PART III.

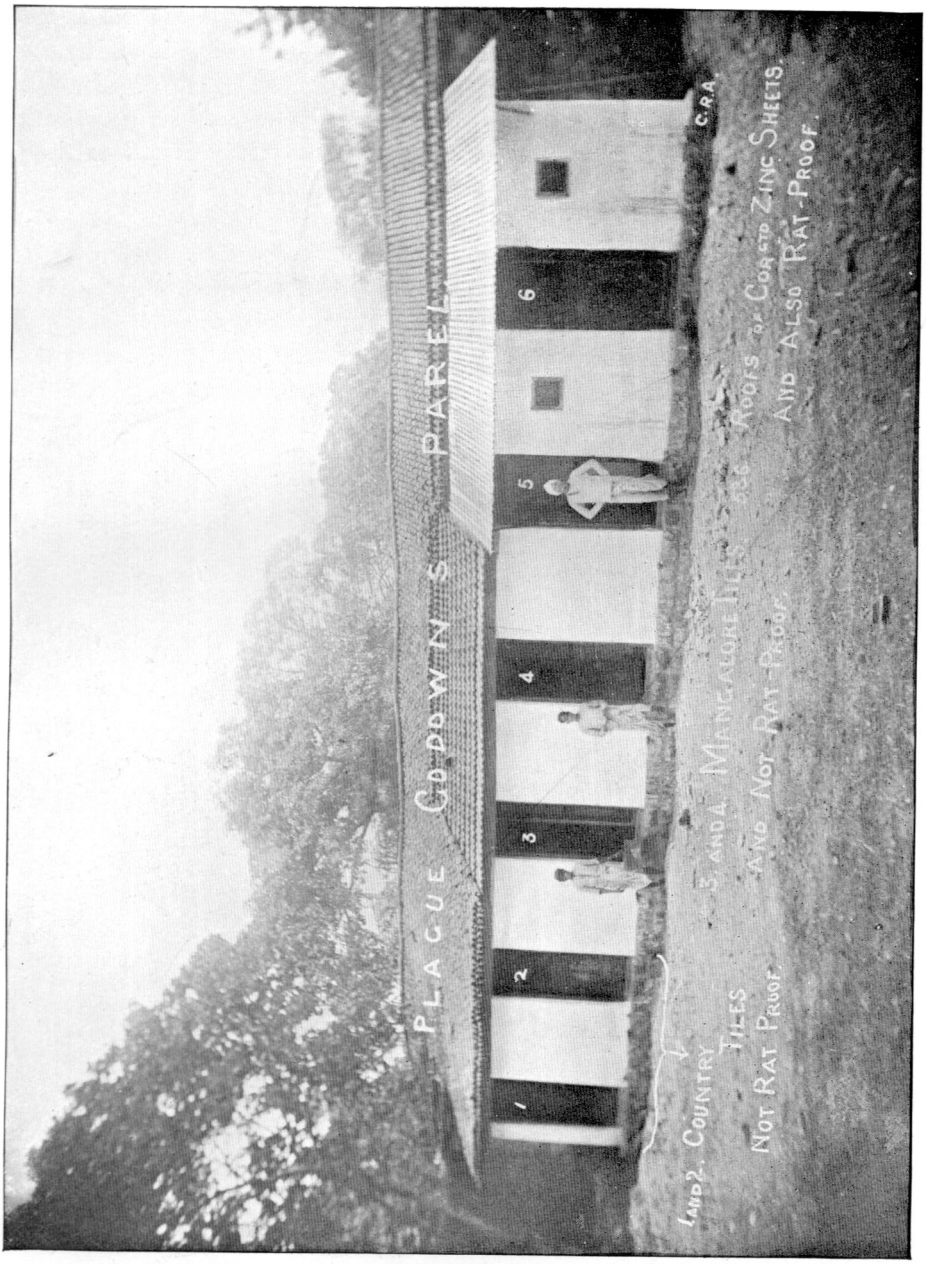
EXPERIMENTAL PRODUCTION OF EPIDEMICS AMONG GUINEA-PIGS.

The following experiments, which had as their object the determination of the relative importance of the Indian rat flea, *Pulex cheopis*, and of actual close contact in the absence of fleas, in the dissemination of plague from animal to animal, were carried out in a series of small "go-downs" or cabins, which were built especially for this purpose. These go-downs were designed by Lt.-Col. Bannerman and Captain Liston, and built under their supervision. They were completed before the present Commission began work in May, 1905. For a proper understanding of the experiments which are to be detailed below, a description of the structure of the go-downs is necessary.

Guinea-pigs and also two monkeys were used for these experiments, as it had been shown by Liston (1905) that the rat flea we have to deal with in India readily attacks these animals in the absence of its natural host.

Construction of go-downs.

The photograph (Plate V) and plan (Fig. 1) show that they are six in number and are built in a row. It will be seen that they are numbered 1, 2, 3, 4, 5 and 6, from left to right. The walls, 9 inches thick, are built of brick and mortar, while the floors are of concrete on the top of a high plinth. The walls and floors are therefore impervious to rats. The interior of each go-down measures 7 ft. × 6 ft., while the inside height of the back wall is 9 ft. and of the front wall 7½ ft., thus giving the roof a slope of about 1 in 4. Each go-down is entered by a wooden door fitting tightly and measuring 6½ × 2½ ft. The door opens inwards into an inspection chamber, that is to say, a space measuring 3¼ ft. × 3 ft., separated from the rest of the interior by means of wire netting, carefully fitted on to a wooden framework and extending up to the wire netting, which, as we shall see immediately, covers the inside of the roof. This netting, as also that under the roof, is made of stout wire, and has a mesh of half-an-inch. A door, also of wire netting, gives access from this inspection chamber into the interior of the go-down. The size and shape of



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the space available for animals is well shown in the accompanying plan.

In the case of go-downs 1, 2, 3 and 4, with the exception of the doors, there is no opening in any of the walls. For the purpose of ventilation, in the case of go-downs 5 and 6, there are small windows, measuring one foot square, in the front wall. These windows are closed by a double layer of wire netting carried on a wooden framework, let into the masonry of the wall.

While the go-downs, as far as the above description is concerned, are all exactly alike, the essential difference in their construction consists in the structure of the roofs. In the case of Nos. 1 and 2,

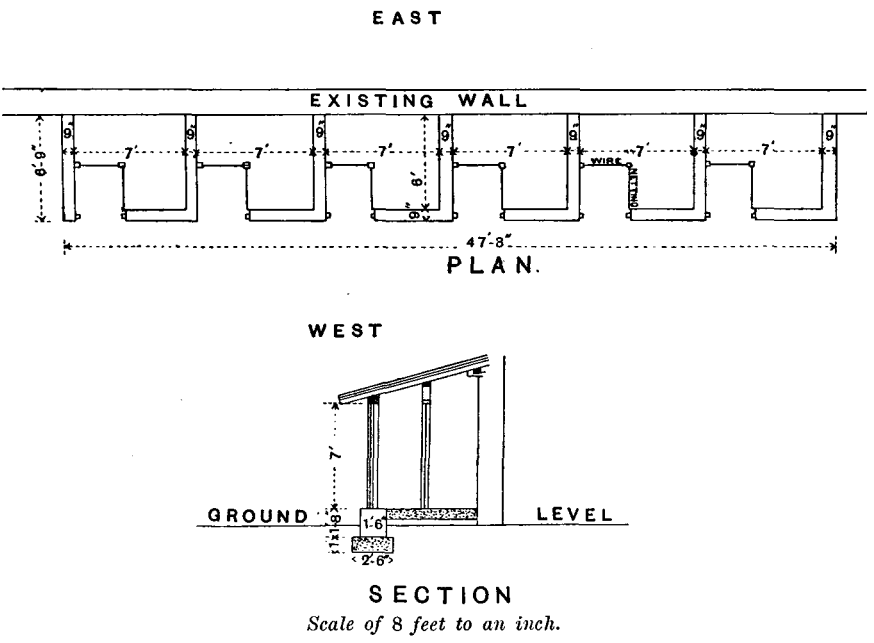

Fig. 1. Go-downs in connection with the Plague Research Laboratory in the Old Government House at Parel.

the roofs are of ordinary country tiles, placed in four layers on the top of wooden lathes. Immediately on the inside of this roof, there is a wire netting carried on a wooden framework, which framework is carefully built on all sides into the masonry of the walls. In the roof of No. 1 a certain amount of light is allowed to penetrate through a small glass window in the tiles. This opening does not exist in the

roof of No. 2. Further, in the case of No. 1 there is a second wire screen 10 inches below the one immediately under the tiles. This screen is also carried on a wooden framework built into the walls. While, therefore, rats can inhabit and build their nests in the tiles of the roof, they are completely shut off, in the case of No. 1 by a double screen of wire, in the case of No. 2 by a single screen of wire, from the interior of the go-downs. The object of the double screen of wire in No. 1 is to prevent any possible contact between experimental animals in the go-down and the wild rats inhabiting the roof.

The roofs of go-downs Nos. 3 and 4 only differ from those of the first two in having a single layer of flat Mangalore tiles instead of country tiles. In the roof of No. 3 there is a ventilator, also of Mangalore tile, through which a small amount of light is allowed to penetrate into the interior of the go-down. This ventilator does not exist in the roof of No. 4. Further, the roofs of both these go-downs are separated from the interior by a single layer of wire netting. Mangalore tiles, in comparison with country tiles, afford a poor shelter to rats. We find, therefore, that the rats inhabiting the roofs of Nos. 3 and 4 go-downs are not nearly so numerous as in the case of Nos. 1 and 2. That rats inhabit the roofs of these go-downs has often been a subject of observation. They have frequently been seen on the top of the wire during the day while the go-downs were being examined. This is especially the case in No. 2, where as many as twelve rats have been seen at one time. The species seen was always *Mus rattus*. Further, rats' nests have on several occasions been found on the wire netting. Rat dung has also often been found in the go-downs themselves.

We come now to go-downs Nos. 5 and 6. The roofs of these go-downs are made of a single layer of corrugated iron which is fastened down with cement to the top of the walls all round. It is evident, therefore, that no rat can penetrate inside the roof of either of these go-downs. The wire netting under the roof is in the case of No. 5 of single layer, but in the case of No. 6 there is a double layer as in No. 1.

Before we pass on to the description of the experiments carried on in these go-downs, it is well to emphasize the fact that the only essential difference between them is in the structure of the roofs. This difference, however, is of such a nature that the natural supply of fleas, depending as it does on the number of rats inhabiting the roofs, and the amount of light, varies in the different go-downs. In

the case of go-downs Nos. 1 and 2, the roofs of which offer good protection and shelter to the wild rat of Bombay, the flea supply is abundant and regular; in the case of go-downs Nos. 3 and 4, the roofs of which offer only poor protection to rats, the flea supply is more or less scanty; while into go-downs Nos. 5 and 6, the roofs of which are absolutely impervious to rats, no fleas should be able to gain access unless carried in through the door on the experimental animals themselves, or by the attendant when feeding these animals. As a matter of fact, it was found impossible to keep these two go-downs absolutely free from fleas, and, as we shall see later on, at one time, owing either to breeding or to a sudden migration from without through a flaw in the cement of the roof, a fairly abundant supply of fleas obtained access to go-down No. 6.

The following two tables show the number of fleas caught on guinea-pigs placed in each go-down on two occasions (May and October), the fleas caught being removed on each day for six successive days. They show that the number of fleas varies in each instance with the accessibility of the roof to rats: the influence of light and darkness is also well brought out.

TABLE I. *Three guinea-pigs in each go-down.*

Go-down Date....	31. v. 05	1. vi. 05	2. vi. 05	3. vi 05	5. vi. 05	6. vi. 05	Total
No. 1. Country tiles with small roof-light	1	7	8	8	22	8	54
No. 2. Country tiles with no roof-light	14	32	41	46	46	49	228
No. 3. Mangalore tiles with small roof-light	2	5	3	5	16	9	40
No. 4. Mangalore tiles with no roof-light	7	7	14	12	15	15	70

TABLE II. *Six guinea-pigs in each go-down.*

Go-down Date..	27. x. 05	29. x. 05	30. x. 05	31. x. 05	1. xi. 05	2. xi. 05	Total
No. 1	11	1	14	12	11	*43	92
No. 2	14	16	9	16	20	15	90
No. 3	3	1	0	0	0	1	5
No. 4	2	2	2	1	0	0	7
No. 5	0	0	0	1	0	0	1
No. 6	0	0	0	3	0	1	4

* The roof-light had been shut up before this count was made.

Finally, it is necessary to add that the back wall of the go-downs also serves as the back wall of a series of houses, which, along with

attached runs, are used for the housing of those guinea-pigs and rabbits of the laboratory which are kept for breeding purposes. The roof of these houses, continuous with the roofs of the go-downs, is constructed of country tiles, and in consequence offers a safe habitation for wild rats.

The experiments, under varying conditions, are still in progress and will be continued for some time, but the results so far obtained are sufficiently striking to place on record. At a later period observations indicating their significance in the epidemic spread of plague among human beings will be recorded.

All animals dying in the course of these experiments were submitted to a careful and detailed post-mortem examination. This examination included a microscopical examination of smears of the bubo, of the spleen and of the heart's blood. Further, cultures were taken, and if there was the slightest doubt of the diagnosis, cultural and animal inoculation tests were made. It is, however, considered unnecessary to detail the results of these examinations in each case.

Series A.

Experiments in which epidemic plague did not occur when healthy guinea-pigs lived in close contact with plague-infected guinea-pigs under conditions where access of fleas was prevented, but in which under otherwise similar conditions plague spread among the healthy animals in places where fleas were abundantly present.

Simond, Gauthier and Raybaud, and Liston never succeeded in infecting animals from one another when healthy and plague-infected animals were confined together in the same cage, *if fleas were excluded and the animals were not allowed to devour the cadavers of their dead comrades.*

The experiments of the Commission have been conducted upon a somewhat larger scale than has been previously possible.

25 to 50 guinea-pigs were confined together for periods of one to two months in a go-down in company with a number of animals which had been inoculated with plague. The go-downs were not cleaned out during the whole time, and the animals took their food from the floor which was contaminated with the excreta of their sick companions. Under these circumstances, as may be seen from the recorded experiments, an occasional guinea-pig succumbed to plague, but the disease

did not spread epidemically, except in the case of Experiment VI, in which, notwithstanding the precautions taken, fleas in large numbers found access to the animals.

Experiment I.

On May 31st, 1905, there were placed in No. 6 go-down, rendered as flea-proof as possible, 50 healthy guinea-pigs and 10 guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. Of these 10 guinea-pigs 5 had been inoculated subcutaneously and 5 by the cutaneous method.

By the 6th June, all the animals inoculated subcutaneously had died of plague. As regards those cutaneously inoculated, one died under chloroform when being searched for fleas. This was subsequently proved to be plague-infected. By the 10th June, three of the remaining four had died of typical plague, while the fifth developed a local ulcer, but recovered.

Among the healthy uninoculated animals two died, one on the 28th June and the other on the 18th July. Both these animals were proved bacteriologically not to have died of plague. The other 48 guinea-pigs remained healthy, until the experiment was abandoned on the 18th July. It is to be noted that this experiment was made during the non-epidemic season in Bombay.

Experiment II.

On Nov. 25, 1905, there were placed in go-down No. 5, which had been rendered as flea-proof as possible, 50 healthy guinea-pigs and 10 guinea-pigs which had been inoculated with a virulent culture of *B. pestis*.

By the 30th November all these inoculated animals had died of plague. On the 7th of December it was noted that one of the uninoculated animals was ill. A flea census was at once made and one flea was caught on 25 guinea-pigs examined. It is evident, therefore, that the go-down was comparatively, but not absolutely, free from fleas. The sick guinea-pig died of plague on the 11th December, with buboes in the submaxillary and cervical regions. From this date nothing further happened in the go-down until the 1st January. So, from the 30th November when the last inoculated guinea-pig died, until the 1st January, only one out of 50 uninoculated animals had died of

plague. The subsequent history of the 49 remaining animals is given in Experiment VII below.

Experiment III.

In describing the construction of the go-downs it was pointed out that in the case of Nos. 1, 2, 3, and 4, rats had taken up their abode under the tiles which covered these four cabins, and although they were unable to enter these cabins they ran about and slept upon the wire netting which was stretched below the sloping roof. By this means, these go-downs were kept supplied with rat fleas, for as the insects left their hosts they fell down into the go-downs.

The first experiment in the presence of rat-fleas was made in go-down No. 2, during the months of June and July, that is to say, during the season when in Bombay only sporadic cases of plague are occurring, and when there is no general mortality from plague amongst the wild rats. Experiment I served as the control to this experiment.

On June 13th, 50 healthy and 10 inoculated guinea-pigs were placed in this go-down. The inoculated animals had all succumbed by June 29th. One of the uninoculated animals died of plague on the 2nd July; a second animal died on the 3rd July; a third on the 4th July, and a fourth on the 12th July, the cause of death being in all cases proved to be plague. All the other animals remained healthy until the experiment was abandoned in August.

Experiment IV.

This experiment was made during the period when the epizootic was just commencing, Experiment II serving as a control.

On the 25th November, 1905, 49 guinea-pigs were placed in No. 2 go-down. These animals were allowed to run free in the go-down. On the same day 10 guinea-pigs were added which had been inoculated subcutaneously with a virulent culture of *B. pestis*. These inoculated animals were also allowed to run free. By the 1st December all the inoculated guinea-pigs had died of plague. After an animal had died, the body was allowed to remain in the go-down for about 24 hours.

A death among the uninoculated animals took place on the 2nd December, and by the 19th December, that is to say in 17 days, not a single guinea-pig was left alive, the cause of death being in every instance acute plague. From the last two animals 400 fleas were

recovered, 326 from one, which was moribund, and 74 from the other, which had already died. These fleas were returned to the go-down.

Experiment V.

On 16th December five guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were placed in go-down No. 1. In four days, that is by the 19th December, these five animals had succumbed to acute plague. On the 20th December there were placed in this go-down 26 healthy guinea-pigs. These animals were allowed to run free. An epizootic of plague of the most rapid description at once broke out amongst these healthy animals, the first dying on the 25th December and the last on the 1st January. 115 fleas were taken from the last five animals when moribund.

Experiment VI.

In this experiment, which was intended to be one in which fleas were excluded, 25 healthy guinea-pigs were confined in go-down No. 6 together with some others inoculated with plague, and more inoculated animals, 32 in all, were added, a few each day, during one month. Unfortunately, fleas were not completely excluded and after five weeks their number was found to have increased considerably. The experiment is therefore one which during the first month was carried out in the nearly complete absence of fleas, but later in the presence of fleas in abundance.

On the 16th December five guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were allowed to run free in No. 6 go-down. On the 20th December, two of these animals having already died and the other three being sick, 25 healthy uninoculated guinea-pigs were added and allowed to run free in the go-down. The last of the inoculated animals died on the 24th December. On the 26th December four more guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were added to the go-down. These animals in their turn died, and as soon as they were all dead four other inoculated animals were again added. In this way the supply of plague infected animals was kept up for a period of about three weeks.

The following table shows the total number of plague infected animals and the dates on which they were added:

No. of inoculated guinea-pigs	Date when put into go-down
5	16th December
4	26th December
4	29th December
4	1st January
4	4th January
*7	9th January
*2	10th January
*2	14th January
Total 32	

* These guinea-pigs were naturally infected. They were taken from a run in which only breeding animals were kept and in which an epizootic of plague had broken out. Two of these naturally-infected animals recovered and were removed on the 16th January.

From the table it will be seen that between the 20th December and the 15th January the healthy guinea-pigs were living in close contact with plague-infected animals.

The results of this experiment were not decisive, for on the 3rd January one of the uninoculated guinea-pigs was found dead; a post-mortem examination showed that the animal had died of plague with cervical buboes. On the day on which this animal was found dead a flea count was made, and six fleas were caught on the 29 inhabitants of the go-down. We cannot, therefore, in this case exclude a flea-infection. The remaining 24 uninoculated guinea-pigs remained healthy until the 18th January, when it was noticed that one was sick. A flea count made at once yielded one flea on 24 animals. The sick guinea-pig died of plague on 20th January. On the 26th January it was again noticed that a guinea-pig was sick; *a flea count made on this day yielded* 169 *fleas on the* 23 *remaining animals.*

It is difficult to account for this enormous increase of fleas within a period of eight days. We know that the go-down was never quite free from fleas, so that it is possible that breeding had taken place and that a large batch of pupae had developed into adult insects during this period, or else the fleas may have found access through a crack which was found in the cement used to fill in the corrugations of the galvanised iron roof. Be that as it may, the fact remains that on the 26th January 169 fleas were caught on the 23 remaining guinea-pigs and henceforth in an experiment which was intended as a control in the absence of fleas, the conditions had changed and fleas existed in abundance. The fleas were removed from the go-down, as were also all the fleas which could be caught on the four following days. It was also proved that some of these fleas were infected and capable of transmitting

plague. For the fleas removed on the 26th January were divided into two groups and each group was placed on a healthy white rat in a flea-proof cage. One of these rats remained healthy, but the other died of plague on the 15th February, the buboes being in the right sub-maxillary and left axillary regions.

From the 28th January the plague epizootic among the 23 guinea-pigs made fair progress but at a much slower rate than in go-downs Nos. 1 and 2 (Experiments IV and V). On the 27th February 12 guinea-pigs had died of plague, leaving 11 still in the go-down. From these 11 animals only 10 fleas could be recovered. Five females which were pregnant were removed from the go-down, freed from fleas and segregated; none of these died of plague. As the epizootic seemed to be dying out it was re-started. There were placed in wire cages in the go-down four wild Bombay rats, from which the fleas had not been removed and which had been previously inoculated with a virulent culture of *B. pestis*. These rats all died of plague, the last on the 3rd March. Following this fresh infection, the epizootic amongst the guinea-pigs acquired new life, so that between the 7th and 11th March four of the six remaining animals had died of plague. The other two guinea-pigs remained healthy until the experiment was abandoned on the 21st March.

Before we leave this observation it is interesting to be able to tabulate the flea counts which were made in this go-down during the course of the experiment.

Date	No. of guinea-pigs	No. of fleas	Remarks
3rd January	29	6	Removed
17th January	24	1	Removed
27th January	23	169	Removed and proved infective
28th January	23	173	Removed and added to go-down No. 5 (Expt. VII).
29th January	21	98	
30th January	21	67	
31st January	20	79	
5th February	18	122	Returned to go-down
15th February	15	65	
19th February	14	31	
28th February	11	10	

Series B.

Experiment in which the transference of fleas from guinea-pigs dying from plague started an epidemic in an uninfected go-down, which by the introduction of fresh fleas was maintained.

Experiment VII.

This experiment was carried out in go-down No. 5 which contained 49 healthy guinea-pigs and which had hitherto been practically free from fleas. It had served for the control experiment No. II above. It was now tried to start an epidemic among these 49 guinea-pigs by transferring fleas from a go-down in which an epidemic had just taken place to the flea-free go-down in which they were living.

On the 1st January there were added to this go-down 115 fleas which had been caught on five moribund guinea-pigs in go-down No. 1, a go-down which was at the time heavily infected with plague (Experiment V). Next day 106 more fleas were added from the same source.

Following the addition of these 221 fleas an epizootic of plague started, the first animal dying on the 5th January. The supply of fleas to the go-down was now kept up artificially as follows:

Date	No. of fleas	Source of fleas
10. i. 06	59	Go-down No. 1
12. i. 06	142	Go-down No. 2
15. i. 06	100	Bombay wild rats
18. i. 06	100	,,
21. i. 06	120	,,
22. i. 06	100	,,
24. i. 06	100	,,
28. i. 06	173	Go-down No. 6 (Expt. VI)
29. i. 06	98	,,
30. i. 06	67	,,
31. i. 06	79	,,

The epizootic, thus begun after the addition of fleas taken off animals dying of plague in one of the other go-downs, continued, the supply of fleas being constantly kept up, until by the 14th February not an animal was left alive and every death was proved to be due to plague.

The fleas died or disappeared rapidly, for while between the 1st and 31st January 1359 fleas were added to this go-down, a count of the fleas on the ten guinea-pigs left alive on the 7th February yielded only 104 fleas.

Series C.

Experiments indicating that when an epidemic has occurred amongst a number of guinea-pigs the contagion still remains in the place, and is effective in proportion as the test animals are accessible to, and found to be infested with, fleas.

The experiments of this series were made by placing guinea-pigs for one day in go-downs which had recently been used for some of the previous experiments and in which epidemics of plague had occurred. These go-downs contained many fleas.

Of the guinea-pigs placed in these go-downs some were allowed to run loose, others were confined in cages accessible to fleas and placed two inches from the ground, and a third lot were placed in cages which were suspended from the roof at a sufficient distance (*i.e.* 2 feet) from the floor to prevent any fleas reaching them.

In Experiment VIII infectivity was tested by placing animals for one night in the presumably infected quarters, (1) immediately after the last animal had died, and (2) eight days later. In Experiment IX in another go-down the animals were exposed to infection for 24 hours one week, two weeks and three weeks after the last remaining guinea-pig had succumbed to the epidemic; and in Experiment X five and 13 days after the end of a previous epidemic.

The results of these experiments cannot, however, be relied upon to determine how long a go-down may maintain its infectivity, because of the possibility of the introduction of fresh infected rat fleas from above. (See construction of go-downs.) The epizootic amongst rats in Bombay was at its height about the time of the experiments so that a fresh infection from this source could not be excluded.

Experiment VIII.

On the 1st January, *i.e.* on the same day as the last animals died in Experiment V, two groups of four guinea-pigs each, (A) running free, (B) in a cage placed two inches from the ground, were put into go-down No. 1. They were removed next day and examined, when on group A 95 fleas and on group B 11 fleas were caught. The guinea-pigs were then segregated. All the four guinea-pigs of group A died of plague; of the four of group B, one died under chloroform while the fleas were being removed, two died of plague and the fourth remained healthy.

On the 8th January, that is to say, eight days after the last original

guinea-pig of Experiment V had died of plague, the experiment of 1st January was repeated. On these animals being examined next day, 58 fleas were caught on group A and only one on group B. The guinea-pigs were segregated; two of the animals of group A died of plague, the other two remaining healthy: all the four guinea-pigs of group B remained healthy.

Experiment IX.

On the 27th December, that is to say, eight days after the last animal had died of the plague epidemic in go-down No. 2 (Experiment IV), which had been kept shut up in the meantime, the following groups of guinea-pigs were placed in the go-down:—

(A) four guinea-pigs running about free on the floor;

(B) four guinea-pigs in a wire cage two inches from the ground;

and (C) four guinea-pigs in a wire cage suspended two feet from the ground.

Next day (28th December) groups A and B were freed from fleas under chloroform, removed from the go-down and segregated. 330 fleas were caught on the four guinea-pigs which had been running about and 41 on the four which had been placed in the cage two inches from the ground. Two fleas, which had probably fallen from the roof, were found on the four animals suspended two feet from the ground: these animals were left in the go-down. The fleas were all returned to the go-down. The fate of the animals of these three different groups is interesting. One of group A died under chloroform when being searched for fleas; the other three all died of plague. Two of group B died of plague, the other two remaining healthy. The animals of group C, which were left suspended in the go-down for two weeks, all remained healthy.

On the 3rd January, 1906, that is to say, a fortnight after the last uninoculated guinea-pig of Experiment IV had died of plague, the experiment of 27th December was repeated. From the animals of group A, 361 fleas were removed, while from those of group B, 109 fleas were obtained. These fleas were returned to the go-down. Of the four animals of group A, one died while being searched for fleas under chloroform, one died of plague and the other two remained healthy. All the guinea-pigs of group B remained healthy. We have already mentioned that all the animals of group C, which were left in the go-down for a fortnight, remained healthy.

On the 10th January, that is to say, three weeks after the last

uninoculated guinea-pig of Experiment IV had died of plague, this experiment was repeated for a third time. In this case 117 fleas were obtained from the four guinea-pigs of group A, while only 25 were found on those of group B. These fleas were again returned to the go-down. Three guinea-pigs of group A died of plague, while all the four animals of group B remained healthy.

Experiment X.

On the 19th February, five days after the last animal had died of the plague epidemic in go-down No. 5 (Experiment VII), four guinea-pigs were placed overnight in it, two being allowed to run free and two being placed in a wire cage two inches from the ground; next day only nine fleas were caught on these four animals. They were then segregated but remained healthy.

Again on the 27th February a guinea-pig was allowed to run about free in the go-down for ten minutes. On being examined it yielded ten fleas. During the night of the 27th February, four guinea-pigs were allowed to remain in the go-down, two running free and two in a wire cage two inches from the ground. Ten fleas were obtained from the free guinea-pigs and two on the caged ones. These five animals were segregated but remained healthy.

SERIES D.

To show that fleas removed from infected guinea-pigs, and isolated in test-tubes, can convey plague to healthy animals on which they are allowed to feed.

Experiment XI.

On the last two remaining guinea-pigs at the end of an epidemic in go-down No. 4, which were at the time moribund, 95 fleas were caught. These fleas were placed in three test-tubes, the mouths of which were covered with muslin, and fed daily through this muslin on three healthy guinea-pigs.

Test-tube No. 1 contained five fleas: the animal remained healthy and the fleas died in a few days.

Test-tubes Nos. 2 and 3 each contained about 45 fleas. These fleas were fed upon guinea-pigs. In the case of the experiment with the fleas in tube No. 2 the guinea-pig died of plague on the seventh day, and with tube No. 3 on the eighth day, after feeding began.

A fourth experiment was performed by feeding those fleas which still remained alive in test-tube No. 3 upon another guinea-pig. This

animal died on the fifth day after the fleas were first allowed to feed upon it.

Series E.

To show that plague is not conveyed from mother to offspring in the absence of fleas.

Experiment XII.

An interesting and important observation was made in go-down No. 5 towards the end of Experiment VII detailed above.

Between the 31st January and 11th February, during which time an epidemic was in progress, seven female guinea-pigs, which had littered in the go-down, were removed along with their young, 12 in all. The fleas were removed from all the animals, which were then segregated, the young being kept with their respective mothers. Out of these animals, four of the adults died of plague, while not one of the young contracted the disease, although they were suckled by their mothers right up to the time of the latters' deaths.

Series F.

In which a monkey was safely exposed in a plague infected place where the free access of fleas to his person was prevented, whereas his companion not so protected succumbed.

Experiment XIII.

We have, finally, to record an experiment which was made in No. 2 go-down towards the end of February 1906.

Similar experiments with monkeys are being repeated and the results will be published later.

On the 23rd February three guinea-pigs inoculated with a virulent culture of *B. pestis* were placed in go-down No. 2, which is accessible to fleas. These animals had all died of plague by the 28th February. On the 3rd March two monkeys were placed in the go-down in cages of similar pattern, the only difference being that one monkey was surrounded by a layer of "tangle-foot[1]" six inches wide, while the other one was not thus protected[2]. The cages were of such

[1] "Tangle-foot" is a patent sticky resinous preparation spread on paper. It is much used in India for catching flies.

[2] It had previously been found that a rat flea could not hop farther than about five inches.

a design that no flea could get in from the top and the animal could not touch the tangle-foot. The bottom of the cage was of teak wood and thus prevented any contact of the animal with the ground.

These monkeys were left for two nights in the go-down, when they were removed and segregated. Two fleas were caught on the unprotected monkey, while five fleas were found stuck on the tangle-foot. The unprotected monkey soon became ill, a bubo developing in the right axilla. On the 12th March the animal, having torn the bubo open, was killed with chloroform. A pure culture of *B. pestis* was obtained from the bubo and from the heart's blood. The monkey which had been surrounded by tangle-foot remained healthy.

Note on the situation of buboes in the guinea-pigs which died in the go-downs.

We have now to refer briefly to the situation of the buboes in the guinea-pigs which died of plague in the course of these experiments. We have records of the detailed post-mortem examination in the case of 179 of these animals. The following table shows the position of the buboes:—

Bubo in 1 situation			Buboes in 2 situations		Buboes in 3 situations	No bubo
162 (90·5 %)			13 (7·2 %)		2 (1·1 %)	2 (1·1 %)
Neck	Groin	Axilla	Neck & groin	Neck & axilla	Axilla, neck, groin	
144	15	3	10	3	2	
88·9 %	9·3 %	1·8 %	77 %	23 %	100 %	

It will be seen that in the case of single buboes in the great majority of instances the bubo was situated in the cervical region, and that in the case of multiple buboes the neck glands were involved in every instance. If we accept the flea as the transmitting agent from guinea-pig to guinea-pig in these experiments, these facts are at first sight somewhat difficult of explanation. It has, however, been a common observation by us that the favourite situations of fleas on the body of guinea-pigs are the under surface of the neck and beneath the chin. A certain amount of support is lent to these observations by the results of flea counts which were made on the bodies of 53 live guinea-pigs during the course of the experiments just related. The method was as follows:—In most instances the animal was put into a wide-mouthed stoppered bottle containing a piece of wool soaked in chloroform. Both the animal and the fleas soon became stupefied. The animal was

then carefully removed and the fleas picked off, a note being made of the situation in which they were found. For this purpose the body was divided into the following regions: (1) head and neck; (2) fore legs and axillae; (3) hind legs, gluteal regions and groins; (4) trunk.

In a few instances no chloroform was used, the fleas being simply picked off with the fingers as they moved about. In the case of half the animals the enumeration was begun at the head and neck, and in the case of the other half at the gluteal regions. The following table shows the results which were obtained:—

No. of guinea-pigs examined	No. of fleas found on			
	Head & neck	Fore legs & axillae	Hind legs & groins	Trunk
53	96	17	19	15
	(65·3 %)	(11·5 %)	(12·9 %)	(10·2 %)

While this method of enumeration is no doubt open to fallacy, still the result shows that the commonest situation in which to find fleas is the head and neck.

Summary of Conclusions.

The following conclusions appear to us to be justified as a result of the experiments cited above:—

(1) Close contact of plague-infected animals with healthy animals, if fleas are excluded, does not give rise to an epizootic among the latter. As the go-downs were never cleaned out, close contact includes contact with faeces and urine of infected animals, and contact with, and eating of food contaminated with, faeces and urine of infected animals, as well as with pus from open plague ulcers. This conclusion is justified from Experiments I and II and especially from Experiment VI, in which close contact with infected animals was maintained for nearly a month. In Experiment I the animals were inoculated cutaneously, and in many cases had developed ulcers at the site of inoculation.

(2) Close contact of young, even when suckled by plague-infected mothers, did not give the disease to the former.

(3) If fleas are present, then the epizootic, once started, spreads from animal to animal, the rate of progress being in direct proportion to the number of fleas present. Thus, in the experiments during the months of December, January and February, the epizootic was very rapid in those go-downs, namely, Nos. 1 and 2, Experiments IV and V,

in which the flea population was abundant and was kept up by a natural supply from the roof: it was much slower in Experiment VII, go-down No. 5, in which the flea supply was kept up artificially; and, finally, it was slowest of all in go-down No. 6, Experiment VI, in which there was no definite natural supply of fleas, and from which the fleas were daily removed for a period of six days, after which removal only a comparatively small number could be caught.

(4) An epizootic of plague may start without direct contact of healthy animal and infected animal. Thus, in the case of Experiment V in go-down No. 1 the healthy guinea-pigs were not put in until the last inoculated guinea-pig had died and been removed.

(5) We have in Part II shown by direct experiment that the rat flea can convey plague from rat to rat. Further experiments (Experiments VI, VII and XI) of a similar nature with the fleas removed from infected go-downs are now recorded.

(6) Infection can take place without any contact with contaminated soil. Thus in go-downs Nos. 1 and 2 (Experiments VIII, IX), guinea-pigs placed in wire cages two inches above the ground developed plague; also the monkey in go-down No. 2 (Experiment XIII) was never in contact with the ground.

(7) Aerial infection is excluded. Thus, guinea-pigs suspended in a cage two feet above the ground did not contract the disease, while in the same go-down those animals allowed to run about and those placed two inches above the floor became infected. Further, the monkey surrounded by tangle-foot was exposed as much to aerial infection as the control animal which contracted the disease.

PART IV.

EXPERIMENTS IN PLAGUE HOUSES IN BOMBAY.

We have definitely shown above that the infection of plague can be conveyed from animal to animal by means of the rat flea. We now propose to detail some observations which go to prove, both indirectly and directly, that in a plague-infected house the infection may be due to the presence therein of rat fleas, which are capable of transmitting the disease to animals.

In choosing the houses for our present purpose we took a certain amount of care to ensure that they were really plague infected. Thus for the most part only those rooms were used in which two or more