report for the year 1901 states that bugs are more refractory to the gas than any other house insect. One ounce of potassium cyanide to 155 cubic feet of air space with an exposure of one hour killed 12 out of 15 bugs. The remaining three were very feeble. Eggs of the bugs failed to hatch in three tests after exposure for one hour to gas of the above strength.

He advises the use of one ounce to 100 cubic feet of space and an exposure for two hours. "When there are any deep cracks or crevices in any building to be penetrated, one ounce to 60 cubic feet of space may be used".

(2) Actual procedure to be adopted as advised in the report of the Government Entomologist for the Cape of Good Hope for the year 1901.

The building to be fumigated is made as air-tight as possible. Arrangements should be made for opening doors and windows from the outside to ventilate after fumigation. Each room should be fumigated separately, if possible, but sometines two connecting spaces may be treated as one. In the latter case crevices and splits in doors connecting the two spaces should be enlarged and burrows opened up. Bedding, clothing, etc., should be spread out to let the gas get full effect on them.

The vessels in which the chemicals are to be mixed must be of such a nature that they are not acted on by them. Their size depends on the quantity of chemicals to be used. They must be of such a shape that the depth of combined sulphuric acid and water

will cover the cyanide put in. They may be glazed earthenware vessels or china washhand vessels. Lead vessels are good but heavy and soft. Enamelware till cracked acts well. Kerosine oil tins will serve for two or three different charges. One of these tins can take a charge of 3 lbs of potassium cyanide - the utmost that ought to be put into one vessel. Place the vessel near the middle of the room. Measure out quantities of water, acid and potassium cyanide. The man who measures out the potassium cyanide ought to handle it with rubber gloves as the salt is so poisonous. Mix the acid into the water, at once drop in the potassium cyanide at arm's length, and run out of the room. Many people wrap up the potassium cyanide in soft paper which takes a few seconds to be eaten through, permitting the operator to get out of the room before the gas is given off. The American fumigators usually lower the potassium cyanide by means of a string and pulley from the outside after the door of the room to be fumigated is locked. As the hydrocyanic acid gas rises, being lighter than air, if there are two storeys begin at the top; and if there are many rooms in a corridor begin at the farthest from the exit. An assistant may go in front mixing the acid and water. Great quickness and care are essential as the gas is so poisonous.

Of course if a tenement is to be disinfected all inhabitants must be cleared out first. It is essential to have a clear space round the building to be fumigated. It is recorded in one of the American experiments that persons walking 100 feet from the building could detect the odour of the gas the whole time.

The period of exposure should be two hours at least. It is better not to try ventilation till 12-24 hours have elapsed. So far as we have seen in our experiments (with the 1-2-4 formula)

in 50 minutes the action of the sulphuric acid on the potassium cyanide has been completed. So really the ventilation of the building can be carried out in two hours if the doors and windows can be opened from the outside. It is at the moment of entering the building after fumigation that danger is most to be guarded against.

After opening the doors and windows from the outside certainly half an hour should elapse before any one enters the building, and it is safer to wait three hours. It depends altogether on the character of the building and if there are many doors and windows which can be opened from the outside. We have entered a room five minutes after the delivery of the gas has stopped. There is a very good test suggested to us by Captain Dickinson, the Chemical Analyst of Bombay. It depends on the formation of prussian blue by the gas if there is any free in the room. A paste is made up consisting of ferrous sulphate and caustic potash. A little is put on a rod of glass and the rod introduced into the room. It is exposed to the air of the room for five minutes. If hydrocyanic acid gas is present sodium ferrocyanide is formed. A few drops of pure hydrochloric acid are then run on the paste and a little water containing a few drops of ferric chloride added in a beaker. If there is any hydrocyanic acid gas at all in the room blue colour is formed - deep blue if much gas; faint green blue if a little. If only a yellow colour is present it is quite safe to enter the room. This is a most delicate test. It will detect 1/780 grain of hydrocyanic acid in a very dilute liquid (Watt's Dictionary of Chemistry). It is much safer to rely on this test than on the odour of almonds, as the sense of smell seems to be paralysed by the diluted gas after the first few whiffs. Even

Even a trace of hydrocyanic acid in the air leads to headache and nausea. It is so deadly that a whiff of fairly strong gas kills at once.

It is obvious that the greatest care must be taken during the whole procedure. No naked lights should be left about a building as hydrocyanic acid gas is somewhat inflammable.

THE ACTION ON ANIMALS, INSECTS, PLANTS, FOOD, and FABRICS.

Our experiments have been almost entirely on rats and rat fleas, and will be detailed later. With respect to its action on certain insects, arachnoids and mammals, Lounsbury states that flies are much more susceptible to the gas than fleas,and fleas than bed bugs. Argas persicus - the tick of the fowl - is the most refractory. "The eggs of the bed bug and of the flea (at least of the dog flea, Pulex serraticeps) are devitalized almost, if not quite as readily as the parent insects". Rats and mice are also readily killed.

On the subject of the action of the gas on plants a vast amount of literature is available. Generally speaking the amounts of chemicals recommended above as sufficient for fumigation do not destroy even delicate plants.

The action on foods and water is of importance. It is agreed that dried grain is not made poisonous by the action of the gas[1]. An experiment was performed by us on that point. Into a fairly air-tight room of 830 cubic feet capacity the gas evolved from 340 grammes of 98 per cent potassium cyanide (equivalent to about 1½ ounces to 100 cubic feet air space) was introduced.

[1] Report of Government Entomologist to Cape of Good Hope,1901

The amount of hydrocyanic acid gas was calculated to be about 4.38 cubic feet at 26° Centigrade. It was allowed to act for 20 minutes. Fleas were found dead after 5 minutes' exposure to the air of the room. The test rod showed a plentiful supply of hydrocyanic acid gas to be present by the development of prussian blue. The grains tested were: (1) bajri, (2) wheat, (3) jowari, (4) mula or radish, (5) rice. Bajri, wheat, and rice were put into kerosine oil tins. A pipe bringing in gas diluted with air (the mixture was driven in by a fan from the generating flasks) was led to the bottom of the mass and another pipe was directed on its surface. Mula and jowari were in small amounts and the gas mixture was directed on their surface only.

Monkeys, rabbits, guineapigs, hens and pigeons were fed on the grains for 24 hours; none became ill.

Chapatis were made of the wheat, jowari, and bajri and given to monkeys and hens with no ill-results.

On the other hand moist food-stuffs, such as water, milk, butter, and flesh are said to absorb the poison. Experiments on this subject are detailed on p. 51. Meat and water exposed to the gas are said by Lounsbury to have proved fatal to dogs.

Therefore, during the fumigation of a building, all such moist food-stuffs ought to be removed. Dry food-stuffs can be left alone, but had better be thoroughly aired before use.

Another question is: "Does the gas injuriously affect the germinating powers of the grain?" This is of importance in view of the amount of grain stored in native houses to be used later for sowing.

As all the experience of workers in America and South Africa goes to show that even delicate plants are not injured by the gas when present in sufficient concentration to clear them of insects,

the answer would be that it is not injurious. An experiment we did confirmed this. Certain amounts of the above grains subjected to the gas were planted, and their growth compared with control grains which had not been fumigated. No difference in the rate of growth could be detected. So during fumigation, stored dry grain can be left in the house without fear of rendering it poisonous or of destroying its germinating power.

Unlike Sulphur dioxide gas hydrocyanic acid gas has no action on metals or fabrics.

THE ACTION OF THE GAS ON BACTERIA.

About 4.38 cubic feet of diluted gas were introduced into a room of capacity of 830 cubic feet and allowed to act for twenty minutes on bacteria. Cultures of streptococci, Bacillus typhosus, Bacillus coli, and Bacillus pestis on agar slopes in test tubes with their cotton-wool stoppers removed were put in the room. After the experiment subcultures on agar showed that the plague was overgrown with a yellow contaminating organism. The other bacteria had not been killed by the action of the gas.

This coincides with the experience of the Government Entomologist of the Cape of Good Hope as noted in the Report for 1901: "Cultures of the plague bacillus were found to be unaffected when exposed by substituting a piece of gauze tied over the top for the plug of cotton-wool ordinarily used for stopping culture tubes by one hour 1 to 80 gas - the severest test employed". Owing to the feebly resistant nature of the plague bacillus to desiccation it has been proved by the Plague Commission[1] that highly susceptible animals allowed to run about on floors in Bombay contaminated by plague cultures 24 hours previously, have escaped infection. Further in dead bodies of rats, plague bacilli are rapidly overcome by putrefective organisms. For these reasons the bacillus pestis is, from the epidemiological point of view of the importance only when in the living organism ----- whether of rat or flea and in a much less degree of man and other animals infected.

[1] Journal of Hygiene vol VI 1906

DISINFECTION FOR PLAGUE.

Its harmless effect on the plague bacillus does not condemn hydrocyanic acid gas as a preventive for the spread of plague. Preventive measures for this disease fall under three heads:-

(1) Disinfection of clothing of railway and other travellers. This is equivalent to the destruction of plague germ carriers, namely, fleas.

(2) Disinfection of plague houses. This is equivalent to the destruction of plague rats and of plague fleas.

(3) Disinfection of shipholds and their contained cargoes.

I.- The use of hydrocyanic acid gas for the disinfection of clothing.

There is no question of its great efficiency, its cheapness, and the rapidity with which the operation can be carried out - all matters of importance. It has further the advantage of having no destructive effect on fabrics.

Our experiments were carried out in two ways:-

(a) First method. - Potassium cyanide was mixed with sulphuric acid and water (1 of sulphuric acid to 19 of water) at first in glass flasks and in later experiments in a specially constructed leaden vessel. The mixture was heated over a Bunsen flame, the whole being placed outside the room to be fumigated. Tubes conducting the undiluted gas were led directly into the room in some cases; in others the tubes were led into a large iron vessel from which the gas was pumped by a powerful fan into the room through a large delivery pipe. There was a second opening in the iron vessel through which air was pumped and mixed with the hydrocyanic acid gas. It was the largely diluted hydrocyanic acid gas that was introduced. The quantities of hydrocyanic acid gas produced were calculated as follows: about 82 grammes of pure potassium cyanide taken as 100 per cent will produce

1 cubic foot of hydrocyanic acid gas. We used for one charge 85 grammes of 98 per cent potassium cyanide or the equivalent amount of other strengths of the salt. We were at the time unaware of the necessity of using sodium-chloride-free potassium cyanide/stated by Wilmon Newell in his paper and quoted above on page 30 .

With the 85 grammes of potassium cyanide we mixed 35 c.c. of strong commercial sulphuric acid diluted to 700 c.c. with water (i.e. H_2SO_4 1 in 20 strength).

The room in which these experiments were carried out is comparatively air-tight. It is of the following dimensions: length 12 feet 6 inches; breadth 7 feet 8 inches. The roof slopes upward from a wall 5 feet 8 inches to one of 11 feet 8 inches. The cubic capacity of about 830 cubic feet. The walls are of cement. The floor is of stamped earth. The roof is of corrugated iron made rat proof by being firmly fastened to the walls. There is a large window 2 feet 7 inches by 3 feet 6 inches and, opposite, a large door 3 feet 2 inches by 5 feet 6 inches. The window is capable of being tightly closed by shutters. Through the shutters the delivery pipe was led into the room. The door, which leads into another room, was closed and any cracks or obvious holes sealed over, so that a comparatively air-tight chamber was produced.

In the different experiments varying amounts of gas from 2 to 4.38 cubic feet were introduced into the room for different periods of time.

The results giving details of amounts of gas used, the time of exposure, and the effect on fleas in different situations are given in experiments I to VI.

Rats were introduced into the room simply to discover what

amount of gas would be necessary to kill them. They require more gas and a longer exposure than fleas. But the effect of the gas on them is of no importance for this investigation - namely, disinfection of clothing.

Two points were aimed at: (a) to obtain a sufficient penetration of clothes, and (b) to conclude the whole operations in a short period of time, so that no great delay to the persons waiting for the clothes would occur.

Both objects were attained. Bundles of clothes wrapped fairly tightly up were thoroughly penetrated by the gas. A consideration of the tables will show this. For example, in Experiment I the gas penetrated through four bags, - three of coarse blanket and one of cotton, killing 10 fleas out of 11 placed centrally. It penetrated through three bags, two of blanket and one of cotton, killing all the fleas placed centrally. The gas was led direct from flasks outside the room without the use of a fan. About two cubic feet of gas were introduced into the above described room of 830 cubic feet capacity and the time of exposure was 51 minutes.

Experiment II shows the same condition of affairs as Experiment I, but a shorter exposure of 30 minutes with not so successful a result. The most successful experiment is No.III. About 4.38 cubic feet of gas were pumped into the room by a fan for 20 minutes, and then the delivery of gas was stopped. The gas was distributed by 9 small tubes throughout the room. The fleas in all parts of the room were killed, even though the tubes containing them were wrapped up in several layers of thick sheeting and blanket. But in series 3, where they were surrounded by a durree, blanket, and coat very tightly rolled, eight fleas remained alive out of nine. Fleas put in cages

with bran and sand at the bottom were all killed in this experiment, showing that there is a certain penetration of the gas into these substances,- an important point in the disinfection of houses, as the fleas bury themselves in dust, grain, etc.

If clothes are left loosely in boxes, can we say the gas will penetrate sufficiently to kill? If so, it would obviate the necessity of hanging out the clothes for disinfection.

Experiments IV and V were tried to determine this point. If pipes were led with great care into the boxes, fleas were killed. But it will be noted that failure occurred in some instances, where, perhaps, owing to the tube being kinked, some obstfuction to the entrance of the gas occurred. It is much safer to hang up the clothes on pegs or scatter them loosely about the room.

In Experiment IV it is noted that the room was entered in 30 minutes from the commencement of the operations. The disinfection of the clothes was very thorough, so that it is possible by pumping gas into a room to kill fleas in clothes and deliver clothes back to their owners in 30 minutes. The use of the test rod mentioned on page 36 enables one to enter the room with safety.

(b) Second method,- The fan and pipes are, however, a complicated and expensive means of distributing the gas. Accordingly the method of producing the gas, as detailed on pages 34 & 35 was tried.

A flea-tight and very air-tight godown - one of those used in the Plague Commission's experiments - was taken. Its cubic capacity is about 346 cubic feet. Into a small china vessel four ounces of water were put, 2 ozs. of commercial strong

sulphuric acid were added evolving heat, and then 1 oz. of potassium cyanide was finally put in and the door closed. The gas was left in contact with the clothes for forty minutes and then the door was opened. The room was entered 15 minutes later. The fleas left in different parts of the room were all killed,- the gas even penetrating through four bags, three of blanket and one of cotton, killing all the fleas placed centrally. Fleas were put in various situations in a box full of clothes; some escaped. For details see Experiment X.

So again it was shown that it is necessary to take the clothes out of a box and hang them up. The strength of gas used was less than one-third that suggested by fumigators elsewhere (1 oz. to 100 cubic feet); yet it was completely successful. The action of the sulphuric acid on the potassium cyanide is not completed for 40 minutes probably, as when the door was opened in the above experiment fumes were still being evolved from the generating pot. After 60 minutes the evolution of gas is completed. Accordingly some means must be thought of to cut off the supply of gas, say after 15 minutes, to allow one to enter the rooms safely and give the clothes back quickly to their owners. That the fleas are killed in five minutes was shown in one of our experiments. In five minutes after the commencement of the evolution of gas a tube containing fleas was withdrawn; they were all dead.

In another experiment larvae of rat fleas loose on sand were subjected to the same strength of gas, 1 oz. potassium cyanide, to 346 cubic feet for an hour. They were all killed. Controls were all living at the time of examination. Eggs and cocoons of the rat flea were subjected to a stronger gas, 1 oz. to 100 cubic

feet. The eggs were all killed. Larvae hatched out of control eggs not subjected to the gas but otherwise under the same conditions.

All the cocoons, however, were not killed by the gas.

But the important point is to kill infected fleas. Infected fleas have not been shown to transmit plague bacilli to their eggs.

II. The Disinfection of Plague Houses - that is to say, the destruction of rats and fleas in these houses.

A room was chosen which presents the characters of most native huts in Bombay, - that is to say, not at all air-tight. Its length is 12 feet 6 inches; breadth 15 feet 7 inches; height at one end 5 feet 6 inches; at the other end 11 feet 4 inches. The cubic capacity is 1,640 cubic feet. The floor is of stamped earth and the roof of country tiles. Below the edge of the roof and the smaller end wall a small space communicates with the outside. There is also very free ventilation between the edges of the tiles. There are two large windows which can be well closed with shutters and two doors, one of which communicates with the outside and one with the small room described on page 41.

In the following experiments as many of the large ventilating holes as could be filled up were closed with tightly-packed paper and the room was made as air-tight as most native huts can be made.

In the floor of the room four types of rat burrows were present, each consisted of a small wooden box to contain rats and fleas and one or two passages leading from it to the floor of the room. The boxes measured 21 by 10¼ by 12 inches. They are fitted with hinged lids and are sunk in the ground. Inside each box is a rack to hold test-tubes containing fleas. The cage for the rat can be placed inside the box. The channels consist of a series of short sections of earthenware pipes sunk in the ground.

Each section is made up of concave country roofing tiles placed together to form a somewhat tapering pipe, the narrow end of one section fitting into the broad end of the other. The narrow end of the lowest of these sections projects slightly into the box. These "burrows" measured from $5\frac{1}{2}$ to 12 feet.

There are four types of burrows:-

Type A. - A blind burrow with a single opening.

Type B.- A blind burrow with a single opening inside the room. The box for the cage was outside the room for facility of observation.

Type C.- Two burrows with S-shaped curves on the horizontal, leading to the box containing the cage.

Type D.- Same as type C, but with the curves vertical.

Over the tiles 4 to 6 inches of earth were placed and, after the fleas and rats had been placed inside, the lids of the boxes were carefully sealed down with moist clay.

These burrows and observations chambers were originally devised by Captain Gloster, I.M.S., for his experiments with the Clayton gas apparatus.

The results of experiments in this room are detailed in Experiments VII to IX.

Experiment VII shows the results when the gas was generated outside the room and introduced by tubes without pumping. Some tubes were also led a little distance into the burrows and the mouth of the burrows closed up. The gas is lighter than air and better results were obtained at the upper layer of the room than on the floor. Neither rats nor fleas in the burrows were affected by the gas. At the level of the floor many fleas in cages with sand at the bottom managed to escape by burrowing into the sand,

while those in bran were killed.

In Experiment VIII the gas was introduced by larger calibre tubes and pumped in by a fan; four of the tubes were led into the burrows and the mouth of the opening closed up with mud round the pipe. This was a most successful experiment. Fleas in all situations, - in the room, in cages with sand and bran at the bottom, and in test-tubes in the burrows,- were killed. Six out of eight rats on the floor or 4 feet above floor level were killed and seven rats at higher level were all killed. The rats in burrows A and C were killed. There was some obstruction in burrow D preventing all the gas entering. Burrow B was the observation burrow and was opened during the experiment when the rat was found to be sick. The whole experiment was completed in 40 minutes with an introduction of about 4.38 cubic feet of the gas equivalent to 340 grammes of 98 per cent potassium cyanide, about ½ oz. to the 100 cubic feet space.

Experiment IX.- Rats loose in the burrows were also killed.

Attempts were made to dispense with the complicated apparatus of fans and tubes by generating the gas inside the room, as described on pages 34 & 35. They are detailed in experiments II and 12. At higher levels of the room rats and fleas were killed, but rather unsatisfactory results were obtained at floor level, and the experiment was an absolute failure in the burrows, both rats and fleas escaping.

Therefore, with artificially-constructed burrows and the use of an apparatus with a fan to pump in the gas small rooms can be disinfected thoroughly in, say one hour.

Whether natural burrows can be penetrated by the gas remains to be proved. As far as we can see at present with small isolated huts when the inhabitants are all cleared out there would be no danger. The inhabitants could return with perfect safety in two hours after ventilation was begun, but to make it absolutely sure say twelve hours.

When the houses are placed close together, as in a Punjab village, the whole would require to be evacuated simultaneously for two reasons:-

(1) As rat burrows run from one house to another the gas produced in one house might find ingress into others causing great danger to the inmates. The faint almond odour of the gas and the taste at the back of the throat is only appreciated for the first few moments. The gas then paralyses the sensory nerves and, though dangerous quantities may be present, they would not be appreciated.

(2) For a successful experiment such continguous houses should be done simultaneously in order that no rats may escape.

With tenements in cities like Bombay evacuation of the whole tenement would be necessary for the same reasons. Further, a clear space round is a necessity, as even when only 4 ozs. of potassium cyanide were used in a small godown the odour of the gas was perceived twenty feet away when the door was first opened. See also on this point what is noted above on page 35. For these reasons the wholesale use of the gas is impossible. It ought first to be tried in isolated houses to investigate its action on rats under natural conditions. The investigation should be made by someone familiar with the use of the gas.

. Disinfection of clothes.- As we have stated above, an air-tight godown must be built with arrangements for letting the gas play on the clothes for, say, fifteen minutes, and then turning off the supply of gas to allow of safe entry to the building. This would be necessary if the clothes are to be returned to their owners, in a reasonable time. The advice of an engineer is requisite for the construction of the godown as the danger from the gas is not to underrated. The price of the chemicals is slight.

The cost of potassium cyanide (98 - 100 per cent) is 1 shilling per lb for 28 lbs., or 99 shillings a cwt. if bought in bulk (Merck's quotation). The cost of sulphuric acid is 1d (one penny) per lb. if carboys are bought (Baird & Tatlock's quotation).

. Cost of Disinfecting Houses.- Suppose the room was 1,000 cubic feet capacity and moderately air-tight, 7½ ozs of potassium cyanide and 15 ozs. of sulphuric acid would be required. The total cost of chemicals for disinfection would be between 6 pence and 7 pence plus the pay of a supervisor. China vessels to hold the chemicals would last indefinitely.

The conclusion then is, that so far as disinfection of clothes is concerned we have in hydrocyanic acid gas a very cheap and efficient pulicide. With a specially constructed godown, it would be possible to deliver over to their owners clothes absolutely free of fleas in half-an-hour. There would be no damage to fabrics. If proper precaution is taken and an experienced man acts as supervisor no accident would occur.

With regard to the disinfection of houses the conclusions

arrived at are not so satisfactory. If as satisfactory results can be got with natural burrows, we have an efficient means of clearing houses of plague rats and fleas. But the meams are not without danger. This danger could be largely guarded against in isolated houses with a clear space round them, or in an aggregation of houses with a clear space round them, as in a village, if evacuation of the houses could be insisted on till such time as occupation be declared safe. In crowded localities, as in tenements in cities, the danger is too great to permit employment of the method.

III. Disinfection of shipholds and their contained cargoes.[1]

The disinfection of shipholds, if empty of course, resolves itself into the same problem as that of houses and godowns. But, as it is important if possible to thoroughly disinfect a loaded hold without injury to the contained cargo, I intend in this section to study the effects of hydrocyanic acid gas on certain materials.

I have already stated that the gas, in the concentration necessary for thorough fumigation to get rid of rats and fleas, has no action on metals nor fabrics. I have further remarked that moist food stuffs such as water, milk, butter and flesh were stated by Lounsbury to have proved fatal to dogs after exposure to the gas. Dry grain, however, such as bazri, wheat, jowari, radish, and rice were not rendered poisonous, I proved, nor was the germinating power of wheat, barley, raddish and jowari injured (p.p. 38 & 39).

Certain epidemics of plague in Persian Gulf Ports since 1900 gave rise in the minds of authorities in Teheran to the suspicion that steamers bringing rice from Bombay had carried infected rats

1. Throughout this portion of the work I had the advice of Major Glen Liston M.D. D.P.H. C.S.I.

and fleas. Now in Bombay the measures taken to disinfect ships are of the same nature as ~~is~~ here applied in English ports, namely thorough fumigation by sulphur dioxide. As has already been pointed out, it is impossible however to satisfactorily disinfect a ship by this gas without injuring cargoes containing food stuffs, such as rice.

The Government of Bombay thereupon asked us to further extend the experiments quoted above in order to ascertain the action of hydrocyanic acid gas on rice.

A considerable number of experiments were made on rice to test, first, whether milled rice was rendered poisonous after thorough fumigation with the gas; second, whether the germinating power of the unhusked rice was affected by the action of the gas.

I do not intend to detail these experiments, as they were in the lines of those described above. I deduced from many observations that the dry milled rice was not made poisonous. Given in its natural state after fumigation to pigeons and hens or cooked and given as chapatis or as a cooked rice to ducks, geese, rats, and monkeys no ill effects were noted. Also water, meat, and butter exposed to the gas for 55 minutes (the presence of the gas being assured by chemical and animal tests) given to monkeys and cats proved non-poisonous. Also chemical tests did not reveal the absorption of hydrocyanic acid gas by these substances. These results should be contrasted with those obtained by Lounsbury (p.38).

The germinating power of the unhusked rice exposed to thorough fumigation was tested by the rate of growth being compared with normal unfumigated rice obtained from the same sample. I could detect no ill effect from fumigation.

As this is a matter of great importance, we thought it advisable to send samples of 1st milled rice and 2nd unhusked rice to the Agricultural Chemist to the Government of Bombay, it order that the conclusions we arrived at formerly might be examined independently. We are much indebted to Dr. Harold Mann for his report.

1st That no hydrocyanic acid gas was absorbed by the rice examined by him.

2nd That the food value of the rice had not been affected.

3rd That the unhusked rice germinated equally well after treatment.

These results are in confirmation of our own and are parallel to those obtained with other grains (p.38)

In connection with the disinfection of ship-holds it is necessary to establish two important points.

(1) What diffusion of the gas occurs in a closed space throughout the atmosphere. Glass tubes about 5 feet in length and 3 centimetres in breadth were introduced into an air-tight godown and the effects of the diffusion of the gas during fumigation were tested as follows:-

Some paste composed of Ferrous sulphate and potassium hydrate was smeared inside the glass tubes at varying intervals and after exposure to the gas this was tested for the formation of Prussian blue by the addition of pure hydrochloric acid.

The tubes are placed in the following ways:-

(1) One was placed horizontal about 8 feet from the ground.

(2) One was placed vertical (the lower extremity being about 2 feet from the ground).

(3) One tube was placed obliquely at an angle of about 30° from the horizontal, the lower extremity being about 6 feet from the ground.

(4) One tube was placed obliquely at an angle of about 75° from the horizontal, the lower extremity being about 5 feet from the ground.

The following were the results:-

No trace of the action of the gas was detected along the length of number one, the horizontal tube, nor of number three placed obliquely at an angle of 30° to the horizontal.

A very faint trace of the action of the gas was detected towards both ends (up to about 1 foot) of the vertical tube number (2). At the extreme upper end of the oblique tube number (4) placed at 75° to the horizontal, and at about 1 foot from the lower end a very faint trace was also detected. In these latter cases the gas was probably forced up the tube by its low specific gravity or was reflected down from the roof on to the upper ends. There was no diffusion of the gas through the air in the tubes placed horizontal or at a slight degree from the horizontal.

(2) The degree of penetration of the gas into bags of rice. The method of testing this was as follows:-

Fleas were placed either in muslin bags or in small open glass tubes closed simply at both ends by muslin. That diffusion of the gas occurred through these muslin bags and glass tubes was proved by the fact that when placed in the open in godowns fumigated by the gas, the contained fleas were all killed.

The bags or tubes were then buried in the bags of rice at various depths and the occurrence of even one live flea was taken to prove the fact that the gas had not penetrated to that depth.

The results of various experiments showed that while fleas buried in rice 2 and 3 inches from the surface escaped destruction fleas buried 1 inch deep in both unhusked and husked rice were killed. It should be noted that control experiments performed

with fleas buried in the same situation in an unfumigated godown, showed that the fleas all lived. We are therefore justified in concluding that the gas does not penetrate into rice further than 1 to 2 inches from the surface. The production of the gas was by both methods described above (pp 40 & 43). With regard to the second method (p.43) - the use of a special generating machine with fan - the procedure was modified by eliminating the use of Bunsen burners or oil lamps. A mixture of water and sulphuric acid in the proportions of 2 to 1 evolves sufficient heat in itself to produce the gas from the salt. We found it an advantage to have the gas produced somewhat slowly; the longer the gas acts on rats and fleas the better. This continuous action of the acid on the salt is much helped by using lumps of potassium cyanide of the size of a walnut instead of the powdered salt. If the latter is used the gas is evolved in great volume in a short time and much of it is lost by diffusion in the godown, as it is very light in density.

Further in certain of the experiments to increase the density of the gas in the godown, a pipe of the same diameter as that which conducted the gas from the machine was led back from the top of the godown to the machine. Thus a circulation of the air of the room was established through the machine and as the experiment went on this air became heavily charged with the gas..

As a result of these experiments we requested Government to grant us permission to work on a larger scale.

To approximate the natural conditions of a crowded ship hold, as much as possible, we desired to fill up a small air-tight godown with bags of rice and to allow free rats to burrow about inside. When the rats had settled down the effects of the gas upon them could be noted.

The governing body of the Indian Research Fund Association agreed in the commencement of 1913 to meet the cost of this experiment. Fifty-seven bags of milled rice and three bags of unhusked rice were purchased and fifty-nine of these were put into a very air-tight godown of about 346 cubic feet capacity completely filling it up. The gas was generated in a machine and blown in by a fan as already described (p.40)

These experiments enabled us to make observations on the habits of rats and fleas in grain or rice godowns and among bags of rice in the holds of ships. On this matter depends the problem of effective fumigation. From my experiments I conclude that rats do not burrow into bags but live in the spaces between them making holes in the bags to obtain food only. In one of my experiments when 20 rats had been in a small godown of about 346 cubic feet completely filled with 59 rice bags for a period of 10 days, after fumigation on the eleventh day the rats were all found lying dead between the bags. Further as detailed in experiments 14 and 15, fleas do not burrow deep into rice. As Wade therefore surmised, the administrative problem will be solved if we get a gas which penetrates into "crevices of appreciable dimensions".

We conclude, therefore, that under natural conditions with rats and fleas free in a space filled with bags of rice fumigation by hydrocyanic acid gas will be effective. Some of the experiments from which these latter conclusions are drawn are detailed as Nos. 13, 14 and 15.

Recently Liston and Taylor[1]

[1] "The use and advantages of hydrocyanic acid gas as a disinfectant for plague infected houses and ships". Proceedings of the All-India Sanitary Conference, 1914. By Major W.Glen Liston,C.I.E.,M.D.,D.P.H., I.M.S. - Captain W.D.H.Stevenson,M.B.,I.M.S. and Captain J.Taylor,M.D. D.P.H.,I.M.S.

Liston + Taylor.
have generated the gas by putting the solutions of 50 per cent strength of potassium cyanide and sulphuric acid separately in two separator funnels. By means of stop cocks and rubber tubes, the two solutions were allowed to mix in the machine at any desired rate. They were able thus at the beginning of fumigation to produce large quantities of gas by running in the two solutions rapidly and later by slower mixture they could keep up the concentration of the gas in the room being disinfected.

Experiments on a larger scale have been conducted by them, a barge of hold space 12000 cubic feet being used. They write

"Each hold had a rough floor made of planks laid over the ribs of the vessel so that there was a space of about 9 inches to 1 foot between the outer iron plates and the wooden floor. The hatches were very large, and in our experiments, were simply covered with sail-cloth and tarpaulins which were weighted down around the edge of the hatches. The distributing and return pipes from the generating machine were led under the canvas into the holds. The holds were by no means air-tight but in spite of this no appreciable odour of hydrocyanic acid gas was observed by us while walking about on the barge during the course of the experiments. The generating machine was worked ffom the wharf to which the barge was moored. The holds of the barge were empty and rats were placed in cages in different parts both above and below the wooden floors, and in some experiments they were allowed to run freely in the hold and to take such shelter as was available beneath the planks."

The details of some of their experiments show that fleas everywhere were killed. Of rats above the boarding 100 per cent were killed and below the boarding from 50 to 75 per cent were killed. By lengthening the exposure of the gas to 4 hours and

fumigating with potassium cyanide ½ oz to 100 cubic feet of space better results were obtained, the rats in the cages above and below the floor being all killed, while of rats loose in the hold 87 per cent were killed in the after hold and 96 per cent in the forward hold.

CONCLUSIONS.

(1) Hydrocyanic acid gas is an efficient disinfectant for plague owing to its powerful lethal action on rats and rat fleas. I have shown that rats do not live inside grain bags, but between them, and that fleas do not burrow into grain to a greater depth than the gas can penetrate.

(2) The poisonous nature of the gas can be guarded against by the use of the delicate chemical test described, and to some extent by its smell. It has these great advantages over carbon monoxide.

(3) The gas does not injure fabrics nor metals and does not render the foods tested poisonous. Further the germinating power of grains is not injured by fumigation with the gas.

(4) The cost of chemicals is small - for disinfection of 1000 cubic feet 6d to 7d. Fewer operators will be necessary than those required for working sulphur dioxide and carbon monoxide disinfecting plants.

(5) The apparatus used for the generation and distribution of the gas proved efficient for houses, godowns, and the hold of a small ship; it could no doubt be easily improved upon. For the disinfection of a large ship several such machines would be necessary, but their price would not approximate that of either the sulphur dioxide or carbon monoxide plants.

(6) The quantity of gas necessary for thorough fumigation varies according to the nature of the space to be fumigated as regards air-tight conditions. As a rule a ½ to ¾ of an oz. of potassium cyanide

for every 100 cubic feet, the exposure being from 1 to 4 hours, should be sufficient.

(7) Owing to the low specific gravity of the gas it rapidly disappears in ventilation and rapid entrance can be regained. This is a great advantage over other methods of fumigation.

(8) Finally it has been shown that carbon monoxide is not an effective fumigating agent inasmuch as it has no lethal action on of fleas. The fact that it is cheap and that it has no ill effect on grain, metals, or fabrics does not compensate. As stated above it has been found in Hamburg that ships fumigated with this gas have been reinfected with plague probably by fresh rats finding their way on board catching the infection from the fleas left living. The disadvantages of sulphur dioxide have already been enumerated. Hydrocyanic acid gas from all points of view is the most effective.

EXPERIMENT I [1]

A.

Fleas and rats were placed in the small room described on page 41 of 830 cubic feet capacity. 170 grammes of 98 per cent. potassium cyanide, equivalent to about ¼ oz. of the salt to 100 cubic feet air space, was decomposed outside the room. The resultant gas calculated to be about 2.19 cubic feet at 26°Centigrade was led into the room by tubes without the use of a fan to drive it in. The generation of gas took 51 minutes.

The object of this experiment with fleas was to test the penetration of gas through various layers of cloth stuffs.

Details of covering, etc. enclosing the fleas.	Results after 51 minutes' exposure to the gas	Remarks.
(a) 15 fleas were put in a white cotton bag and that again enclosed in one bag of coarse red blanket.	(a) All fleas were found dead.	
(b) 9 fleas in a white cotton beg were enclosed in two layers of coarse red blankets.	(b) Do.	
(c) 11 fleas in a white cotton bag were enclosed in three layers of coarse red blankets.	(c) 10 dead; 1 alive.	
(d) 11 fleas were put in a fine flannel bag.	(d) All fleas dead.	
(e) 9 fleas were put in a chintz bag.	(e) Do.	
(f) 10 fleas were put in a cashmere bag.	(f) Do.	
(g) 20 fleas were put in a single chintz bag and that rolled in a durree, blanket, coat and trousers.	(g) 7 escaped; 12 alive; 1 dead.	

[1] Note.- In Experiments I to IX inclusive the potassium cyanide was stated to be of 98 per cent. strength.

EXPERIMENT I. (contd).

B.

Rats and fleas were put in various positions on the floor - the rats in cages and the fleas in test-tubes with the glass end removed and both ends covered by some netting to allow of proper diffusion of the gas.

Position	Rats			Fleas		Remarks.
	Dead	Sick	Alive	Dead	Alive	
On the floor	...	1	2	...	...	
4 feet from floor	2	...	1	...	...	
7 do	...	1[e]	...	All	...	[e]Escaped but found outside sick.
10 do	...	1	2	All	...	
15 do	...	1	2	All		

C.

Twenty fleas were placed in each of four cages,-two containing sand at the bottom and two bran. At the end of 51 minutes the cages were examined and guinea-pigs put into the cages on three separate occasions to recover the fleas.

Cages	No. of fleas put into each cage.	Result, i.e. recovered alive after exposure of 55 minutes.	Remarks.
Cage No.1 with bran at the bottom.	20	4	
Cage No.2. with bran at the bottom	20	None.	
Cage No.1 with sand at the bottom.	20	2	
Cage No.2. with sand at the bottom	20	2	

For a control to this, see the end of Experiment III.

EXPERIMENT II.

A small room was used - of 830 cubic feet capacity. The same amount of hydrocyanic acid gas as in Experiment I was introduced without the use of a fan. The exposure to the gas was for half-an-hour only.

Position	Rats			Fleas		Remarks
	Dead	Sick	Alive	Dead	Alive.	
On the floor	1	...	1	...	...	
4 feet from floor	1	...	2	All	...	
6 do	...	2	...	...	...	
10 do	...	2	...	All	...	
12 do	...	1	...	All	...	

Experiment with Fleas.

Details of covering etc. enclosing the fleas	Results after 30 minutes' exposure to the gas	Remarks
(a) 11 fleas were put in a white cotton bag and that again enclosed in one bag of coarse red blanket.	(a) 9 alive;2 dead.	Another cotton bag containing 11 fleas was put between two layers of blanket and two of cretonne - all dead. In this case a pipe discharged the gas on the top of the blanket.
(b) 12 fleas in a white cotton bag were enclosed in two layers of coarse red blanket.	(b) 7 alive;5 dead	
(c) 12 fleas in a white cotton bag were enclosed in three layers of coarse red blanket.	(c)10 alive;2 dead	
(d) 17 fleas were put in a fine flannel bag.	(d) All dead.	
(e) 14 fleas were put in a chintz bag.	(e) All dead.	
(f) 15 fleas were put in a cashmere bag.	(f) All dead.	
(g) 10 fleas were put in a single cotton bag and that rolled in a durree, blanket, coat, and trousers.	(g) All alive.	

EXPERIMENT III.

A small room of 830 cubic feet capacity was used. 340 grammes of 98 per cent potassium cyanide, equivalent to about 1½ ozs. of the salt to 100 cubic feet air space, was decomposed outside the room. The resultant gas, calculated to be about 4.38 cubic feet at 26° Centigrade was delivered into the room by means of a fan. Exposure for 20 minutes.

Details as to conditions under which the fleas were admitted to the action of the gas.	Results of fumigation	Remarks.
(1) 10 fleas in a cotton bag wrapped in several layers of blanket and thick sheet.	(1) All dead	In all these results the fleas were kept for two hours and looked at again - in no case did any flea show signs of life.
(2) 8 fleas in a bag at the foot of a box containing boots, deckshies (copper vessels), and some clothing.	(2) All dead.	
(3) 9 fleas in a bag wrapped in a durree, blanket, and coat rolled tightly up.	(3) 8 alive; 1 dead	Control fleas kept in bags in precisely the same conditions as those shown herein were found at the end of two hours to be all alive.
(4) 9 fleas in a cotton bag on top of a box.	(4) All dead	
(5) 8 fleas in a cotton bag wrapped up in a durree and 3 blankets, fairly tightly rolled up - a pipe discharging gas was put at the mouth of the roll.	(5) All dead	4 cages, 2 with bran and 2 with sand at the foot, were placed on the floor. 20 fleas were put in each. After fumigation guinea-pigs were put three times into each box. No fleas were recovered, so presumably all were killed by the gas.
(6) 9 fleas in a cotton bag hanging 5½ deet from the floor.	(6) All dead	
(7) Bag 'A' as in Experiments I and II, that is, 8 fleas in a cotton bag enclosed in a red blanket bag 5½ feet from the floor.	(7) All dead	
(8) Bag 'B', same as above, but two layers of blanket bags.	(8) 7 fleas; all dead.	In control cages guinea-pigs were put in; recovered 21 out of 40 fleas from the 2 sand cages, and 19 out of 40 from the 2 bran cages.
(9) Bag 'C', same as above, but three layers of blanket bags on the floor.	(9) 8 fleas; all dead.	
(10) 8 fleas in a test-tube on the floor.	(10) 8 fleas; all dead.	

EXPERIMENT IV.

In disinfection of boxes do the clothes need to be taken out of boxes and hung up; or can we thoroughly disinfect clothes by leading pipes into the boxes?

Two experiments were done on this point.

The same amount of hydrocyanic acid gas as in Experiment III was pumped into the small room of 830 cubic feet capacity (about 4.38 cubic feet). The exposure was for 20 minutes. The room was entered in 30 minutes from commencement of operations. By using the test rod a good prussian blue tint showed that a concentrated gas was passed into the room. Nine pipes took the gas into the room from the generating chamber outside. One pipe was led into the bottom of Box I. Two pipes were led into the bottom of Box II.

In Box I from above downwards fleas were put in the following positions:-

Particulars	Result	Remarks
A. - 9 fleas were in a bag covered by coat, trousers, durree, and blanket.	All 9 alive	
B. - Lower down rug, 4 blankets, 1 chudder, and durree, then the bag with 8 fleas.	All 8 alive	
C. - Then a great-coat and 10 fleas below in a cotton bag.	9 alive; 1 dead	
D. - Then a military blanket, then 7 fleas in bag; than a rug on bottom of box.	4 alive; 3 dead	

In Box II from above downwards fleas were put in the following positions:-

Particulars	Result	Remarks
A. - Rug, then bag containing fleas	All dead	No.2 box much less tightly packed than No.1. box.
B. - Blanket, pillow, and then bag containing fleas.	All dead	
C. - Pillow and rug, then bag containing fleas.	All dead	20 fleas in 4 boxes 2 with sand at the bottom and 2 with bran, as before.
D. - Pillow and rug, then bag containing fleas; 2 pillows at foot of box.	All dead	None recovered; all present dead presumably.

EXPERIMENT V.

With Clothes in Boxes.

Same period of exposure 20 minutes. The same amount of hydrocyanic acid gas (about 4.38 cubic feet) as in Experiment III was introduced into the room of 830 cubic feet capacity by means of a fan.
Box I, from above downwards.

Particulars	Result	Remarks
1. Rug and chudder, then cotton bag with 10 fleas in it.	10 fleas; all dead	
2. Then durree and 2 blankets with bag of 7 fleas below.	7 fleas; all dead	
3. Then one blanket, with bag of 9 fleas below.	9 fleas; all dead	
4. Then a blanket in several folds and a bag with 7 fleas below it.	7 fleas; all dead	
5. Then another blanket with 9 fleas below in a bag.	9 fleas; all dead.	

Box II. - Two pipes were led into Box II, from above downwards.

Particulars	Result	Remarks
(a) Pillow, then 10 fleas in bag.	9 dead;1 just feebly living.	The pipes were led into foot of box. Those fleas which were found "feebly living" at the end of experiment remained so for 1½ hours after the experiment.
(b) One rug and 9 fleas in bag below it.	6 dead; 3 feebly living	
(c) Then rug and blanket with 8 fleas in bag.	8 dead, all.	
(d) Then rug and pillow with 10 fleas in bag below.	10 dead, all	

EXPERIMENT VI.

Does the gas kill cultures of micro-organisms and what effect has it on the germinating power of grains? Does it render dry food-stuff poisonous?

The small room of 830 cubic feet capacity was used. About 4.38 cubic feet of hydrocyanic acid gas was introduced by a fan and allowed to act 20 minutes from the start. The test rod showed plenty of gas to be present.

The grains tested were: (1) Bajri, (2) Wheat, (3) Jowari, (4) Mula or Radish, and (5) Rice.

(1), (2), and (5) were put in kerosine oil tins. A pipe conveying the gas was conducted to the bottom of the mass and one was directed on the surface. Mula and jowari were in small amounts and gas played on their surface from pipes directly placed on them.

I. Were the grains poisonous?

No. Tested on monkeys, rabbits, guinea-pigs, hend and pigeons. Fed on it for 24 hours. None dead nor ill. Again chapatis were made of the wheat, jowari, and bajri, and monkeys and hens were fed on them. No ill results.

II. The germinating power of the seeds was not affected. Certain quantities of the above grains were planted and seeds not subjected to the gas were planted at the same time to serve as control in the comparison of the rate of growth.

Both examples of bajri, jowari, and mula grew well; of wheat grew feebly, because much of it was husked.

Cultures of (a) Micrococcus melitensis, (b) Cholera spirillum, and (c) Bacillus anthracis, were placed in the room, but the cotton-wool stoppers were not removed from the test-tubes containing the cultures as it was thought not to be safe to do so. They were after subjection to the gas re-transplanted on agar and showed good growths.

Test-tubes containing cultures of (a) Streptococci, (b) Bacillus typhosus, (c) Bacillus coli communis, and (d) Bacillus pestis, were placed in the room with their cotton wool plugs removed, so that the gas had full action on the germs. After the experiment the germs were recultured on agar. The Bacillus pestis was overgrown with some yellow contaminating organism. The others grew well and seemed to the naked eye to be pure cultures.

So probably the above germs are not affected by the gas.

Fleas after 5 minutes' exposure to the gas were found dead. They were put into tubes and withdrawn after 5, 10, and 15 minutes' exposure, all dead.

EXPERIMENT VII.

The following experiments - VII, VIII, and IX - were performed in the large room. Description of room and burrows will be found on pages 18-20 The cubic capacity is about 1,640 cubic feet. In Experiment VII the gas was delivered into the room without fan by tubes led from flasks outside the room. Potassium cyanide of the strength of 98 per cent to the amount of 255 grammes (equivalent to about .56 ozs. of the salt to 100 cubic feet air space) was decomposed The resultant hydrocyanic acid gas was calculated to be about 3.28 cubic feet at 26° Centigrade.

In 10 minutes after the cyanide and acid were mixed sufficient gas was evolved to give a decided result with the test rod.

The exposure to the gas was for 45 minutes.

Position	Rats			Fleas		Remarks
	Dead	Sick	Alive	Dead	Alive	
Rats on wall	3	3	1	20(all)	...	
Hanging from rafters (10-15 feet)	...	1	1	All	...	
15 feet from floor	...	...	1	All	...	
The tubes were introduced into the burrows 1½ feet.						
Burrow A.	...	...	1	...	All	For description of burrows see page 19
" B.	...	...	1	...	All	
" D.	...	...	1	...	All	

Fleas were placed in 4 cages, 2 of bran and 2 of sand on the floor

Cages	No.of fleas put into each cage.	Results i.e. recovered alive after 45 mins. exposure.	Remarks
Cage No.1 with bran	40	1 was recovered after fumigation	The counts were made by means of guinea-pigs in all these cases.
Cage No.2 with bran	20	None recovered	
Cage No.1. with sand	20	12 were recovered	For a control to this see the end of Experiment III
Cage No.2. with sand	20	14 were recovered.	

In this experiment the gas proved so light that it was most effective at the upper levels of the room; many fleas in cages on the floor escaped, especially those in cages with sand at the bottom in which they buried themselves. Both fleas and rats in the burrown escaped even although the tubes were led into the burrows a little distance.

EXPERIMENT VIII.

Another experiment was tried with the following alterations: (1) the fan was worked; (2) the tubes leading off from the main tube were larger in calibre and their total surface area equal to the surface area of the large tube leading from the generator, so that the full amount of gas was delivered into the room. There were nine pipes altogether: five delivered into the room at different heights and four were inserted into the four burrows, and the mouths of the burrows where the pipes were inserted were choked up.

Three hundred and forty grammes at 98 per cent Potassium cyanide was decomposed. The resultant hydrocyanic acid gas calculated to be about 4.38 cubic feet at 26° Centigrade was delivered into the room of about 1,640 cubic feet capacity. The amount of potassium cyanide used would be equivalent to about ¾ oz. to 100 cubic feet air space. There was 35 minutes' exposure to the gas. By means of the observation rods the room was seen to be sufficiently free from the gas 5 minutes later. Forty minutes, therefore, were taken for the whole experiment. After the fumigation the fleas were examined at once and also three hours later to see whether they had revived.

Position	Rats			Fleas		Remarks
	Dead	Sick	Alive	Dead	Alive.	
On the floor	3	...	1	28(all)	...	
4 feet from ground	3	...	1	12(all)	...	
6 do	2	...	....	...	...	
8 do	2	....	...	...	...	
12 do	3	...	...	10(all)	...	
On the rafters	...	...	...	11(all)	...	
Burrow A.	1	...	...	15(all)	...	For description of burrows, see page 19
" C	1	...	...	11(all)	...	
" D-Obstruction in pipe found	...	...	1	...	12(all)	20 fleas were put in each of the four cages (2 of sand and 2 of bran).
" B .-Observation burrow opened in midst of experiment.	...	1	...	10(all)	...	After fumigation none were recovered by guinea-pigs and therefore were presumably killed. For control see Experiment III.

EXPERIMENT IX.

Into the same room of about 1,640 cubic fe t capacity about 6.57 cubic feet of hydrocyanic acid gas were introduced, equivalent to about 1.2 ozs. potassium cyanide to 100 cubic feet air space. The exposure was for 40 minutes. Rats were let loose on the floor and put loose into the burrows to simulate natural conditions.

The gas was driven into the room by the fan. The disposition of pipes was similar to that in Experiment VIII.

Position	Results			Remarks
	Dead	Sick	Alive.	
6 on the floor	3	1	2	For description of burrows see page 19
Burrow A, 3 put in (Blind burrows)	3	...	...	
Burrow B, 3 put in (Blind burrows)	3	...	...	
Burrow C, 4 put in	1	...	3 escaped	
Burrow D, 3 put in	2	...	1 escaped.	

EXPERIMENT X.

The following experiments - X, XI, and XII - were made without the use of fan or tubes. The gas was produced in the room itself. First of all the sulphuric acid (commercially strong, specific gravity 1.83) was mixed with water (2 parts of sulphuric acid to 4 parts of water) generating heat. The mixture was made in a china vessel when small amounts were used and in a kerosine oil tin when large amounts were necessary. Then one part of potassium cyanide (100 per cent Burgoyne Burbidges' Double Salt, for analysis see pages of Report) was put in and the room at once quitted and the door shut.

In Experiment X a small, very air-tight godown was chosen, of about 346 cubic feet capacity.

One oz. potassium cyanide 2 ozs. sulphuric acid and 4 ozs. water were mixed. The exposure was for 40 minutes. The doors were then opened, but fumes of gas still were seen to be coming off.

The room was entered 15 minutes later. The fleas were examined at once and also two hours later.

Position	Fleas		Remarks
	Dead	Alive.	
15 fleas in a muslin bag 8 feet from the ground.	15	...	
6 fleas in a flannel bag on the floor.	6	...	
9 fleas in a cashmere bag	9	...	
10 fleas in a cotton bag enclosed in one rough blanket bag 5 feet from floor.	10	...	
7 fleas in a cotton bag enclosed in two blanket bags 5 feet from floor.	7	...	
11 fleas in a cotton bag enclosed in three blanket bags.	11	...	

EXPERIMENT X (contd)

A box was put into the room with fleas in bags in different positions.

Position	Result	Remarks.
On the top of all was a bag with 8 fleas.	8 dead (all)	50 fleas were put in each of four cages, 2 with sand at foot and 2 with bran. After the fumigation was over flea counts were made on guinea-pigs three times at intervals of several hours and none were recovered. All, therefore, were presumably killed. For controls, see the end of Experiment III.
Then came a durree, trousers, and a coat in folds.		
Then came No.2. bag with 10 fleas	Immediately after the godown was opened all the fleas looked dead, but two hours later four were found dead and six alive.	
Then came a pair of trousers, a coat, and a durree.		
Then No.3 bag with 7 fleas.	4 alive; 3 dead.	
Then came several layers of a chudder with No.4. bag below it.	9(all) living two hours after opening the godown.	
Then came a blanket in 8 layers with No.5 bag below.	7 alive; 3 dead, two hours after opening the godown.	
Then several layers of a rug with No.6. bag below.	5 dead; 4 alive, two hours after opening the godown.	

The following experiments - XI and XII - were made to determine whether the simple process above described would disinfect the large room of 1,640 cubic feet capacity described on pages containing burrows, so dispensing with the fan and complicated arrangements of generator and tubes. It will be seen that the burrows were not penetrated even with the 1 oz. of KCN to 100 cubic feet used in Experiment XII.

EXPERIMENT XI.

Eight ozs. of potassium cyanide (Burgoyne Burbidges' Double salt 100 per cent) 16 ozs. of sulphuric acid specific gravity 1.83 and 32 ozs of water were mixed - a proportion of about ½ oz. to 100 cubic feet.

The exposure was for 50 minutes. Fifteen minutes later the room was entered and was examined at once on opening the godown and also three hours later.

Position	Rats			Fleas		Remarks
	Dead	Sick	Alive	Dead	Alive.	
On the floor	2	1	1	20(all) dead	...	
4 feet from the floor	...	...	2	8 dead	...	
5 do	5	...	...	30 dead	...	
6 do	1	...	...	...	...	
10 do	1	...	2	21 dead	5	
Rafters	...	...	...	8 dead	6	
Burrow A)Blind(burrows	...	...	1	...	7	For description of burrows, see page
" B)Blind(burrows	...	...	1	...	7	
" C)Two way(burrows	...	...	1	...	8	
" D)Two way(burrows	...	...	1	...	7	

EXPERIMENT XII.

Same room. 16½ ozs. of potassium cyanide (same salt as in Experiments X and XI), 32 ozs. of sulphuric acid of specific gravity 1.83 and 64 ozs. of water - about 1 oz. of KCN to 100 cubic feet of space. Exposure 50 minutes.

Position	Rats			Fleas		Remarks
	Dead	Sick	Alive	Dead	Alive	
On the floor	1	2	1	21	...	
4 feet from floor	2	1	2	9	...	
6 do	3	...	1	35	...	
10 do	2	...	1	16	2[1]	[1] These appeared dead on opening the godown, but recovered 3 hrs. later
Rafters	...	...	...	2	9	
Ledge near open space between roof and smaller end wall.	...	...	...	7	2	
Burrow A) Blind burrows	...	...	1	...	6 (all)	For description of burrows, see page
" B) "	...	...	1	...	9 (all)	
" C) Two way burrows	...	...	1	...	9 (all)	
" D) "	...	...	1	...	8 (all)	

EXPERIMENT XIII[1]

The amount of the chemicals used were 5 ounces of potassium cyanide, 10 ounces Sulphuric acid and 20 ounces of water.

The gas was produced within the godown, as described in page 40.

The experiment commenced at 8 a.m. and the door of the godown was not opened for ventilation till 3 p.m. and the bags removed and the godown inspected next morning.

30 rats (all) were found dead, and all the fleas buried 1 to 2 inches in the rice bags were killed.

Some penetration of the bags had then occurred.

Ten fleas had been buried as a control, in a bag of rice not exposed to fumigation, for the same length of time as the fleas in the experimental godown. Of these one was found living.

The rats which were killed had been allowed to run about in the godown for some hours. The godown was packed full of rice bags as described on pages 55.

[1] The three subsequent experiments were incorporated in a report published in the Proceedings 2 All-India Sanitary Conference 1914.

In the following experiments attempts were made to observe the action of the gas on fleas set free on the top of rice in bulk in the godown. The floor of the godown was covered over with rice, both husked and unhusked to the depth of 3 or 4 inches. It was thought that fleas might burrow into the rice to some depth and so escape destruction by the gas. Two godowns were taken and the rice spread over the floors of both. The same number of fleas were set free in both. One godown was fumigated, the other was used for control observations. Guinea-pigs which were thoroughly freed from fleas were introduced into both rooms to act as a flea trap and a flea count thereafter made on the guinea-pigs.

EXPERIMENT XIV.

170 fleas were introduced in both godowns at 8-30 a.m. One hour later one of the godowns[1] was fumigated (5 ounces of Potassium cyanide, 10 ounces Sulphuric acid, and 20 ounces of water). Both godowns were opened at 12 noon. At 1 p.m. two guinea-pigs (freed from fleas) were placed in both rooms and allowed to remain till 10-30 a.m. next day. They were thereafter chloroformed and examined.

From the control godown, which had not been fumigated, 8 fleas were recovered.

From the fumigated godown no fleas.

As a side issue, 2 porous bags containing fleas were buried 1 inch deep one in the unhusked and one in the husked rice. All the fleas were found dead at 1 p.m. on the day the gas was introduced (fleas under the same conditions in the control godown unfumigated were found alive at the same time.)

Again therefore penetration of the rice by the gas to within one inch had occurred.

[1] of about 346 cubic feet capacity and very air-tight.

EXPERIMENT XV.

This is a repetition of Experiment VI but 300 fleas were put into both godowns.

They were introduced at 9-15 a.m. One godown was fumigated at 11-30 a.m. The rooms were opened at 1 p.m. 4 guinea-pigs freed from fleas were introduced into both godowns at 2 p.m. They were taken out for examination at 11-30 next morning and again placed in the godowns for 2 hours more, when they were again inspected.

32 fleas were recovered from the control non-fumigated godown and none from that fumigated.

It was concluded therefrom that when rice is in bulk, fumigation would kill fleas, as they evidently do not burrow into the rice.

INTRODUCTION

ANTI-PLAGUE VACCINES.

The preparation of an efficient anti-plague vaccine is of general importance from the public health point of view. It is on the prophylactic results of the use of anti-plague vaccine that the Indian Government largely depends in its yearly campaign against plague. The extent to which this vaccine is adopted depends on two factors (1) the zeal of the local authority, District Commissioner Civil Surgeon or Sanitary Commissioner, and the extent of his belief in its efficacy and (2) the attitude of the general population - vaccination everywhere being voluntary.

An index of the growing popularity of this method of combating plague is the increase in the figures of the amounts of vaccine issued by the Plague Laboratory at Parel, Bombay. The amount of the vaccine issued depends of course on the severity of an epidemic in any given year. The following figures taken from the Reports of the Bombay Bacteriological Laboratory for 1910-11-12 give the number of doses issued for the last 7 years:-

For 1906	... ...	176,651 doses
1907	... ...	620,923
1908	... ...	583,315
1909	... ...	593,164
1910	... ...	625,690
1911	... ...	1,211,170
1912	... ...	727,377

The fall in the demand in 1912 was due to the comparative mildness of the disease in that year. Two outstanding outbreaks of plague were combated chiefly by the use of vaccine in 1911. In Hyderabad City out of a population which remained in the city

during the outbreaks, of about 187,000, approximately 78,085 persons were inoculated. In Salem City in the Madras Presidency out of a population of 73,000 persons, 53,500 were inoculated. These inoculation campaigns will be considered further in their proper place. At present all I wish to do is to point out to what an extent inoculation can be carried out in a population who voluntarily submit to it.

It is accordingly of great importance to consider what are the best methods to employ in the preparation of a vaccine. In our consideration of the merits of the various methods which have been adopted it is not sufficient to consider merely the relative efficacy or prophylactic power of any particular vaccine. It is owing to the question having been regarded only as of academic interest that many of the methods have been advocated. From a practical standpoint the following requirements must be kept in view. The vaccine must, besides being efficient, be absolutely safe, it must be capable of being turned out safely in large quantities, and it must not offend the religious and social prejudices current in India. The cost of production must also be kept low. It is from these critical standpoints that I wish to discuss in detail the various vaccines employed.

Yersin, Calmette, and Borrel[1] were the first to take up the question of immunisation of animals (rabbits were used) by killed bacillary cultures. The bacilli were killed at a temperature of 58°C. at one hour's exposure. These authors[2] were specially interested in the production of a curative immune serum. The methods which have been

[1] Annales de l'Institut Pasteur, Tome IX 1895, "La Peste Bubonique".

[2] "Sur la peste bubonique" (sero therapie) by Dr. A. Yersin, Annales de l'Institut Pasteur, 1897.

advocated subsequent to the work of Yersin Calmette and Borrel may be classified as follows. I take them in the order which I think is most convenient, considering at the outset those methods which do not appear to be very practicable or important.

(1) Immunisation by means of the clear filtrates of bouillon cultures of plague, containing protective substances and toxines in solution, probably endo-toxines set free by bacteriolysis.

(2) Vaccines which consist of the dead bodies of bacilli grown in living organisms; in Klein's method and possibly S. Mullanah's.[1]

(3) Methods which depend on the action on dead bacillary bodies of both immune and non-immune sera, so as to obtain a soluble endo-toxine - Shiga's method and Besredka's method.

(4) A method of using for vaccination natural and artificial plague aggressins; the method of Hueppe & Kikuchi and Strong's method.

(5) Analogous with No.4. but prior to it, a method which uses as a vaccine the sterilised peritoneal exudate of guinea-pigs injected intraperitoneally with virulent plague bacilli, the exudate being claimed to contain along with the plague bacilli protective substances - the method of Terni and Bandi.

(6) Methods which involve the use of avirulent living plague bacilli; the method of Kolle and Otto and of Strong.

[1] S.Mullanah's preparations were really curative extracts of organs in which plague bacilli grew. Strong (Phillipine Journal of Science vol.VI, 1907 and Rowland (Journal of Hygiene vol.X, No.III, 5th Plague number), both class Mullanah's work along with Klein's.

(7) A vaccine composed of the nucleo-proteid obtained from the bodies of plague bacilli as in the method of Lustig and Galeotti.

(8) Vaccines composed of true antigenic substances obtained from the bodies of plague bacilli, as in Rowland's work.

(9) Those in which the vaccines consist of the bodies of dead bacilli and their products in cultivation; whether these have been produced by growth in bouillon, as in Haffkine's method, or upon Agar as recommended by the German Plague Commission.

(1)

Immunisation by means of the clear filtrates of bouillon cultures of plague, containing protective substances and toxines in solution probably endo-toxines set free by bacteriolysis.

There has been much difference of opinion as regards the toxines of plague bacilli. Some have held that the toxines are soluble products and may be found in the clear filtrates of bouillon cultures. It is however, the more common view that the toxines and immunising bodies are intrabacillary and only find themselves in solution or very fine suspension on bacteriolysis.

I should state here that the exact relationship between the toxines present in the vaccine and the substances capable of producing immunity (antigenic substances) is not known. I will enlarge further on this in dealing with Rowland's work (p 102-104)

Yersin Calmette and Borrel[1] found that the filtrates of bouillon cultures of the plague bacilli had no toxic action, Roux, according to Metchinkoff[2], found that filtrates from a bouillon culture precipitated by ammonium sulphate have a powerful toxin, 0.25 milligrammes proving fatal to rabbits.

Markl's[3] results were published in 1898 and 1901. He found that filtrates of bouillon cultures were highly toxic; that the toxine particularly occurred in the case of old cultures that had been grown at a low temperature - at 20°C. although they occurred also in young filtrates. The toxine developed in cultures up to about

1 Annales de l'Institut Pasteur, 1895 "La Peste Bubonique", (Tome IX)

2 "Sur la peste bubonique" Annales de l'Institut Pasteur, Tome XI-1898

3 The information on the opinion of Markl is obtained from "Toxins and anti-toxins" by Karl Oppenheim, and from G. Dean's "Researches on certain problems of plague immunity": Studies on Pathology Aberdeen University 1906.

the 2nd month of growth, when it became stationary, and it then gradually decreased in amount. Plentiful admission of air was essential for the production of immunising substances; a high temperature of incubation had an injurious effect on the development of toxines. According to Markl the plague bacillus produces a soluble toxine, which is secreted by the bacilli and is not due to a solution of intracellular bodies owing to the destruction of bacilli. According to Oppenheim[1], Kossel and Overbeck in 1901 produced an immunity by means of the filtrates of cultures. Kolle (1903)[2] was of opinion that the toxine was bound to or contained in bacillary bodies and that toxines present in the old filtrates were due to the solution of intracellular bodies on the death of bacilli. He stated that on immunisation the anti-plague sera had no anti-toxic effects beyond the normal toxine - neutralizing effects of normal horse sera. Strong[3] produced imperfect immunisation of monkeys with "free receptors" of the plague bacillus. He used 48 hours' agar cultures suspended in distilled water killed at 60°C. and thereafter filtered. Two out of nine monkeys proved immune.

G. Dean's[4] researches. Working with the filtrates of broth cultures, he found that the maximum toxicity of the culture is synchronous with the death and degenerative changes in bacilli, and that probably therefore the toxic substances were set free by a process of autolysis. He found great and inexplicable variations in the toxicity of various broth culture filtrates. Neutral broth appeared to be a more favourable medium for the development of

[1] "Toxins and anti-toxins"

[2] The information re Kolle's views is derived from G. Dean's "Researches on certain problems of Plague Immunity - Studies on Pathology".

[3] 'Studies on Plague Immunity' Phillipine Journal of Science 1907.

[4] "Researches on certain problems of Plague Immunity - Studies on Pathology"- Aberdeen University 1906.

toxines than an alkaline one. For mice the lethal dose was

.1cc of a 2 months' growth on neutral broth

.5cc of a 2 months' growth on alkaline broth;

.1cc of a 10 days' growth on neutral broth failed to kill. For rats .1cc of a 10 weeks' old neutral broth killed when given subcutaneously.

Dean found that the injection of large doses of these toxic filtrates produced an anti-toxine He quotes a paper read by Captain Douglas, I.M.S. before the Pathological Society of London in 1906 showing that, contrary to the view held by the German and English Plague Commissions, the supernatant fluid obtained from Haffkine's prophylactic has powerful immunising properties.

Rowland[1] states that the toxic substances present in the filtrates of Markl and Dean are probably identical with the protein he obtained by his extractive processes, a protein derived from the bodies of bacilli and present in old cultures. He did not find these in 4 days' cultures; a slight amount was present in older cultures of 14 days, and a much larger quantity in 13 months' old cultures.

Experiments conducted by myself with the assistance of Khan Bahadur Senior Assistant Surgeon R.J. Kapadia.

In the following experiments I attempted to test the toxicity of the filtrates of a 6 weeks' old bouillon vaccine. In Experiment I, and in Experiment II to compare the immunising value of the filtrate with the immunity conferred by the whole vaccine of Haffkine. My results from these two experiments, which I acknowledge should be amplified considerably, are (1) that the filtrates of the 6 weeks' old

[1] Journal of Hygiene vol.X No.3.

bouillon culture of Haffkine's prophylactic possesses a toxicity equal to that of the whole vaccine, and (2) that the filtrates of bouillon cultures possess equal immunising power to that of the whole vaccine.

It should be noted that this does not denote that the plague toxine is a soluble one as Markl stated, but that in all probability during the process of cultivation free endo-toxines are liberated in the supernatant fluid. If further experiments show that these two conclusions are true, then a simplification of the present Haffkine's prophylactic could be employed, viz: the use of the absolutely clear broth without bacillary bodies.

Experiment I:-

To compare the toxicity for rats of -

(a) the whole bouillon vaccine of Haffkine consisting of both bacillary bodies and substances in solution in the broth.

(b) the filtrates of the bouillon vaccine of Haffkine after filtration through a Chamberland filter; and

(c) the filtrate from the whole vaccine of Haffkine after the action of normal serum upon the vaccine in the manner described by Besredka[1]; containing according to that author endo-toxines set free by the agglutinative action of the serum. 10 c.c. of the culture bouillon were acted upon by 40 c.c. normal horse serum and then filtered through a Chamberland filter.

[1] "Etudes sur le bacille de la peste", Annales de l'Institut Pasteur, Tome XIX - 1905.

The same bouillon culture of plague was used throughout for the three series. It had been growing for exactly 1½ months, was then sterilised, and carbolic acid added to dilution of 0.5 per cent. The original source of bacilli was from the heart blood of a guineapig dead of plague within 5 days after cutaneous scarification. The vaccine was used the day after sterilisation. The filtrates were obtained by filtration through Chamberland filters and were culturally proved sterile. The rats used for experimentation were the Indian wild mus rattus obtained from Madras. These rats have been shown by the last Plague Commission to possess no natural immunity to plague[1].

Results:-

(1) 2 rats were injected intraperitoneally with 4 c.c. of the whole vaccine - 2 dead in 24 hours.

2 rats were injected intraperitoneally with 3 c.c. of the whole vaccine - 2 dead in 24 hours.

2 rats were injected intraperitoneally with 2 c.c. of the whole vaccine - 2 dead in 24 hours.

2 rats were injected intraperitoneally with 1 c.c. of the whole vaccine - 2 living in 24 hours.

2 rats were injected intraperitoneally with 0.5 c.c. of the whole vaccine - 2 living in 24 hours.

(2) 2 rats were injected intraperitoneally with 4 c.c. of filtrate (b) (proved sterile) i.e. filtrate of whole culture vaccine. 2 dead in 24 hours

2 rats injected with 3 c.c. intraperitoneally 1 do

2 rats do 2 c.c. do 2 do

[1] Journal of Hygiene 7th Report of Plague Commission Supplement II - 1912.

(3) 2 rats were injected intraperitoneally with 4 c.c. of filtrate (c) proved sterile on agar. living.

2 injected with 3 c.c. intraperitoneally living

2 do 2 c.c. do living

It must be remembered that the vaccine in series 3 (filtrate C) was diluted in Horse serum - one to four. But if Besredka's claim that large amounts of endo-toxines are set free in the bouillon by the action of the serum be true, 4 c.c. of the mixture in series 3 (= .8 c.c. of the filtrate) should have been highly toxic.

In case the small amount of carbolic acid present throughout in (a) & (b), 0.5 per cent, should be deemed responsible for the death of the rats, two rats were injected intraperitoneally with 3 c.c. sterile broth plus carbolic acid 0.5 per cent, and two rats were injected intraperitoneally with 2 c.c. sterile broth plus carbolic acid 0.5 per cent. None of the rats died.

CONCLUSION -

That the filtrate of a 6 weeks' old bouillon culture of the plague bacillus possesses a toxicity for rats equal to that possessed by the whole culture when sterilized.

Experiment II -

To compare the immunising value of the whole vaccine of Haffkine and of the filtrate of the whole vaccine.

The vaccine was the same as used in Experiment I but 5½ months old, and Madras rats, possessing no natural immunity to plague, were chosen for our experiments.

(A) 10 rats were immunised by the whole vaccine - 8 survived the immunisation.

(B) 10 rats were immunised by the filtrate of the whole vaccine - 8 survived the immunisation.

(C) 10 rats were kept as controls.

17 days later all the rats were inoculated sub-cutaneously with .0018 milligramme of the spleen of a rat which had died of plague and which in smears showed enormous numbers of bacilli.

Of series (a) on the 8th day after the infection by plague 4 survived out of 8 - 50 per cent immunity.

(b) on the 8th day after the infection by plague 5 survived out of 8 - 63 per cent immunity.

(c) on the 8th day after the infection by plague 9 survived out of 10 - 10 per cent immunity.

CONCLUSION.-

The filtrates of the bouillon culture possessed at least equal immunising powers to that of the whole vaccine. The small number of rats experimented upon do not permit any conclusion to be drawn between the 50 per cent immunity of (a) series and the 65 per cent of (b) series. The difference may lie within the limits of experimental error. But undoubtedly the difference between on the one hand series (a) and (b) and on the other series (c) is quite conclusive of the development of considerable immunity. I therefore conclude that the observations of Douglas are correct and that the supernatant fluid of Haffkine's prophylactic possesses powerful immunising properties.

(2) Vaccines which consist of bacilli grown in living organisms and thereafter killed.

(a) Klein's method.

Klein[1] detailed a new process of plague inoculation which he employed on mice, rats, and guineapigs. He removed the lymphatic glands, liver, spleen and lungs of a guineapig which had died of subacute plague. These he minced up and dried at a temperature of 45° to 47° centigrade over sulphuric acid. He stated that the desiccation killed all the plague bacilli. The material then in thin dried scales was finally powdered in a mortar. For injection, a definite quantity of the powder was weighed out, transferred to a sterile mortar, and rubbed up into an emulsion with sterile warm distilled water. The immunising dose for rats he gave as 10 milligrammes, and he thought it advisable to repeat this dose after an interval of 9 to 10 days. This amount afforded absolute protection to rats against B. pestis when tested 1 to 13 weeks after immunisation. In his comparisons of the efficacy of this vaccine with Haffkine's, he made the statement that 10 cubic centimetres of Haffkine's prophylactic is necessary to immunise an adult rat. In my experience two or three cubic centimetres of Haffkine's prophylactic will cause acute toxaemia and death in full grown mus rattus in a large number of cases (p. 119) and a dose of one quarter or one half cubic centimetre is sometimes sufficient to immunise more than 50 per cent. of rats against subcutaneous injections of virulent plague, pp. 127. 128. The probability is that Klein worked with a very feebly toxic vaccine prepared after the manner of Haffkine's. It was sterilized at 70° C. a temperature which we now know destroys a large amount of the immunising bodies and toxines present in the vaccine. The comparison between the efficacy of these two vaccines

[1] Report to the L.G.B. 1906, p.392.

is therefore fallacious. Undoubtedly the procedure adopted by Klein has given a strongly immunising vaccine but there are several serious objections to its use.

(1) It would be impossible to prepare it in a large enough scale to produce a million doses a year as we require in India, if for no other reason than that the expense would be prohibitive.

(2) With the greatest care and with the employment of a large skilled staff it would be found impossible to insure the sterility of the vaccine. Contamination from the skin of the animals whose viscera are employed and from the air in the manipulations to which the organs are subjected before the final stage is reached would almost certainly occur if the vaccines were to be prepared in any large amounts.

(3) Klein states that the process of drying certainly destroys the vitality of the plague bacilli. This, of course, could be insured by culture before the vaccine is despatched for use, but the procedure would require constant care, and accidents might occur and living germs be sent out.

(4) A vaccine which consists of an animal extract, as Klein's does, would certainly meet with the greatest opposition to its use in India from the religious and social point of view. The vaccine has never been employed, to my knowledge, to immunise human beings.

(b) S. Mullanah's method.

Strong[1] and Rowland[2] both state that Mullanah advocated somewhat similar methods to that of Klein for the preparation

[1] Phillipine Journal of Science vol.VI, 1907

[2] Journal of Hygiene vol. X, No.3.

of anti-plague vaccine. Mullanah[1] proposes a curative glandular extract, and, so far as I can see, he does not advocate it as a prophylactic vaccine. A paper of Mullanah's in the Centralblatt fur Bacteriologie, I originale, t. XLII 1906[2] is epitomised by Besredka in the Bulletin de l'Institut Pasteur, vol. V, 1907 and appears to contain an account of the same work as is found in the Lancet above quoted. Besredka considers the number of observations made by Mullanah as too few to draw conclusions from. The hypothesis he works upon is that the organs where the plague poison exercises its concentrated effects probably are the centres where the anti-bodies are manufactured. He carried out his work in the Hygienic Institute at Hamburg in 1905 to 1906. His method was as follows:-

Healthy rabbits were inoculated with Haffkine's prophylactic to produce a slight immunity. Later, weak culture of living plague bacilli were given subcutaneously, and finally virulent germs in increasing doses were given until the animal could withstand one or two loops of very virulent plague germs. Lastly, the animals which were quite sound in health were killed with chloroform, generally 15 days after the last intravenous injection. The glandular juice of such organs failed to show any bacteria and cultures were sterile. The liver, spleen, and lymphatic glands were powdered in a precisely similar fashion to that used by Klein. The powder was found to be curative in doses of one hundred to two hundred milligrammes when administered subsequently to infection by plague. He states that an average of 28 grammes of powder are yielded by one rabbit. The same objections hold against this method as against Klein's.

[1] Lancet, 26th January. 1907.

2 Not consulted [illegible]

(3)

Methods which depend on the action on dead bacillary bodies of both immune and non-immune serum.

Besredka's method.

A method of immunisation by combining the effect of immune serum with killed cultures of plague bacilli was used by Calmette and Salembini.[1] According to Besredka the serum exercises a deteriorating effect on the bacilli so that prolonged immunity is not produced. According to Rowland[2] both Shiga and Gosio tried similar methods. Besredka[1] utilising the discovery of Ehrlich and Morgenroth that a cell in contact with its own antibody fixes that to the exclusion of any other substance with which it finds itself in contact, tried to see if by reducing the quantity of serum to the minimum he could limit its unfavourable action. After mixing the immune serum with the bacilli which had been killed at a temperature of 60°C. he found sedimentation and agglutination of the bacillary emulsion occurring. The serum was decanted off and the sensitised bacilli were carefully washed with saline. These sensitised bacilli in suspension formed his "vaccin-antipesteux". He says that the advantages accruing from the use of this vaccine are (1) that the immunity conferred takes place within 48 hours without the formation of a "negative phase". (2) that there are no appreciable local or general phenomena after administration. (3) that the animals injected elaborate a specific antibody. (4) that the vaccines

[1] Referred to in the Annales de l'Institut Pasteur, Tome XVI 1902 "De l'Immunisation active contre la peste, le cholera et l'infection typhique" Par Besredka.

[2] Journal of Hygiene Vol. X No.3.1910.

properly sealed in tubes preserve their immunising properties a long time.

Later Besredka[1] substituted the use of normal horse serum for immune serum. He found that the sedimented pasty deposit of bacillary bodies was atoxic. The clear supernatant fluid contained a soluble pest endo-toxine which was very stable to heat and was neutralised by antipest serum. .02 centigrammes of plague bacilli + 1 c.c. normal saline + 4 c.c. horse serum would give sufficient endo-toxine to kill 20 mice, While 5 decimiligrammes of bacillary deposit unacted upon by serum kills mice, the atoxic deposit obtained after the action of horse serum can be injected in doses of 1 centigramme into mice without killing them. A further simplified method of producing soluble endo-toxines and an atoxic residue is the following:-[2]

A 48 hours' growth on agar is diluted with normal saline solution, killed at 60°C. for one hour's exposure and dried in a vacuum. One gramme of the dried microbes is mixed with .30 to .45 grammes sodium chloride and titrated in an agate mortar to an impalpable powder for one hour. 1 to 2 c.c. distilled water is added drop by drop until the strength of normal saline solution is reached. Agglutination of bacilli follows which is helped by heating. The soluble endo-toxine is contained in the supernatant fluid and the deposit is atoxic. The sensitised bacilli deprived of their toxic action formed his new 'vaccin anti pesteux'.

Still later, Besredka[3] returned to his first method - the use

[1] "Etudes sur le bacilli typhique et le bacilli de la peste". Par le Dr. Besredka. Annales de l'Institut Pasteur, Tome XIX, 1905

[2] 'Des Endotoxines solubles typhique pesteuse et dysenterique' Par le Dr. Besredka. Annales de l'Institut Pasteur, Tome XX, 1906.

[3] 'De la vaccination par les virus sensibilises' par le Dr.Besredka Bulletin de l'Institut Pasteur, Tome VIII, 1910 - p.241.

of a vaccine rendered atoxic by immune serum of high agglutination power termed a 'vaccin sensibilisé'. The special points in its favour, according to its author, are as compared with the vaccine of Haffkine that it is atoxic for mice in doses 30 times larger than lethal doses of Haffkine's vaccine and from his own personal observation that it is atoxic to man. There is no local or general reaction such as is found after the use of Haffkine's vaccine. Immunity establishes itself in 48 hours and is as durable as that obtained by other vaccines[1].

According to his first method, to which he has returned finally, the immune serum causes agglutination and bacteriolysis of the bacteria, thus allowing the endo-toxines to be liberated. The protective substances or immune bodies are however retained by the bacillary deposit which is now atoxic and sensitised. He found too that normal horse serum can perform the agglutination and liberation of the endo-toxines. According to S. Rowland[2] there is present in normal horse serum a natural amboceptor for plague bacilli. Rowland[2] in investigating these questions raised by Besredka found "no difference discernible between the lethal dose of the organisms that had been soaked (sensitised) in the anti-plague serum, and those that had been treated simply with salt solution" (physiological). Further he found that "on sensitising the plague bacilli in an anti-toxic serum no permanent neutralisation of the endo-toxines takes place". He states "I am therefore unable to confirm Besredka's statement that sensitised organisms yield an atoxic vaccine".

[1] Review by Besredka in Bulletin de l'Institut Pasteur, Tome X, 1912 p.529

[2] Journal of Hygiene Plague Supplement No.2. 1913.

(4)

A method of using for vaccination natural and artificial plague aggressins.

The methods of (1) Hueppe and Kikuchi

(2) Strong.

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(A) Vaccination by natural aggressins.

Aggressins or "produits soluble predisposants" are supposed to act as either inhibiting normal phagocytosis or as neutralising the normal lysins which cause bacteriolysis. They are said to intensify the toxic action of certain bacteria as of anthrax, staphylococcus pyogenes, and the tubercle bacillus. Hueppe and Kikuchi[1] in 1905 in the Centralblatt fur bacteriologie described the following method of immunisation. The peritoneal exudate, made sterile, of animals infected peritoneally with plague, when injected in two doses at 9 days' interval, will preserve guineapigs from death by the production of anti-aggressins. They claimed that their method was inoffensive and the most efficacious up-to-date. Strong[2] states that at the time of the appearance of the work of Hueppe and Kikuchi he had been experimenting on the same lines. He obtained natural aggressins in the following way. The abdominal exudates of guineapigs dead of plague were collected on the day of death and mixed with an equal volume of saline solution; carbolic acid was then added to form a .5 per cent solution. The mixture was then heated for two hours at 45°C. and subsequently centrifuged; the clear fluid contained the natural aggressins. Its sterility was demonstrated.

[1] Referred to in Bulletin de l'Institut Pasteur, Tome III, 1905. The original paper in the Centralblatt has not been consulted.

[2] Phillipine Journal of Science, Vol.II, 1907.

15 guineapigs received intraperitoneally 2 to 5 c.c. of exudate. All survived. Two months later the immunity was tested when 26 per cent survived In another series 33 per cent survived.

(B) Immunisation by artificial aggressins

Strong[1] obtained what he calls the 'free receptors' of the plague bacillus in the following fashion. Virulent cultures on agar were suspended in distilled water (30 milligrammes of the growth to 1 c.c.) The emulsion was then placed on an electrical shaking machine for 5 days. The suspension was thhn heated for one and a half hours at 45°C. and carbolic acid added to form .5 per cent solution. It was then centrifuged. The clear fluid contained the artificial aggressins. The results were very unfavourable, only 11 per cent guineapigs and 12.5 per cent monkeys proving immune after treatment with it.

(5)

The method of Terni and Bandi[2]

This vaccine is to some extent of the same nature as those dependent upon the immunising effects of natural aggressins. It was published under the title "Un Nuovo Metodo Di Preparazione del Vaccino Antipestoso" from the Laboratorio Micrografigo Municipale di Messina in 1899. My information is gathered from that paper and also from Terni's account of the experiences at Rio de Janeiro. Guineapigs and rabbits were injected intraperitoneally with plague cultures. They died within 36-48 hours and their peritoneal exudate was crowded with bacilli and also, according to these authors, with substances protective against plague. Some sodium chloride in physiological

[1] Phillipine Journal of Science. Vol. II, 1907.

[2] "Un Nuovo Metodo Di Preparazione del Vaccino Antipestoso" Nota preventiva dei dottori C.Terni e L.Bandi.

dilution was added to the thick exudate and the whole was incubated at 37°C. for 12 hours to increase the development of the germs. Thereafter the emulsion was killed by fractional sterilisation at 50°- 52°C. for 2 hours on two successive days. Carbolic acid to 0.5 per cent. strength, Sodium chloride to 0.75 per cent. strength, and sodium carbonate to 0.25 per cent. strength were added. After dilution etc. about 50 c.c. can be prepared from the exudate of one guineapig of 300 grammes weight. Doses of this vaccine (in 1/10 to 1/5 c.c. can be given to guineapigs of 300-400 grammes and to sewer rats of 200 grammes without killing them. The bacilli used to test the immunity were very virulent and were derived from Bombay and Oporto Strains. They killed in doses of 1/10 c.c. of a broth culture. The protective value of the vaccine according to these authors is greater than that obtained by the use of Haffkine's vaccine and the immunity is obtained quicker; in 4 or 5 days while Haffkine's is in 10 or 12 days. The duration of immunity is at least for two months. The views of these authors regarding Haffkine's vaccine were wrong (see p.p. 145-147) An interesting point is that these authors state that protective substances may be demonstrated in the serum of vaccinated persons in 8-10 hours. This may be compared with the result I obtained in conjunction with Kapadia (pp 143-144), and with Rowland's results in the Journal of Hygiene Plague Supplement II, 1912 p.368.

Terni[1] describes the employment of this method in the Rio de Janeiro epidemics of 1899 to 1909. Only 622 cases of plague altogether occurred in the city. From a footnote to "Lymphatite e Peste Bubonica" it seems that the records of vaccination in Brazil rose to over 46,000 persons vaccinated. Only 7 of these fell ill with plague with two deaths and these occurred in persons who had only

[1] "Lymphatite e peste bubonica"
Records of a Conference held at Rio de Janeiro 1900 by C.Terni.

been vaccinated 4 days and therefore were not immunised. In the City of Rio de Janeiro consisting of 779,000 inhabitants, 622 cases of plague were verified. Among the uninoculated the mean of attacks would be 0.83 per cent whilst among the vaccinated it amounted to 0.15 per cent.

As far as one can judge this method would be impracticable for India as it is too difficult a method of preparation. A million doses per year could not be issued. The Rio de Janeiro epidemic, (too) was not in sufficiently large a scale to form any judgment of the merits of this method.

(6)

Methods which involve the use of avirulent living plague bacilli, - the method of Kolle and Otto and of Strong.

W. Kolle and R. Otto in 1903[1] attenuated cultures of plague by growing them at the high temperature of 40° to 41° C. These attenuated bacilli in one million times the dose with which virulent bacilli kill animals are innocuous and behave as saprophytes in the animal body. They compared the use of this vaccine with those of Haffkine and Lustig and got much better results. 72 per cent of rats treated survived an injection of plague fatal to control non-vaccinated animals.

In 1905 Strong reported to the Manilla Medical Society, Phillipines, upon vaccination with attentuated living cultures of plague. These and further experiments are described by him in detail

[1] Referred to in the Bulletin of Pasteur Institut, Tome II - 1904
the original papers have not been consulted.

in a later paper[1]

Strong worked with 3 avirulent strains of plague bacilli, two of which he obtained from Prof. Kolle of Berne. These were (1) Pest avirulent, of which 1 to 2 agar slants injected subcutaneously did not kill guineapigs. (2) The Massen Alt slightly less avirulent. (3) Pest avirulent Manilla obtained first in 1903, the virulence of which was diminished by growth in broth at 41° to 43° C. Guineapigs usually withstood one agar slant.

The animals immunised were rats(specially Mus decumanus) and guineapigs. Monkeys were also used, as inoculation in them resembled inoculation in human beings, these animals showing individual variations in susceptibility. The test dose of plague was a 48 hours' culture (in its 1st or 2nd transfer to agar) of bacilli kept at a virulent level by passage from guineapig to guineapig. This virulent strain killed guineapigs in 3 to 7 days by "cutaneous inoculation".

Strong conducted comparative experiments to test the immunisation produced by this method with that produced by Haffkine's, Klein's, and that of Hueppe and Kikuchi. He found that while he obtained immunisation of 70 to 88 per cent of animals by his method, Haffkine's produced 25 to 26 per cent immunity, Klein's produced 30 per cent and that of Hueppe and Kikuchi 26 to 30 per cent. These results will be further criticised by me when dealing (dwelling) with Haffkine's prophylactic. I will detail the experiments of Strong with the living attenuated vaccine.

(1) 49 monkeys immunised by the strain Massen Alt; 4 succumbed to the effects of vaccination. Of those which survived, 70 per cent were found to be immune to plague.

[1] "Studies in Plague Immunity" by R. Strong
Phillipine Journal of Science - 1907.

(2) 47 guineapigs immunised intraperitoneally or subcutaneously by the Massen Alt strain, Six died from the effect of the vaccination, and it should be noted that some of these showed plague bacilli in their organs; of those which survived 88 per cent proved immune to infection.

Experiments with the strain Pest avirulent. 71 guineapigs received either subcutaneously or intraperitoneally this vaccine, and again it should be noted that in one animal vaccinated intraperitoneally in which death occurred, bacilli were obtained from the heart blood. Five out of these 75 died out from the effects of vaccination and 72 per cent proved immune to infection. All the controls of these experiments, 115 in number, died.

At this stage I wish to emphasise the fact that death sometimes occurred from the effects of vaccination, and that living plague bacilli were found in the organs of some of the animals found dead. Strong assures us of the safety of his method, but in view of his results I do not think that this vaccine can be safely recommended for use in any large scale. Instead of vaccinating, it might propagate plague in some cases.

Douglas and Bullock[1] write, "very great care would be necessary in recommending a method like this on a big scale in plague-stricken communities, as from unforeseen circumstances the virulence might be increased and plague induced".

Further in his comparison of this vaccine of living plague bacilli with Haffkine's prophylactic, as I will show later, he must have used a very weak brew of Haffkine's prophylactic as his results of 26 per cent of immunisation is much lower than those which I have obtained in my experiments. It should be noted that

[1] "System of Medicine" by Albutt & Roleston.

Haffkine's prophylactic was sterilised at a temperature of 65°C. to 70°C., temperatures which we know are very injurious to both the toxic and immunising value of the vaccine. It has always been our custom of late years in the preparation of this vaccine for India to sterilise Haffkine's prophylactic at no higher temperature than 55°C.

(7)

A vaccine composed of the nucleo-proteid obtained from the bodies of plague bacilli.

The method of Lustig and Galeotti[1 & 2]

These authors published their methods in various European Medical Periodicals, including the British Medical Journal of 1897. They determined to isolate the active toxic principles from the plague bacilli and their products in growth. These were obtained in the form of nucleo-proteids. They suspended the surface growth of plague from agar plates in 1 per cent caustic potash. They added to this solution dilute acetic or hydro-chloric acid till a faintly acid reaction point was obtained. A precipitate then formed which was collected in a filter, washed and dried in vaccuo in the presence of sulphuric acid. Thus a relatively pure nucleo proteid was obtained very toxic to certain animals. .36 milligrammes immunised a rat with no unfavourable symptoms. For use they recommended that this precipitate be dissolved in .5 per cent solution of carbonate of soda, and this solution of the nucleo proteid should be passed through a Chamberland filter to guarantee

[1] British Medical Journal - 1900 February 10th

[2] British Medical Journal - 1897 April 24th.

sterility. The animals experimented upon in 1897 were black and white mice with successful immunising results. In July 1897 in Bombay monkeys were vaccinated with complete immunity to bubonic infection. At that time they also tried this vaccine in doses of 2 to 3 milligrammes on themselves and one or two other operators with the usual reactionary symptoms. Criticising Haffkine's prophylactic they state that any immunising power which it may possess is due to the presence of such nucleo proteids either in the bodies of the bacilli or dissolved in alkaline broth. It should be noted however that when Haffkine's prophylactic is used with a slightly acid reaction, immunising properties are still present in it.[1] Further they state that Haffkine's prophylactic contains in addition to immunising bodies certain harmful albumoses and it is impossible to determine a proper dosage. This criticism, I think, is very much to the point. They also state, with its use abscesses are apt to occur, owing to non-sterile vaccine and that it offends the Vedic prescription. That these two latter statements are not now applicable will be shown when I discuss the modern preparation of Haffkine's prophylactic. The undoubted advantages of Lustig and Galeotti's nucleo proteid are that it may be administered in exact doses, can be prepared in large quantities, stored in the dry state without appreciable deterioration for months, and that it can be issued in sterile solutions. About its efficacy there is considerable discussion and difference of opinion. The Indian Plague Commission criticised it adversely. Klein[2] agrees with the criticism of the Indian Plague Commission, although it is not known on what grounds.

[1] G. Balfour Stewart, British Medical Journal - 1904

[2] Local Government Board Report 1905-06 - p.394.

Mauro Jatta and Romano Maggiora[1] compared the immunising values on rats and guineapigs of (1) Haffkine's (2) Lustig and Galeotti's and (3) a vaccine prepared by themselves at the laboratory of Pianosa. This vaccine is a little different in method from Haffkine's. The bacilli are grown in broth with plenty aeration from 3 to 4 days at 35°C. The growth is killed at 75°C. and thereafter carbolised (.5 per cent). They found that their own vaccine gave the best results.

Prof. Shibayama[2] stated that the preparation of Lustig and Galeotti's vaccine caused a loss of a great deal of material and that ten thousand doses a day could not be prepared. But the fact that it could gradually be prepared and stored without deterioration overcomes this objection. Galeotti[2] says that recent information from Prof. Tavel of the Institute of Infectious Diseases, Berne, is to the effect that the Swiss Federal Bureau of Health undertook comparative experiments on the immunising effects of different plague vaccines and came to the conclusion that while all of them, if properly prepared, can immunise animals most susceptible to plague, yet the superiority lay with that of Lustig and Galleoti on the following respects; first - its specific quality; second - the facility of dosage; and third - the durability of its immunising properties. The vaccine has been used in San Nicola La Plata in 1900, but the epidemic was of so mild a nature that its efficacy was not properly tested.[3] Galeotti[2] also states that the Serum Institute of Berne has prepared large quantities of this vaccine, and it has been especially in use in Australia at Perth, Adelaide, and Sydney. But no details as to the results are given.

[1] Cited in Bulletin of Pasteur Institut, Tome III - 1905, p.226.
Original paper not consulted.

[2] Report of International Plague Conference Mukden 1911.

[3] Strong - Phillipine Journal of Science 1907.

(8)

S. Rowland's work on the preparation of a true antigenic substance obtained from the bodies of plague bacilli.

Rowland in a most exhaustive series of experiments has attempted to isolate an ideal vaccine which would be composed of plague antigen alone. In his first paper[1] he discusses various methods of killing the plague bacilli so as not to injure the toxicity or immunising power. He finally settled on the use of chloroform which does not appreciably damage the antigen, in combination with the physico-chemical desiccating action of anhydrous sodium sulphate. To test the immunity produced he employed a strain of plague bacillus which showed a uniform virulence - 1/10th c.c. of a 72 hours' culture proving fatal for rats. For his test of immunity he employed sub-cutaneous inoculation, which destroyed 70 to 86 per cent control non-vaccinated rats. The test dose of plague was always given 14 days after inoculation.

Method of preparing the antigen -

A four days' growth on agar was killed by chloroform water. After centrifugalisation a nucleo-proteid was found in solution containing 29.5 per cent of the nitrogen content of the bacillary emulsion. This substance A as he denotes it was feebly toxic and immunising for rats.

The deposit of bacilli left after the extraction of substance A was mixed with anhydrous sodium sulphate, powdered in a mortar and alternately heated and cooled. Water was added till a saturated solution of sodium sulphate was produced. The clear fluid was decanted off. It contained a nucleo-proteid called substance B, slightly alkaline highly toxic in doses of 5 milligrammes causing

[1] Journal of Hygiene 5th Plague No.Vol.X - No.3. - Novr. 1910.

70 per cent mortality to rats. It was also highly immunising (.01 milligrammes immunising 80 - 90%) The debris of the bacillary bodies left after abstraction of substances A & B was non-toxic and had no immunising value.

In a later paper[1] Rowland shows that the nucleo-proteins present in substance A. is identical with that in substance B. Its low toxicity and immunising value is due to its combination with chloroform which causes a deleterious action. When the chloroform is removed from the substance A it regains its toxicity. Toluol has a less deleterious action than chloroform on the plague nucleo-proteids and can kill plague bacilli if left in contact with them for 1½ hours.

Heating to 60°C. has little or no deleterious action on the nucleo-proteids as contained inside plague bacilli, but when applied after abstraction from the bodies of bacilli is very destructive. He found that there is in the bacilli a proteolytic enzyme capable of hydrolysing proteins, and that this enzyme is carried over with the nucleo-proteins in the sodium sulphate method. Heat destroys this enzyme. The action of the enzyme in splitting up the proteins and setting free nitrogen, not precipitable by tannic acid, destroys the toxicity of the nucleo proteids of plague, but even after 2 months' autolysis there is no loss of immunising value and only a slight loss after 3½ months.

He compares these results with Ruffer's and Wilmore's (British Medical Journal 1908 - vol.II, p.1176) on the hydrolysis of the endotoxin of the dysentery bacillus. They found that by digesting the endotoxines for three days with pepsin and hydrochloric acid toxicity was reduced to 1/30th and the immunising value not greatly diminished. By a series of experiments Rowland showed that hydrolysis within the first few hours increases the immunising agent, that this remains fairly constant after one month or even two and a half months' autolysis,

[1] Journal of Hygiene 6th Plague No.
Plague Supplement No.1. December, 1911.

while the toxicity steadily diminished by the process.

Therefore though both the toxic agent and the immunising antigen are bound up with the nucleo protein they are distinct from each other. Probably the toxin portion is bound up with the basic protein moiety, and the antigen with the nuclein part, as this latter is known to be highly resistant to hydrolysis.

In a later paper[1] Rowland points out that in his process of extraction there is little or no loss of the antigen content bound up with the nucleo protein. He contrasts the result of immunising with his antigen (60 per cent. rats immunised by doses of .0001 milligrammes) with the result obtained by the nucleo protein of Lustig and Galleoti where .36 milligrammes is necessary as an immunising dose. He tried to separate the antigen from the protein body by means of filtration through gelatine but failed. The matter rests in this position at present.

(9)

HAFFKINE'S ANTI-PLAGUE VACCINE. (Prophylactic).

On the outbreak of plague in Bombay in 1896, Mr. W. M. Haffkine who was then working in Calcutta on inoculation against Cholera was deputed by the Government of India to proceed to Bombay to investigate the cause of the outbreak of plague and to devise some method of dealing with this disease.

After three months' work, Mr. Haffkine reported in January 1897 the discovery of a prophylactic. This discovery has been published in the Indian Medical Gazette of 1897[2] and British Medical

[1] Journal of Hygiene Plague Supplement II - January 1913.

[2] Indian Medical Gazette, June 1897.

Journal of 1897[1]. A full account of his views will be found in the Proceedings of the Royal Society 1899[2] with discussion on his views, reported in the British Medical Journal of July 1st 1899.

Mr. Haffkine was guided by the following considerations in the preparation of his prophylactic fluid. These considerations formed a provisional hypothesis but they are of interest. From his experience in cholera he states that the inoculation of the bodies of spirilla cultivated on solid media results in the production of merely bactericidal powers and not of anti-toxic, with the result that the susceptibility of the individual inoculated to plague is reduced along with the absolute mortality from this disease; the case mortality however is not affected. He hoped in the case of plague by using a vaccine consisting of both the bodies of bacilli and their "intensified extra-cellular toxins" to produce both effects, viz: the reduction of susceptibility and also of the case mortality. His vaccine consisted of, at first, a bouillon culture of plague bacilli initially virulent. The bacilli were allowed to grow in broth for one month, and to exhilarate what he described as a stalactite growth he introduced drops of ghee (clarified butter) on the surface of the broth. Thereafter the whole vaccine consisting of the broth, the dead bodies of bacilli, and their products was killed at an exposure to 70°C. for one hour. The sediment of bacilli caused local inflammation after injection with little general reaction, while the inoculation of the clear broth fluid produced no noticeable local effect but gave rise to high fever. After experimenting on rabbits, Haffkine inoculated himself along with the other members of the staff of his Laboratory and a few other medical practitioners of the city of Bombay. Gaining confidence from these results he tried the vaccine on a large scale

[1]British Medical Journal, May 1897.

[2]Proceedings of the Royal Society, Vol.66 of 1899.

on the prisoners of the House of Correction at Byculla, Bombay. The prisoners were given the option of being inoculated during a severe epidemic. The dose of vaccine given was 3 c.c. Of 172 non-inoculated, 12 developed plague and 6 died. Of 147 inoculated, 2 developed plague and none died. This was a good experiment where both inoculated and non-inoculated were under even conditions. The second experiment was at Umarkhadi Jail, Bombay, where of 147 inoculates there were 3 cases of plague all of whom recovered, while of 127 non-inoculates 10 developed plague with 6 deaths. I will detail one further experiment in a village near Baroda called Undhera, where the results were very strictly enquired into by independent witnesses including the Director-General, Indian Medical Service. This experiment was carried out by Mr. Haffkine and Major Bannerman, I.M.S.[1] In this village half the members of each house comprising half the number of males, females, and children were inoculated, and half were left as controls. Odd figures that happened to be in a family were compensated by odd figures in other families. The plague was very severe in this village, 79 cases having occurred before inoculation was started. Of 64 persons not inoculated 27 cases with 26 deaths occurred. Of 71 inoculated 8 cases with 3 deaths occurred. Mr. Haffkine calculated from these and other figures that the difference in mortality from plague among inoculated and non-inoculated sections of communities averaged over 80 per cent, often approaching 90 per cent

J. Balfour Stewart[2] made some experiments to determine the efficacy of the different constituents of Haffkine's prophylactic. He arrived at the conclusion that in a 10-day old living broth culture of plague there were certain substances elaborated capable of reducing the severity of an attack of plague. This observation cannot be considered conclusive. He tried the prophylactic properties of

[1] Proceedings of the Royal Society of Edinburgh Vol.24, part 2, 1902-3.
[2] British Medical Journal, 1904.

(1) the filtrate, and (2) the deposits of the prophylactic, both separately and together. He came to the conclusion that the suspension of microbes conferred bacteriocidal powers to the person immunised while the filtrate alone conferred the power of preventing or modifying an attack of plague.

The preparation of the plague vaccine was started in the Bombay Bacteriological Laboratory on a large scale in 1897. By means of the work of Gordon and Gibson a suitable medium for preparing broth cultures was prepared by hydrolysing goat's flesh by hydrochloric acid with the aid of heat. After subsequent neutralisation and dilution this produced a valuable medium; it is still used for the production of the vaccine. It obviates the necessity and use of peptone, and thus with the avoidance of cow's flesh it does not run counter to the religious and social customs in India.

At first the bottling of the vaccine was done by hand and the bottles were closed by rubber corks. In 1898[1] Haffkine modified his procedure by enriching the broth growth, adding to it a 4-days' growth of an agar culture. He was of opinion that the addition of the young growth of these microbes would reduce the case-incidence of the disease. However this result was not gained[2]. In November 1902 some cases of tetanus occurred in Mulkowal, a village in the Punjab. It is impossible to be certain how one phial of the vaccine was contaminated, whether in the Laboratory or at the time of the use in the district. However, owing to this accident of 19 deaths several changes had to be introduced in the procedure of the preparation of the vaccine.

There are some points which I wish to describe in detail. For a full description the Bombay Bacteriological Laboratory's handbook

[1] Report of the Plague Research Laboratory for 1896-1901

[2] do do 1905 by W.B.Bannerman, M.D., D.Sc., I.M.S.

"The preparation and use of anti-plague vaccine" and the annual reports must be consulted.

As a virulent strain of plague is essential for the preparation of the vaccine the following points are especially insisted upon. The bacilli are initially obtained from a septicaemic human case of plague. A pure culture is obtained, which on "cutaneous" infection of a guineapig produces death within 5 days. Further in broth, stalactites must be produced within 10 days. Large flasks, containing a litre of (broth faintly alkaline) and shallow to allow aeration, are inoculated from the stalactite growth. These flasks are then incubated at room temperature in Bombay (average mean temperature varying from 72°F. in winter to 85°F. im summer) in the dark from 1 month to 3/ or more months. It is on the influence of the length of time of "brewing" or "incubating" on the efficacy of the vaccine that I describe experiments further on. After incubation the broth flasks containing the growth of bacilli are heated to 55°C for ½ hour thereby killing the bacilli. Formerly Mr. Haffkine used to kill the germs at 70°C. but it was found in so doing much of the immunising value of the vaccine was destroyed also. After cooling, carbolic acid is added to .5 per cent strength. It was found by the German Commission that carbolic acid in this strength added to the cooled vaccine does not destroy the immune bodies. Later the vaccine is bottled by a special device in vacuumised ampules, and thereafter the glass is sealed by heat. Examinations are made - aerobic and anaerobic - during the various stages to ensure (1) the purity of the plague growth and (2) the ultimate sterility of the vaccine used. It is difficult to see how precautions could be carried further, and the fact that since the introduction of these improvements up to the end of 1912, over

10 millions of doses[1] were issued within a single instance of ill results following inoculation, although the whole of the inoculation proceedings are under the strictest supervision, would point to the success of these measures. The vaccine is now issued in sealed ampules, the dosage being 4 c.c. for adult males and 3 c.c. for adult females.

The value of inoculation can be gauged by the statistics reported by the Bombay Bacteriological Laboratory year by year. I will quote only two or three of these which have been most carefully observed.

It is recognised that statistics, unless most carefully compiled, with a due regard to the avoidance of fallacies, can be the most fallacious method of calculating results. There is now in India no special staff for the verification of the plague inoculation statistics. Those supplied to the Bombay Bacteriological Laboratory come from the various Civil Surgeons, Sanitary Commissioners, and Plague Officers to whom the prophylactic is issued, and they are personally responsible for their correctness. To guide these authorities in some measure in the correction of statistics, instructions have been issued by the Laboratory which draw special attention to the necessity of furnishing accurate dates regarding (a) the commencement of the epidemic of plague (b) the termination of the epidemic (c) the commencement of inoculation work (d) the termination of inoculation work. Inthe comparison of the incidence of plague among inoculates and non-inoculates it is important that no cases of plague should be included prior to the commencement of inoculation, as to do so would swell the numbers of the

[1] Bombay Bacteriological Laboratory Report for 1912.

uninoculated. Further in any prolonged epidemic it is necessary to estimate the fluctuation of population in any given town, month by month or week by week. Otherwise the numbers of the non-inoculates would be estimated too high, as many of the inhabitants of plague infected places evacuate the town during an epidemic.

It must be remembered that as an epidemic advances the relative proportion of inoculates to non-inoculates is constantly changing and accordingly monthly or weekly statistics are necessary.[1]

Those statistics which are now quoted are obtained from the annual reports of the Bombay Bacteriological Laboratory for 1911-12 (the last one however is still unpublished, but was received by the Laboratory.) They were all carefully compiled by local authorities, and fallacies were kept in view and avoided as far as possible.

[1] A circular letter on some such lines was issued to Civil Surgeons by the Director of the Laboratory.
Report of the Bombay Bacteriological Laboratory 1912.

Statement showing attacks and deaths from Plague among the Inoculated and Uninoculated of Salem Town from the commencement on 11th August 1910 to the end on 31st March, 1911.

Number of weeks and months	Total inoculated	Total uninoculated	Inoculated		Uninoculated	
			Attacks	Deaths	Attacks	Deaths
3 weeks August	...	219,000	...	...	77	64
4 " September	1,375	266,500	...	...	299	250
4 " October	27,585	183,200	14	11	270	255
4 " November	65,132	71,450	163	86	585	508
4 " December	95,239	29,750	156	65	379	338
4 " January	137,853	17,150	93	59	78	75
4 " February	181,787	22,290	7	4	4	4
4 " March	205,746	33,280	1	1	1	1
31 weeks Total	714,717	842,620	434	226	1,693	1,495
Average for the period[1]	27,489	27,181	...	...	...	...
Ratio per 1,000 of the average strength for the period.	...	...	15.8	8.23	62.3	55.00

[1]Derived by dividing the aggregates by the number of weeks during the period total number of inoculated by 26 (number of weeks) and total number of uninoculated by 31. Inoculation started in the third week of September. The statistics of Salem town were compiled week by week and the figures given are the aggregates of the weekly population. This table appeared as a summary along with the original Salem statistics in the 1911 Annual Report of the Bombay Bacteriological Laboratory.

It should be noted that in the calculation of these figures, numbers of non-inoculates are given before the commencement of the campaign of inoculation, but to balance this, figures are given during March when the epidemic really had ceased.

(2) Kirkee:-

No of weeks & months		Population	Attacks	Deaths.
1912.				
2 weeks August	Inoculated	869	3	2
	Uninoculated	8931	32	22
4 weeks September	Inoculated	1680	6	3
	Uninoculated	4440	53	36
4 weeks October	Inoculated	2490	11	3
	Uninoculated	2820	67	50
4 weeks November	Inoculated	2703	1	0
	Uninoculated	1594	5	2
1 week December	Inoculated	2713	0	0
	Uninoculated	3580	1	1
Total	Inoculated	10455	21	8
	Uninoculated	21365	158	111
Average population for the period:-	Inoculated	2788	..	..
	Uninoculated	5697	..	..
Ratio per 1000 of the average strength for the period.	Inoculated	..	7.5	2.9
	Uninoculated	..	27.7	19.5

The fluctuation of monthly population of this cantonment during the prevalence of plague was as follows:-

1912 -	August	9800
	September	6120
	October	5310
	November	4297
	December	6293

The number of inoculations made month by month is as under:-

1912 -	August	869
	September	811
	October	810
	November	213
	December	10.

Note:-

The commencement of plague epidemic - 19th August 1912.
The termination do 10th December 1912.
The commencement of inoculation 19th August 1912.
The termination do 10th December 1912.

The fluctuation of the population month by month was carefully considered in conjunction with resident Indians. The total population of Kirkee Cantonment at the commencement of the epidemic was 9,800 (exclusive of troops).

I do not intend to swell the report by quoting many further cases. One however from the Bijapur District of Bombay, showing the effect of inoculation on individual households in villages, is interesting. No plague cases are included occurring prior to the commencement of inoculation, and those households alone are considered where signs of plague infection occurred after inoculation and after the lapse of the period of incubation (10 days).

	Population	Attacks	Deaths.
Ilkal:-			
	Inoculated 41	9	-
	Uninoculated 38	37	36
Herur:-			
	Inoculated 41	3	1
	Uninoculated 28	9	7
Gorubal:-			
	Inoculated 42	3	2
	Uninoculated 43	20	19

A point should be borne in mind that attacks among inoculates occurring within 10 days of inoculation should not be taken into account in weighing the value or otherwise of inoculation as such would probably be infected prior to inoculation.

From the results of a large series of inoculations, such as Salem and Kirkee, the Director of the laboratory concluded that inoculates were fully three times less likely to be infected with plague than those not inoculated and that where they are infected they were twice as likely to recover from the disease as the uninoculated.

[1] The Report of the Bombay Bacteriological Laboratory 1911.

The method of Haffkine has been subjected to much criticism. Kolle and Otto[1] state that the loss during the process of immunisation of rats with Haffkine's prophylactic, a measure of the toxicity, was 38.5 per cent and with killed agar cultures (method of German Commission) 33.3 per cent.

The immunity of rats surviving immunisation by Haffkine's method = 22.2 per cent

Do Do with killed agar cultures = 21.9 per cent.

Of 20 guineapigs inoculated with Haffkine's prophylactic two died from toxaemia and only 10 per cent showed immunity.

Of 20 guineapigs thrice inoculated with Haffkine's prophylactic in doses of 1, 1.5 and 3 c.c., six died of toxaemia and one out of 14 was immune = 7 per cent.

Strong[2] experimenting with monkeys found that 3 out of 8 survived infection = immunity of 37.5 per cent - 2 of those which died received 30 and 20 c.c. of the prophylactic.

Of another seriss of 9 monkeys inoculated with 10 - 15 c.c. one month later two showed immunity = 22 per cent.

Of 20 monkeys immunised by killed agar cultures, one to two agar slopes being given which Strong thinks superior to bouillon cultures, 23 per cent showed immunity. In the total series of 15 guinea-pigs and 55 monkeys inoculated with killed cultures, 26 per cent guineapigs and 25 per cent monkeys proved immune.

Klein's[3] criticism of the methods of preparation are not now to the point; as I have pointed out, changes have been made which

[1] Cited by Strong (Phillipine Journal of Science "Studies in Immunity 1907) Original paper not consulted.

[2] Phillipine Journal of Science 1907.

[3] Local Government Board Report 1905-1906. Appendix B.No.1.

fully ensure the sterility of the vaccine. He is of opinion that the carbolic acid added to the vaccine is somewhat harmful to the organism and that it is not present in sufficient quantities to prevent the growth of sporing germs. This question was submitted in 1904 to a Commission in India, and their findings were reported upon by the Lister Institute of London. The report runs as follows:- "This Institute is in entire agreement with the Commission as to the value of 0.5 per cent carbolic acid in restraining tetanus growth, when added to plague prophylactic".[1] Klein found that 10 c.c. of the prophylactic were necessary to immune full grown rats, and 5 c.c. human beings (though on what experience of human immunisation he lays down this figure is not stated). It should be stated that Klein worked with a prophylactic sterilised by heating for 1 hour at 70°C. Such an exposure would destroy much of the toxines and immunising agents present in the vaccine.

Wyssokowitz and Zabolotny[2], working with a bouillon vaccine killed at 60°C., found that immunity was established in 7 days and lasted a long time.

My own experiments with Haffkine's prophylactic.

In another section of this paper I have recorded experiments with the filtrates of old bouillon cultures which confirmed the observations of Dean and Douglas that these filtrates contained endo-toxines in solution and that they were of strong immunising value.

[1]Appendix A. Report of the Bombay Bacteriological Laboratory 1905.

[2]"Researches sur la peste bubonique" Annales de l'Institut Pasteur, 1897.

In the following series of experiments I was assisted by Khan Bahadur Senior Assistant Surgeon R.J. Kapadia.[1]

They were devised as an attempt to answer the questions as to the period after carbolisation and sterilisation during which the vaccine retained its immunising powers. During the course of these experiments various other matters of interest evolved which will be referred to.

The rats used for experimentation were wild Indian Mus Rattus. They were obtained from Madras, as the Plague Commission has shown that the Bombay rats possess by this time (1910) a high natural immunity to Plague - the Madras rats possessing little if any.

A number of fallacies were unavoidably associated with these experiments.

First of all, the various samples of vaccines we had in stock were manufactured from different strains of plague bacilli. All the bacilli were originally obtained from the blood of human septicaemic plague cases and they were passed through a guineapig and obtained from the heart blood of that animal on death, only those strains being chosen which killed the guineapigs by the 5th day after by cutaneous inoculation. But, nevertheless, that virulence of these bacilli possibly - nay probably - varied. Again the vaccines were incubated at room temperature, which varies little throughout the year in Bombay. Charts of the daily mean temperatures in Bombay (Journal of Hygiene vol.VIII "on the Seasonal Prevalence of Plague") show that the lowest mean temperature is about 72°F. and the highest about 85°F. As the vaccines are prepared throughout the year they were subjected to this incubation range of temperature. The most important factor in the toxicity and immunising values of the vaccines is the length of time the germs are allowed to incubate, the period of "brewing" as I call it hereafter. This is a point

[1] Throughout this series of experiments we had the advantage of the advice of Surgeon General W.B. Cameron R.N. Ret. then the Director of the Laboratory Bacteriological of Bombay.

which only came out as a result of the consideration of these experiments now detailed. Variations in this respect will be seen from the tables to be from 8½ months to 6 weeks. Before we were aware of the deleterious effect of prolonged brewing we were inclined to favour the employment of vaccines brewed from periods over 3 months, as experience showed that the local and general reaction after the inoculation of such long-brewed vaccines was much less than after the use of short-brewed vaccines. This reaction is one of the great drawbacks to the success of inoculation campaigns as people dread it. Unfortunately, as we shall see, the immunising power diminishes with the toxicity with length of exposure to "brewing". The vaccines are sterilised between temperatures of 55°C and 58°C. for ½ hour.

After sterilisation the vaccines were kept - what I shall call hereafter "matured" - at ordinary room temperature, some of them were retained in the Laboratory. Others examined had been despatched to various parts of India, subjected to the more or less great variation of temperature met with, and thereafter returned to the Laboratory. Instructions were also sent out with the vaccine that it should be kept as much as possible, in dry, cool, and dark places. The test dose of plague employed varied from about .002 to .008 milligrammes of a spleen of a rat just dead of plague, smears of the spleen showing the presence of plentiful plague bacilli. Attempts were made to count the number of bacilli so injected by dilution processes and subsequent growth on agar slopes, but owing to the auto-agglutination of plague bacilli in suspension such irregular counts were met with that the attempts had to be abandoned.

In considering the results of the immunity experiments, those animals alone, which showed plague bacilli in smears from their organs or bubos, are counted as having died of plague.

I

The toxicity to rats of Haffkine's anti-plague vaccine.

As I have already pointed out, Kolle and Otto found that 38.5 per cent of rats died as a result of immunisation. I cannot find out what doses of vaccine were employed, nor the variety of rats used. This is all-important.

Series I - We found that of 20 rats inoculated subcutaneously in the groin with 2 c.c. of a brew 19 days old, i.e. matured 19 days after sterilisation, 9 died, 7 of them with the post-mortem signs associated with plague, namely, necrotic spots in the liver and an enlarged and modelled spleen. Cultures and smears of these organs showed that no plague bacilli were present, and that the post-mortem appearances resulted from the action of the toxine.

Deaths from toxaemia in this series, then, were 45 per cent, or, if the two in which no typical post-mortem appearances were found are excluded, 35 per cent.

Again of 20 rats inoculated subcutaneously in the groin with 2 c.c. of a vaccine exactly 3 years old, 5 died of toxaemia - 25 per cent.

Of 20 control rats not inoculated none died during the same period.

Series II - of 40 rats inoculated with 0.5c.c. vaccine 3 weeks old (period of incubation or brewing 5 months) 20 per cent died in 19 days.

Of 40 rats inoculated with 0.5 c.c. of a vaccine 2 years 7 months old, 15 per cent died in the same period.

Of 40 rats inoculated with 0.5 c.c. of a vaccine 7 years 4 months old, 10 per cent died in the same period.

Of 40 rats inoculated with 0.5 c.c. of a vaccine 10 years old 7.5 per cent died during the same period.

Of 40 rats non-inoculated and used as controls 7.5 per cent died during the same period.

Allowing for margin of probable error the toxicity was still marked in the vaccine 2 years 7 months old, and was higher in fresh vaccine.

II

Immunity produced among rats by the use of Haffkine's anti-plague vaccine.

1. From comparison between series III and IV it is probable that as the toxicity produced is greater by inoculating 2 c.c. vaccine than by inoculating ½ c.c. vaccine, so comparing the number of rats which survive subsequent infection by plague the immunity is greater also with the larger amount of vaccine used. The vaccines in the series III and IV were of approximately the same age and length of brew.

Series III - of 13 rats immunised with 2 c.c. of 19 days 'old vaccine (period of incubation 5 months) and infected 18 days later by subcutaneous injection of .006 milligrammes of a spleen of a rat dying from plague and rich in bacilli, 11 remained alive on the 9th day = an immunity rate of 54.6 per cent. 14 out of 16 control non-immunised rats died on the 9th day after infection by the same dose of plague an immunity rate of 12.5 per cent.

Series IV.- 28 rats survived out of 40 immunised with ½ c.c. of a vaccine 3 weeks old (incubation period 5½ months). Of these 28 rats injected subcutaneously with .007 milligrammes of a plague spleen 19 days after immunisation, 12 remained alive on the 11th day - an immunity rate of 44.5 per cent.

6 out of 36 non-immunised rats (controls) survived the same period of time after the same dose of plague - an immunity rate of 16.7 per cent.

This point is brought out still better by the following experiment.

TABLE No.1.

The same vaccine in varying quantities was used to immunise a series of rats.

The history of the vaccine was as follows.

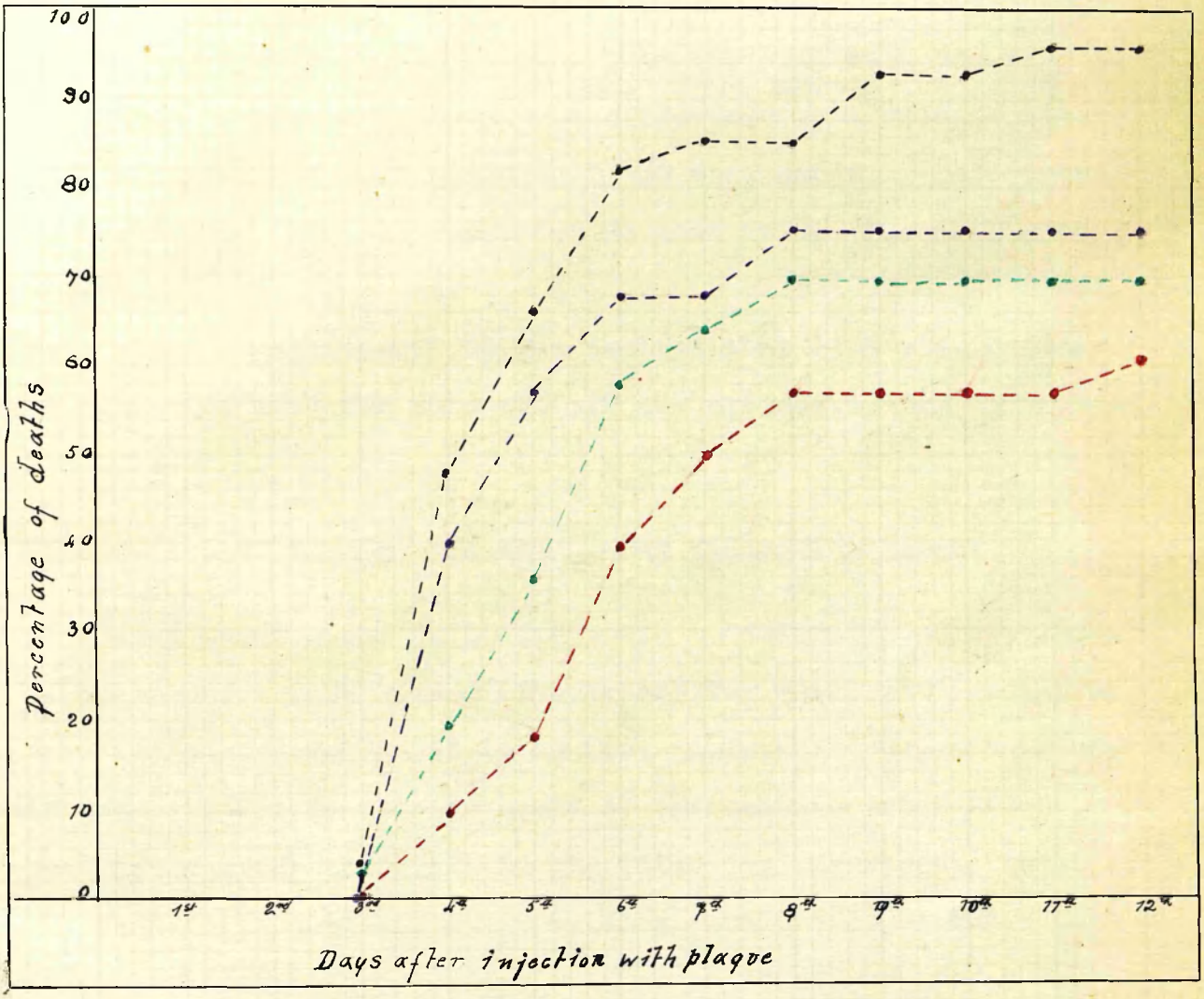
It was 15 days old at the time of experiment and had been stored in the laboratory.

	No.of rats	Dose of vaccine administered subcutaneously.	No.of survivors after immunisation & captivity for 14 days	Date of injection of plague bacilli	Amount of Plague spleen injection	Numbers of Survivors on different days after injection		Percentage of Immunity on different days after injection	
						on 4th day	on 12th day	on 4th day	on 12th day
Series A	40	¼ c.c.	28	14 days after immunisation	.004 mgm	25	11	89.3	39.3
Series B	40	1/10 c.c.	36	do	do	29	11	80.6	30.6
Series C	40	1/100 c.c.	28	do	do	17	7	60.7	25
Series D	40	Controls not immunised	27	on the same date as above	do	13	1	52.8	3.7

Chart I

To illustrate Table I

Curves showing the percentage death rate day by day



Red line - Series A

Green line - Series B

Purple line - Series C

Black line - Series D

CONCLUSION - Even 1/100 c.c. of a fresh vaccine produces a definite immunity in rats.

Chart I. Illustrates these results in a graphic manner.

All vaccines however will not produce immunity with such doses. In one experiment no immunity whatever was produced by a vaccine less than even one year old when given in 1/100 c.c. doses. Unfortunately the number of this brew was not noted, so that it was impossible to investigate it.

From various experiments it was decided that 0.25 c.c. formed a good immunising dose for rats and did not produce a high death-rate from toxaemia.

2. The effect of time on the immunising value of vaccines.

As I have pointed out it is difficult to determine this when working with vaccines which were brewed (incubated) for different periods of time, as that factor was found to be most important, determining the initial toxicity and immunising power of the vaccine after sterilisation. But keeping this in mind, the following experiments have given us some idea as to how long vaccines retain their immunising power.

TABLE No.2.

The immunising dose was 2 c.c.

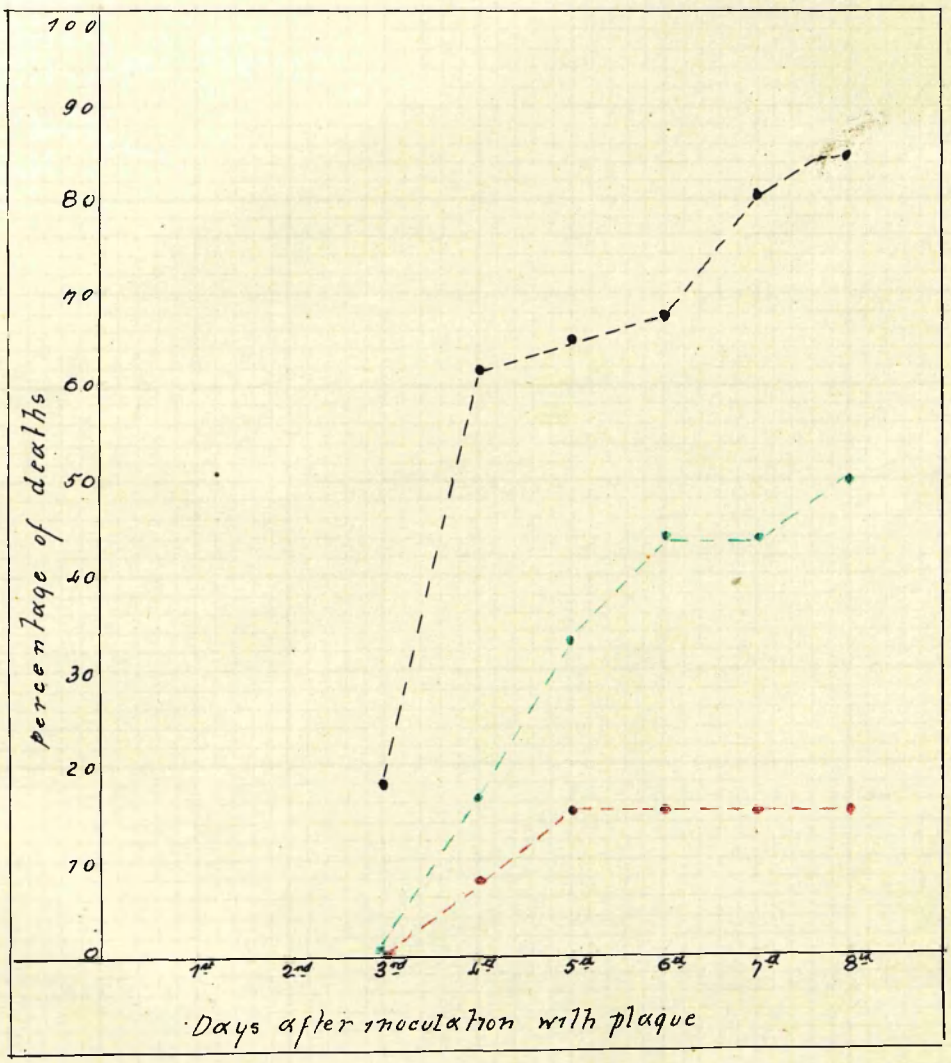
Vaccine A. was 19 days old, had been kept in a dry place in the laboratory at room temperature. It had been incubated for 5 months.

Vaccine B. was exactly 3 years old. It was found impossible to trace its history.

Chart II

To illustrate Table II

Curves showing the percentage death rate day by day



Red line - rats immunised by vaccine A

Green line - " " " vaccine B

Black line - control rats non-immunised

The dose given of a spleen of a rat dying of plague was .006 milligrammes, and the rats were injected 18 days after immunisation.

Number of rats	Vaccine	Survivors after immunisation and captivity	Survivors after infection by plague	Rate of Immunity per cent
20	A	13	11	84.6
20	B	18	9	50
20 controls not non-inoculated	-	16	2	12.5

Using then this large dose of vaccine for producing immunity even a 3 years old vaccine showed considerable immunising power.

Chart II illustrates the daily death rate.

TABLE No.3.

Series A received 1/4 c.c. vaccine dated				12th Jany.1903	The conditions regarding storage of which it was impossible to trace the conditions etc.
Series B	"	"	"	23rd Octr.1907	
Series C	"	"	"	6th May 1910	It had been stored in the laboratory at room temperature in a dry place.

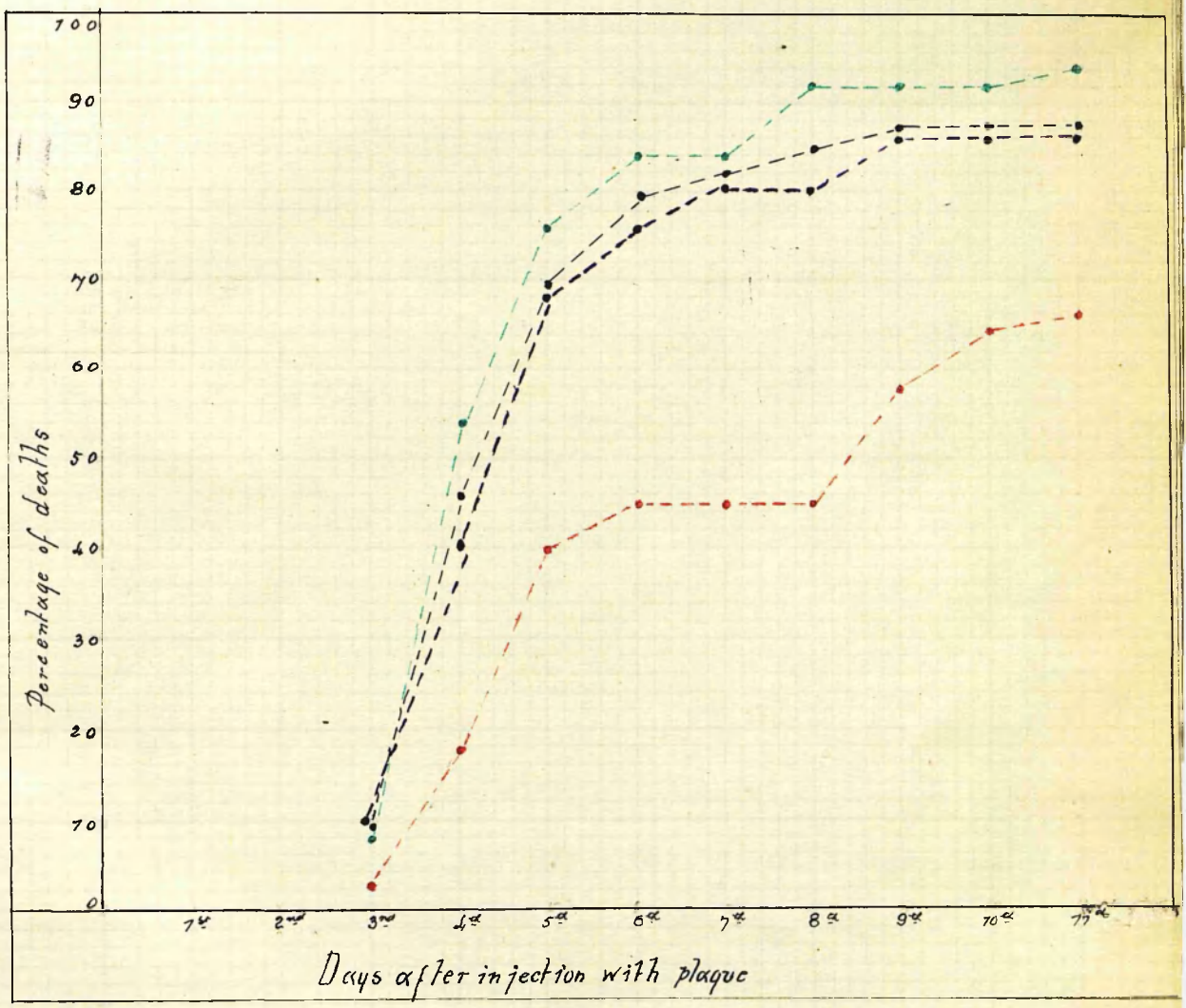
Series D served as controls and were uninoculated.

The first two were very old vaccines, while that used on series C had the following history. It had been matured 6 months before use (was 6 months old) and had been brewed for 5 months 15 days. The numbers of rats in each group which survived immunity was as follows:-

Chart III

To illustrate Table III

Curves showing the percentage death rate day by day



The curve in Green is that of rats which recieved vaccine of 1903 - Series A

The curve in Purple is " " " " " vaccine of 1907 - Series B

The curve in Red is " " " " " vaccine of 1910 - Series C

The curve in Black is " " " " no vaccine - Series D

Series A - 37 rats immunised with brew matured 8 years (Brew No.3163 of 12-1-03)

Series B - 36 " " " 3 years (Brew No.2198 of 23-10-07)

Series C - 39 " " " 6 months(Brew No.5339 of 6-5-10)

Series D - 39 rats not inoculated.

Twelve days after the date of inoculation the rats were infected with living plague bacilli, the dose used being, .0034 milligrammes of the spleen of a plague-infected rat.

Deaths, proved to be due to plague by the occurrence of bacilli in smears from their organs, occurred among the rats on the 3rd day after infection, and continued till the 11th day when the following survivors remained in each group.

Series A - 3 rats = 8.1%

Series B - 4 rats =11.1%

Series C - 12 rats =30.8%

Series D - 5 rats =12.9%

Chart III - shows the percentage death rate day by day from plague. From this result it is evident that with 1/4 c.c. used as the immunising dose the old vaccines used on Series A and B were valueless in producing immunity. The comparatively fresh vaccine used in group C was of considerable immunising power. The immunity is best gauged, not only by the difference of percentage number of deaths but also by the delay of death, a point well brought out in Chart III.

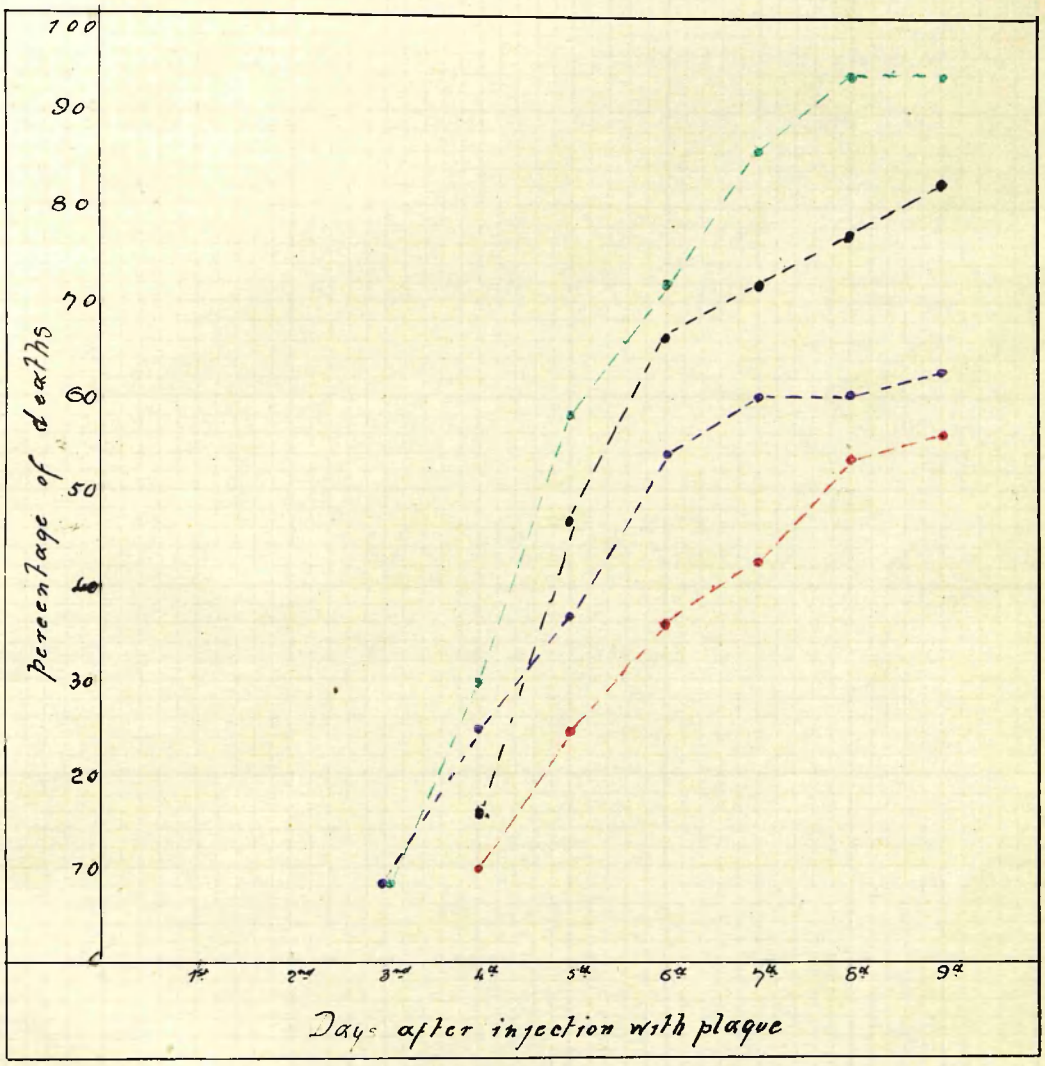
TABLE IV.

It is interesting to contrast an experiment detailed in the next Table No.4. where exactly the same vaccines were used, except that

Chart IV

To illustrate Table IV

Curves showing the percentage death rate day by day



The curve in Green is that of rats which received vaccine of 1903 - Series A

The curve in Purple " " " " " " vaccine of 1907 - Series B

The curve in Red " " " " " " vaccine of 1910 - Series C.

The curve in Black " " " " " " no vaccine - Series D

IV

the vaccine of 12-1-03 was numbered 3162.

As it was carried out 5⅓ months earlier, the vaccine numbered 5339 was only 3 weeks old instead of 6 months as in Table III. The immunising dose of the vaccine in this experiment was 0.5 c.c. - twice the dose given in Table III. The conditions regarding storage of this vaccine have been noted under Table III.

Nineteen days after the date of inoculation the rats were infected by living plague bacilli, the dose used being .007 milligrammes of a plague-infected spleen.

Series A received	1/2 c.c. of brew	No.3162 of 12-1-03	about 7½ years old at the date of the experiment.
Series B	"	No.2198 of 23-10-07	about 2⅓ years old at the date of Experiment.
Series C	"	No.5339 of 6-5-10	3 weeks mature at the date of experiment.

Series D were control non-inoculated rats.

The numbers in each group which survived immunisation were:-

Series A - 36. Series B - 35. Series C - 28. Series D - 36.

After infection by plague the following survived:-

of Series A - 34 out of 36 =	a percentage immunity of	5.6
of Series B - 22 out of 35 =	" "	37.1
of Series C - 16 out of 28 =	" "	44.5
of Series D - 30 out of 36 =	" "	16.7

These results are very parallel to those obtained in Table III. The percentage immunity especially in series B is however distinctly higher, a result which may be due to two causes; the vaccine was 5⅓ months younger in the experiment detailed in Table IV and also the immunising dose was twice as strong.

A vaccine 2½ years old, when used in amounts of 0.5 c.c. for rats still showed immunising powers.

Chart IV. shows the percentage death rate day by day illustrating Table IV.

TABLE V.

In the following experiment a curious fact is that a vaccine 1 year and 2 weeks old had equal potency to one 3 weeks old. The vaccine 3 weeks old had however been brewed (i.e.the incubation period before sterilisation had been) 8 months 13 days, while the more mature vaccine of 1 year 2 weeks had been brewed only for one month and 22 days.

Here is seen for the first time the deteriorating effect of prolonged brewing on the immunising antigens of the vaccine - that is of autolysis carried beyond a certain period.

Series A were inoculated with 1/4 c.c. vaccine 3 weeks old stored in the laboratory.

Series B " " " " 1 year 2 weeks old stored in the laboratory

Series C " " " " 2 years 8 months (had been sent to and returned from Indore.

Series D were non-inoculated and served as controls.

On the date of infection with living plague, 14 days after the date of immunisation, the following number of rats were present in each group.

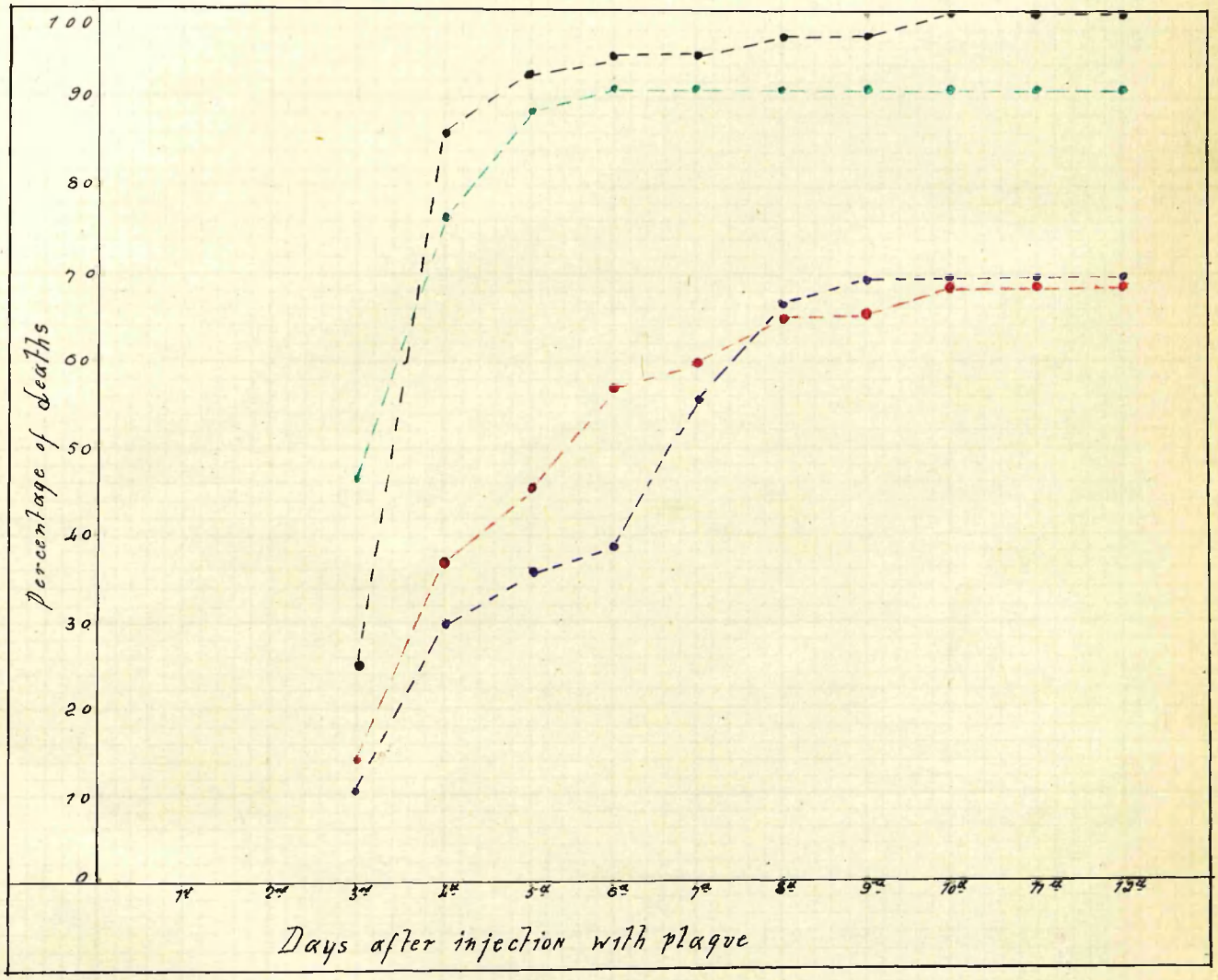
Series A - 35 rats. Series B - 36 rats. Series C - 34 rats.
Series D - 39 rats.

The dose of plague spleen used was .0026 milligrammes and by the 10th day the numbers in each group were reduced to the following:-

Chart V

To illustrate Table V

Curves showing the percentage death rate day by day



Red line denotes percentage daily death rate of Series A

Purple line " " " " " " Series B

Green line " " " " " " Series C

Black line " " " " " " Series D

Series A.11 rats survived - a percentage immunity of 31.4

Series B.11 " " - " " of 30.5

Series C. 3 " " - " " of 8.8

Series D.20 " " - " " 0

The infection of plague was severe as is seen from the fact that no control non-inoculated rats survived.

Chart V. illustrates the daily percentage death rate from plague.

TABLES VI and VII - confirm the above observations that vaccines, the incubation periods of which are for a short period (about 2 months), are more potent in producing immunity than vaccines brewed for longer periods.

TABLE VI.

Series A - were inoculated with a 1/4 c.c. of a brew 10 days old.

Series B - " " " " " 23 "

Series C - " " " " " 2 months old

Series D - served as controls and were not inoculated.

All the vaccines had been stored in the laboratory and the sources of bacilli were from human cases of plague.

The following numbers of rats were alive in each group after immunisation.

Series A - 32 rats. Series B - 29 rats. Series C - 34 rats.

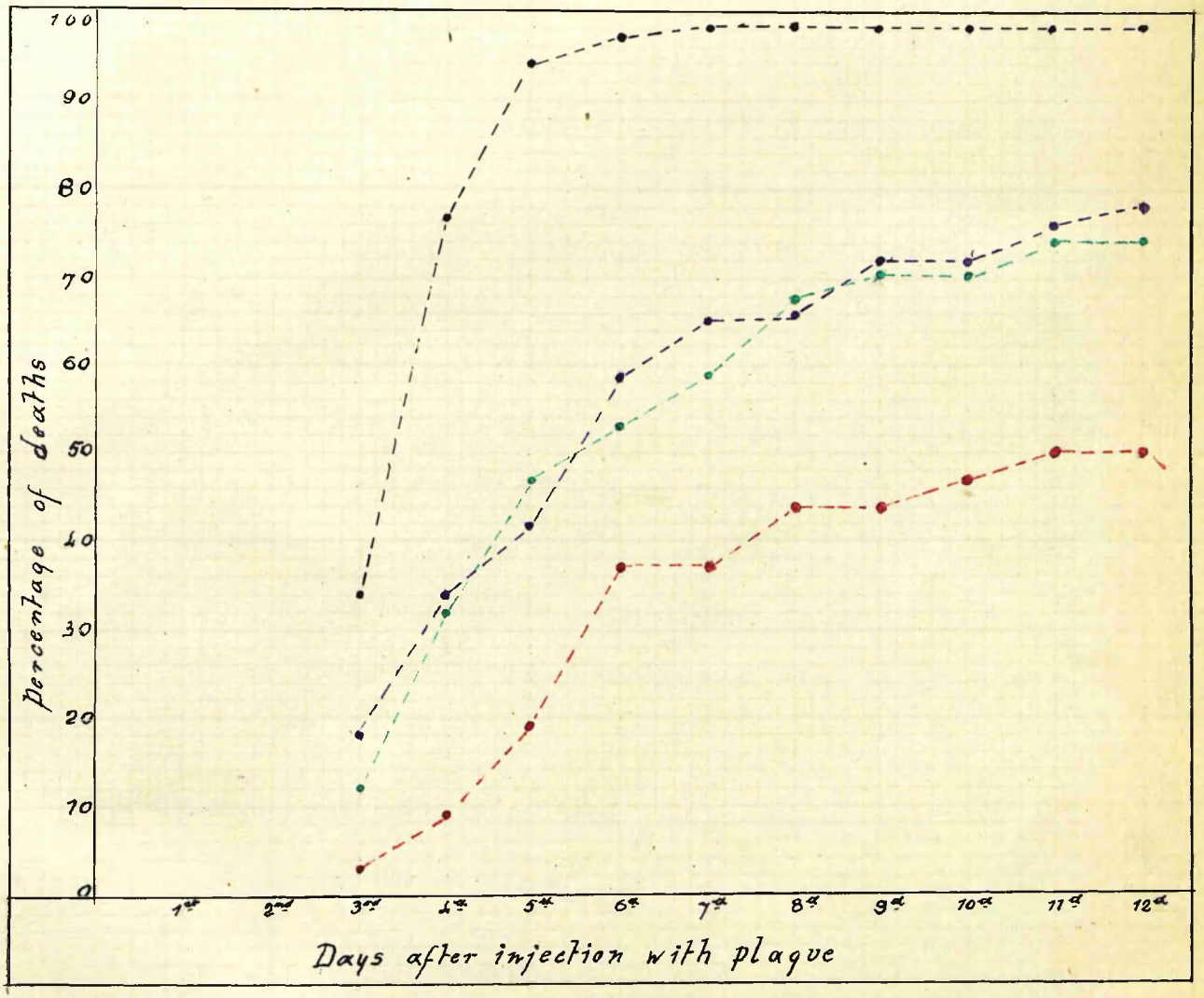
Series D. - 36 rats.

13 days later these rats were inoculated with living plague bacilli, .0019 milligrammes of a plague spleen being used.

Chart VI

To illustrate Table VI

Curves showing the percentage death rate day by day



Series A Red line inoculated with brew, 2 months incubated, and 10 days old

Series B Purple line " " " 4 months incubated, and 23 days old

Series C Green line " " " 8 months 4 days incubated, and 2 months old

Series D Black line control non inoculated rats

All the brews had been kept in the laboratory and their primary source was human plague

By the 8th day after infection the number surviving in each group was as under:-(no more control rats dying after the 8th day).

Series A - 18 rats survived - a percentage immunity of 56.2

Series B - 10 " " " 34.5

Series C - 11 " " " 32.3

Series D - 1 " " " 2.8

The vaccine used in Series A and B differed little from one another in respect of maturity, but the vaccine used on Series A had been brewed for two months, while that used on Series B had been brewed for 4 months. The vaccine used on Series A is manifestly much superior.

The vaccine used on Series C was also quite recent (2 months maturity). It had been brewed for 8 months and 4 days. Its immunising power was equal to that used on Series B.

According to this experiment the immunising antigens then degenerate during incubation between the 2nd and 4th month of autolysis.

Chart VI. illustrates the percentage death rate day by day.

TABLE VII.

Series A were inoculated with 1/4 c.c. of vaccine matured only 24 days, but brewed for 8 months and 5 days. The vaccine had been stored in the laboratory.

Series B were inoculated with 1/4 c.c. of vaccine matured one year and two weeks, but brewed for 3 months. The vaccine had been stored in the laboratory.

Series C were inoculated with 1/4 c.c. vaccine matured one year

and 2½ months, but brewed for one month and 17 days only. The vaccine had been despatched up country and retained there for 9 months.

Series D were inoculated with 1/4 c.c. of vaccine matured for 1 year 11¼ months, but brewed 3 months 5 days. The vaccine had been stored in the laboratory.

Series E were not inoculated and served as controls.

On the 13th day after immunisation a dose of .0022 milligrammes of the spleen of a plague rat was given to all the rats which now numbered 27 in Series A; 26 in Series B; 24 in Series C; 27 in Series D; 26 in Series E.

On the 7th day after infection by which time the control rats reached the limit of death rate the following rats remained alive in each group.

In series A	12	survived - a percentage immunity of		44.4
In Series B	10	"	"	38.5
In Series C	16	"	"	66.6
In Series D	8	"	"	29.6
In Series E	2	"	"	7.7

The most efficient vaccine was that used on Series C, which although old (maturity being 1 year 2½ months) had been incubated only for a period 1 month 17 days.

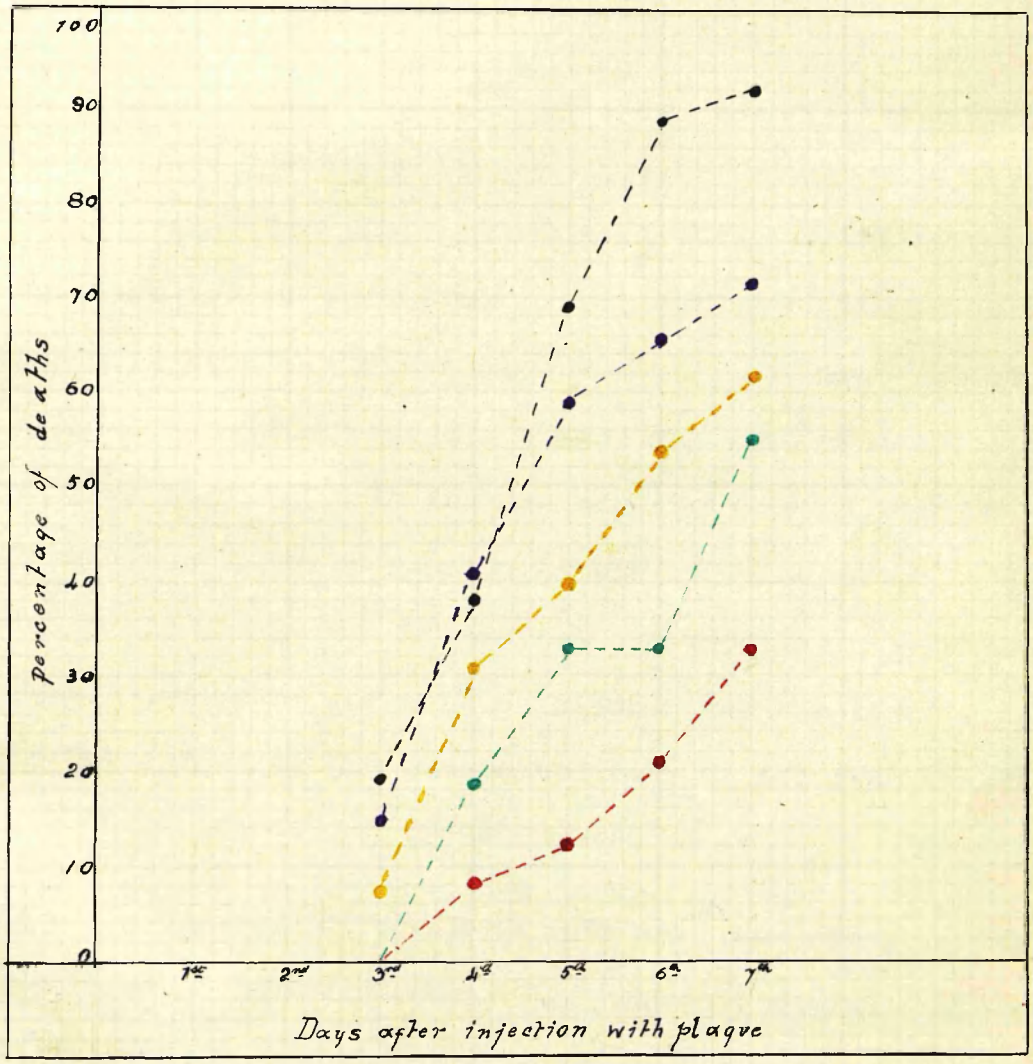
The vaccine used in Series B was of the same maturity, but had a longer incubation - 3 months.

It is interesting to note that an old vaccine of over 1 year's maturity if its incubation period is shorter can be more potent than a fresh vaccine, such as that used in Series A.

Chart VII

To illustrate Table VII

Curves showing the percentage death rate day by day



Green line denotes Series A

Yellow line " Series B

Red line " Series C

Purple line " Series D

Black line " Series E

It should also be noted that a vaccine 2 years old (as that used in Series D) can still show some immunising powers.

According to this experiment then, the immunising antigen degenerates during incubation between 47 days and 3 months of autolysis.

Chart VII. illustrates the daily percentage death rate in this experiment.

CONCLUSION -

(1) It will be noted that in every case the Tables are illustrated by Charts which show the percentage death rate day by day. A consideration of these charts will show that incubation not only lessens the percentage of death, but that in those inoculated rats which develop plague the date of death is deferred; or putting it in another way the disease does not kill the inoculated so rapidly as the non-inoculated. The curve of the death-rate of control non-vaccinated rats reaches it summit somewhere, as a rule, between the 4th and 6th day, while the curves of the inoculated rats do not reach their summits till the 8th or the 12th day.

(2) The absolute immunity afforded to rats varies according to the dose of vaccine administered. In Table II the death rate diminished from 87.5 per cent in non-inoculated rats to 15.4 per cent in inoculated rats (immunised with doses of 2 c.c. vaccine), - a difference of 72.1 per cent, while in Table III when the rats were inoculated with 1/4 c.c. of doses of vaccines, the difference of immunity at most between the control rats and the rats immunised with the most potent vaccine used in that experiment

was 17.9 per cent. This point is specially brought out in the experiment recorded in Table I.

(3) The gain in immunity to rats inoculated with potent vaccines of course is dependent not only on the strength of the vaccine as well as the dose given, but also on the number of virulent living plague bacilli administered, and their virulence, to test the immunity. Hence in our experiments the immunity produced varies from 27.8 per cent in Table IV and 30 per cent in Table V to 53.4 per cent in Table VI and 58.9 per cent in Table VII.

These results should be compared with those of Kolle and Otto and of Strong referred to above.

Further, except on the supposition that he was working with a non-potent vaccine, Klein's statement that an adult rat cannot be immunised with less than 10 c.c. of Haffkine's prophylactic cannot be understood.

(4) The toxicity of the vaccine for rats varies with 2 factors:- (1) the length of time the vaccine had been incubated - the period of brew; (2) the length of time the vaccine was stored after sterilisation and carbolisation - the period of maturity.

Of course other factors also influence the toxicity of the vaccine, as the initial virulence of the germ and the circumstances under which the vaccine was stored - exposure to light and heat. The strains of bacilli used were all obtained from septicaemic cases of plague and they were cutaneously rubbed into guineapigs which they killed within 5 days. The conditions of storage are noted in the tables.

With regard to the period of brewing it is interesting to note that Markl[1] stated that the toxic power in broth cultures increased up to about the 2nd month of incubation, after which it became stationary and then gradually decreased, a point which I have now proved true regarding the immunising power also. With regard to the influence of "maturity" on the toxicity of the vaccine, it will be seen from Series I to IV that it gradually diminishes it. In the human subject the reaction, both local and general after inoculation, is very much milder with well-matured vaccines than with vaccines used when fresh.

As the fear of this reaction deters many from being inoculated during an epidemic, it is now the custom of the Bombay Laboratory to issue vaccine matured for some months, 3 at least. There were occasions, however, during the year 1911 when the demand for the vaccine was so great that fresh prophylactic had to be issued as it was prepared. In the month of October, 1911, 220,348 doses were issued.

The Laboratory had many complaints subsequently of the severity of the local reaction caused.

(5) With regard to the influence of the two factors of (1) length of incubation and (2) length of storage on the vaccines, I think it is proved from Tables I to VII that the immunising value runs somewhat parallel to the toxicity and that vaccines brewed from 6 weeks to 2 months are very much more potent to protect from infection than those brewed more than 3 months.

[1]Cited by Oppenheimer "Toxins and Anti-toxins" 1906.

Autolysis in my experience diminishes the immunising value, parallel with the toxicity somewhere between the 2nd and the 3rd month of "brewing". This is contrary to the findings of Rowland (pp 103, 104)

The length of storage does not diminish the immunising value at nearly the same rate, as it does the toxicity of the vaccines. Vaccines over 2 years old were proved to possess some protective value, and vaccines of 1 year 2 months had strong protective powers, even although they may have undergone transit up country.

(6) As a consequence of these experiments the Laboratory, which during 1910 had issued vaccines of long brew (4 months and over) on account of the mildness of the reactions associated with their use, decided to adopt an incubation period of not more than three months, leaving the subsequent maturing for 3 months after sterilisation to diminish the toxicity and local & general reactions caused by the toxines. It would be better still to reduce the incubation period to not more than 2 months.

(7) Practical results of these experiments.

It will be seen that the use of the vaccines issued from the laboratory prior to 1910 gave good results - results which were at least equal to those obtained by Mr. Haffkine in the early days. The analysis of the Salem statistics has already been given as they were obtained after most careful investigation. The figures also are large and the chances of error in deduction from them is accordingly reduced.

From these statistics we were assured that inoculates were fully three times less likely to be infected than non-inoculates, and when infected the inoculates were twice as likely to recover as the non-inoculates.

I have made the following calculation from figures abstracted from the books by the staff of the laboratory of the brews issued to the Salem authorities during the epidemic of 1910-11 to the Collection of Salem and the Chief Plague Officer of Salem; 293 brews (representing about 58,600 adult doses). Of these brews the period of incubation or "brewing" was:-

Of 22 brews	less than	4 months	but more than	3
24	"	5	"	4
95	"	6	"	5
46	"	8	"	7
90	"	9	"	8
16	"	10	"	9

The brews had been kept in the laboratory after sterilisation before despatch - were "matured" - for the following periods:-

12 brews	for less than	10 months	but over	8
36	"	8	"	6
42	"	6	"	4
11	"	4	"	2
99	"	2	"	1
93	"	1 month		

It will be seen that in no case was the vaccine brewed for less than 3 months and the majority were brewed from 5 to 9 months. It follows then that the toxicity and immunising power of these brews would be less than if they had been issued after brewing for 3 months or less.

In 1911 the demand for the vaccine all over India was very largely increased, 1,211,170 doses being issued instead of 625,690 in 1910. It had been resolved, as stated above, to issue from the laboratory vaccines brewed not longer than 3 months, as much as possible, and in order to diminish the toxicity, as far as possible, of this potent vaccine to issue mature vaccines alone. But towards the end of 1911 owing to the great demand for the vaccine, it was necessary to issue it without maturing. It was therefore highly toxic as well as potent. The consequence was that many complaints were received from all quarters regarding the severity of the symptoms both local and general which resulted. These symptoms consisted of swelling and redness of the arm at the locus of injection, sometimes an arthritis, and general malaise and fever of variable duration.

These experiences were disturbing as they naturally led to unpopularity in the use of the vaccine. The interesting question is whether in the field an improvement on the statistics will be noted by the use of vaccines brewed under 3 months. By the issue of the more potent vaccine the incidence of plague among the inoculates should, one would imagine, be still further diminished. In this connection some statistics, which seem to have been very carefully compiled, have just come to hand. "A Report on the recent Epidemic of Plague in the City of Hyderabad and its suburbs" by Lt. Col. H. E. Drake Brockman, I.M.S., Director of the Medical Department and Plague Commissioner, H.H. The Nizam's Dominion 1913.

At Col. Drake Brockman's request, Captain J. Taylor I.M.S., a member of the Plague Research Commission, investigated

the plague work done including the inoculation campaign statistics. He elicited the following facts:-

The population of Hyderabad City is 387,133, of which approximately 200,000 evacuated the City during the early stages of the epidemic. The total number inoculated was 78,085 (all done voluntarily and at a cost of 3 annas 5 pies per head about 3½d)

Of the inoculates 162 died of plague; of the non-inoculates 16,901 died of plague.

Captain Taylor estimated that the death rats per mille of the inoculates was 3.16, while of the inoculates it was 81.70.

"The Relative chance of escaping death from plague if inoculated was 25.8 to 1.

Relative chance of recovering if once infected, if inoculated, 4½ to 1".

Great precautions were taken with the records; the work was done under Col. Drake Brockman's own personal supervision and no unauthorised persons were allowed to perform the operation, the result being that "beyond one arm much inflamed, which was in the case of a European who foolishly played hard tennis directly after being inoculated, I cannot honestly remember any untoward result of the operation out of the whole lot of men, women and children who were inoculated".

These results are very striking. The vaccine issued was brewed for periods mostly under 3 months, and the protection afforded evidently is much superior to that obtained before by the use of longer brewed vaccines.

From records abstracted by the staff of the laboratory from the books, I have made the following analysis:-

884 brews(making a total of about 176,800 adult doses) were issued to the Chief Plague Medical Officer Hyderabad, the Medical Store Keeper H. H. Nizam's Government Hyderabad, the Director H. H. The Nizam's Medical Department, the Municipal Commissioner Hyderabad, and to the Resident Surgeon, Hyderabad in 1911.

Of these brews the period of incubation or "brewing" was of

103	brews	less	than	1	month		
44	"	"	2	months	but	over	1 month
658	"	"	3	"	"	2	"
65	"	"	4	"	"	3	"
12	"	"	5	"	"	4	"
2	"	"	5				

The brews had been kept in the laboratory after sterilisation before despatch - were "matured" for the following periods:-

1	brew	less	than	12	months	but over	10
29	brews	"		8	"		6
422	"	"		6,	"		4
248	"	"		4	"		2
39	"	"		2	"		1
145	"	"		1			

It will be necessary to collect the experiences of some more years before we can definitely state the effect in practice of this reduction of the incubation period of the vaccine. But these Hyderabad figures are very encouraging.

THE ABSENCE OF A "NEGATIVE PHASE" AFTER INOCULATION WITH PLAGUE PROPHYLACTIC.

The question of the existence of a negative phase after inoculation with plague vaccine has again risen since the discussions of the delegates to the International Plague Conference at Mukden in April 1911. By a 'negative phase' I mean a period of definite hyper-susceptibility to plague infection and not merely a latent period during which no immunity is being produced.

During the discussions it was obvious that the opinions of the delegates were divided on the subject. That it is one of great importance every inoculator will agree. Can the vaccine be safely administered to persons living in plague-infected houses, and again, — an aspect of the problem that especially puzzled the workers in Harbin according to Dr. Wu, a Chinese delegate,—what length of time should elapse between inoculation of the plague staff and the date they are allowed to start work among plague cases? This, of course, is of special importance in an epidemic of Pneumonic plague where transmission of the infection to attendants is so common.

On reading the official report of this Conference, one is struck by the lack of any data from which it might be possible to deduce observations. Dr. Paul Haffkine, for example, Director of the Russian Plague Hospital at Harbin, talks of the "usual period of the negative phase which is 12 days". [1] But when asked by Dr. Richard Strong, the Professor of Tropical Medicine at Manilla, to give his evidence, he replies, "The human body is weakened by inoculation. I only found 4 cases in which plague was contracted 12 days after inoculation. All the others who got infected were infected in the first days after inoculation. Therefore it seems probable that there is a negative phase".

[1] On page 124.

He seems to have forgotten that those who, he says, were infected in the first days after inoculation, may have been incubating the germs before they were inoculated at all. He frankly stated when pressed that his opinion was an "hypothesis".

The general opinion of the Conference was that the subject required further evidence, and I propose to give such evidence now.

Before I do so, however, let me detail the previous literature and experiments on the subject.

In a paper entitled "A discourse on Preventive Inoculation", delivered at the Royal Society, London, on June 8th 1899, Haffkine of the Plague Laboratory, Bombay, stated that his experience in anti-cholera inoculation entitled him to give a reassuring answer to this question.

From the Undhera statistics Haffkine deduced:-

"Inoculation has again acted, so to say, immediately; or as we have adopted to generally formulate the result, has acted within the time necessary for the subsidence of the general reactionary symptoms produced by the inoculation".

Bannerman in the "Proceedings of the Royal Society of Edinburgh", 1901, Vol.24, Part 2, to which paper we are indebted for reference to much of the literature on this subject, also states that:-

"Protection begins to be effective after the lapse of 24 hours and goes on steadily increasing for some considerable number of days thereafter".

On the other hand, we have the views of the Indian Plague Commission Report of 1898-99, Vol.5, page 262, that inoculation does not appear to confer any such degree of protection within the first few days after the inoculation has been performed.

We can find references to only two animal experiments on the subject:-

First,- Calmette's experiments recited in brief in the British Medical Journal of October 27, 1900:-

> "With M. Salimbeni he had shown during the recent epidemic in Oporto, that animals during the period of immunisation with heated cultures were extremely sensitive to very small doses of the virus, doses which were rarely mortal to non-vaccinated animals. It followed that 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 be fatal."

The cultures used by Calmette and Salimbeni were killed at a temperature of 70°C. and therefore were of little, if any, immunising value, as it is well known that such a temperature to a large extent destroys the plague toxine. The dose given to mice was one half cubic centimetre and 1 to 2 cubic centimetres for guineapigs. These writers stated that with such a dose "immunity only establishes itself at the end of 8 to 10 days and scarcely lasts more than 2 weeks".[1]

These results were confirmed by Mauro Jatta & Romano Maggiora (Bulletin de l'Institut Pasteur Tome III 1905).

According to Professor Zabolotny, Professor of Bacteriology, Medical Institute, St. Petersburg, "Calmette's investigations were only done with a few animals." Report of International Plague Conference, Mukden 1911. I cannot find out how many animals were used.

Second,- Some experiments were reported in the Bombay "Bacteriological Laboratory's Annual Report" for the year ending 31st March 1905. There, the uncertainty of the knowledge at that time about this question of

[1]Annales de l'Institut Pasteur, Tome XIII, 1899.

"negative phase" was expressly implied. It is pointed cut that prior to certain experiments there detailed, carried out by Captain Liston, I.M.S., the dose of prophylactic for persons living in infected households was half that for others not so in contact with plague.

These experiments, however, although they were held to be sufficient evidence to justify the administration thereafter of the same full dose to those living in infected houses as to those not in contact with plague conditions are not conclusive, (1) because of the small number of guineapigs experimented upon, and (2) because the average number of days the animals lived after inoculation with plague is taken as the index of the immunity produced by the prophylactic. Of this assumption there is no proof, although it is probably correct that prolongation of the incubation period of the disease indicates an increase in the acquired immunity to the disease.

I will now detail two fresh experiments carried out by me in conjunction with Senior Assistant Surgeon R.J. Kapadia during the months of March and April 1911.

Both the experiments were made on rats (mus rattus) caught in Madras. These rats the Plague Research Commission have shown are highly susceptible to plague differing in this respect from the rats now procurable in Bombay.

Experiment I,- The total number of rats experimented with, namely 280, was divided up into 8 groups of 35. The plague prophylactic vaccine employed throughout the experiment had the following history. The source of the bacilli was from human plague passed through a guineapig. It was brewed at room temperature for 2months. It was kept in the cold room in darkness from 24th February 1911, the date of manufacture.

One "A" group received on 30th March 1914, 14 days before it was inoculated with plague ... ... 0.25 C.C.of the vaccine

One "B" group received on 3rd April 1911,10 days before it was inoculated with plague ... ... do

One "C" group received on 4th April 1911,9 days before it was inoculated with plague ... ... do

One "D" group received on 6th April 1911,7 days before it was inoculated with plague ... ... 0.25 C.C.of the vaccine

One "E" group received on 8th April 1911,5 days before it was inoculated with plague ... ... do

One "F" group received on 10th April,1911,3days before it was inoculated with plague ... ... do

One "G" group received on 12th April 1911,1 day before it was inoculated with plague ... ... do

One "H" group was kept as a control receiving no treatment.

Certain of these rats in the various groups died from handling or effect of the toxin, leaving the following numbers in the various groups alive on the 13th April 1911:-

A	B	C	D	E	F	G	H
32,	33,	34,	29,	33,	33,	35,	35.

On the 13th April all these groups received simultaneously the same dose of plague,- namely, an emulsion of spleen from a rat which died of plague, the spleen smears showing large number of plague bacilli. Each rat in these groups received .0019 of a milligramme of the plague spleen.

Every rat which died was sectioned and smears from the various organs examined for plague bacilli, and unless these were found the death was not ascribed to plague.

At the close of the experiment the following number of rats remained alive in each group:-

A	B	C	D	E	F	G	H
14,	12,	12,	11,	15,	20,	13,	4.

The following table gives the incidence of deaths in the various groups day by day.

TABLE I.

Date on which death occurred	A Group		B Group		C Group		D Group		E Group		F Group		G Group		H Group	
	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague
15th April 1911	...	...	1[1]	...	1[1]	...	...	...	2[1]	...	...	...	...	...	...	1
16th " "	...	...	...	...	...	1	...	1	...	3	1[1]	...	...	...	...	4
17th " "	...	7	...	4	...	4	...	3	...	5	...	3	...	3	...	11
18th " "	...	3	...	4	...	6	...	3	...	...	...	1	...	8	...	6
19th " "	...	2	...	5	...	4	...	5	...	...	...	2	...	3	...	6
20th " "	...	1	...	1	...	4	...	3	...	...	...	...	...	2	...	1
21st " "	...	1	...	...	...	...	...	...	...	3	...	2	...	4	...	...
22nd " "	...	...	...	3	...	...	...	1	...	1	...	1	...	2	...	...
23rd " "	...	2	...	3	...	2	...	2	...	1	...	3	...	...	...	...
24th " "	...	2	...	...	...	...	...	...	...	3	...	...	...	...	...	2
Totals ..	18		20		21		18		16		12		22		31	
	Out of 32		Out of 32, 1[1] not counted in total numbers.		Out of 33, 1[1] not counted in total numbers.		Out of 29		Out of 31, 2[1] were not counted in total numbers.		Out of 32, 1[1] not counted in total numbers		Out of 35		Out of 35,	

It will be seen that even in the group "G", which received the vaccine one day before inoculation with plague, the number of deaths is less than in the control group "H", which received no prophylactic treatment.

On the whole the best result is in group "F", which was inoculated with plague three days after receiving the dose of vaccine.

Experiment II,- The total number of rats experimented with was 280, divided up into 7 groups of 40 rats each. The same vaccine was employed as in Experiment I. One group was kept as a control - receiving no preliminary prophylactic dose. The remaining 6 groups were treated as follows:-

One "A" group received on 25th April 1911,3 days before inoculation with plague ... 0.25 c.c. of vaccine

One "B" group received on 26th April 1911,(5 p.m.) 46 hours before inoculation with plague. do

One "C" group received on 27th April 1911 (5 p.m.) 22 hours before inoculation with plague do

One "D" group received on 28th April 1911(6-30 a.m.) 8½ hours before inoculation with plague do

One "E" group received on 28th April 1911(10 a.m.) 5 hours before inoculation with plague do

One "F" group received on 28th April 1911(1-30 p.m.) 1½ hours before inoculation with plague do

One "G" group, control rats, received no prophylactic.

Certain of the rats in the various groups died from handling or the effect of the toxine, leaving the following numbers alive at the time of inoculation with plague at 3 p.m. on the 28th April 1911:-

A	B	C	D	E	F	G
39,	40,	39,	40,	40,	40,	40.

At 3 p.m. on the 28th April, all the groups were inoculated with doses of an emulsion of a plague rat spleen, each dose containing .0009 of a milligramme of spleen-tissue.

At the close of the experiment, the following number of rats remained in each group:-

A	B	C	D	E	F	G
11,	20,	14,	14,	9,	7,	6.

The following table shows the incidence of deaths in the various groups day by day (Table II attached).

CONCLUSION[1]

These two experiments show conclusively (1) that there is no "negative phase" or period of increased susceptibility of rats to plague after the administration of anti-plague vaccine even within one and a half hours after the operation. From recent work done by the Plague Commission in England showing that vaccines which immunise guineapigs are not efficacious in an equal degree in the case of rats and vice versa; and in view of our ignorance of the methods by which immunisation is effected, it would be unwise to confidently apply these results of experiments among rats to immunity work among human beings. Taken, however, in conjunction with the deductions drawn from a huge number of statistics of inoculation work in India, it is probably safe to state that inoculation is not only harmless, but in fact beneficial to persons living in actual contact with plague conditions. (2) That the production of immunity among rats commences immediately and increases in amount till the 2nd or 3rd day after anti-plague vaccination.

[1] Subsequent to the publishing of this paper, the main facts of which appeared in the Bombay Laboratory Report for 1911. I became aware that Terni and Bandi (Un nuovo methods di preparazione del vaccine antipestoso 1899)demonstrated protective substances in the serum of vaccinated persons in 8-10 hours after inoculation by their method. These results are confirmed by Rowland. Journal of Hygiene Plague Supplement II 1912 p.368. Terni and Bandi however state that after inoculation with Haffkine's prophylactic protective substances do not appear till the 10th or 12th day.

TABLE II.

Date on which death occurred	A Group		B Group		C Group		D Group		E Group		F Group		G Group	
	Not Plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague	Not plague	Plague
29th April 1911	...	...	1[1]	...	2[1]	...	...	...	1[1]	...	...	...	...	...
30th " "	...	...	...	...	...	...	...	1	...	...	...	...	...	...
1st May "	...	1	...	2	...	1	...	2	...	...	...	2	...	5
2nd " "	...	4	...	6	...	7	...	4	...	7	...	8	...	7
3rd " "	...	9	...	4	...	5	...	10	...	9	....	10	...	6
4th " "	...	4	...	1	...	3	...	5	...	5	...	4	...	5
5th " "	...	3	...	2	...	1	...	3	...	5	...	2	...	6
6th " "	...	4	...	2	...	1	...	...	...	1	...	2	...	3
7th " "	...	1	...	2	...	4	...	1	...	3	...	3	...	...
8th " "	...	2	...	...	...	1	...	...	...	...	...	2	...	2
Totals		28		19		23		26		30		33		34
		Out of 39		Out of 39 1[1] not counted		Out of 37 2[1] not counted		Out of 40		Out of 39 1[1] not counted		Out of 40		Out of 40

It then declines somewhat in amount and remains more or less steady from the 4th day onwards to the 14th - as far as our experiments have been conducted.

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