## PART II.

#### TRANSFERENCE OF PLAGUE FROM RAT TO RAT.

# Section A. Transference from rat to rat by proximity without contact in the presence of fleas.

In the following experiments, which are a repetition of those of Gauthier and Raybaud (1902, 1903), it is shown that, in the presence of the common Indian rat flea (*Pulex cheopis*, Rothschild), plague may spread from a plague-infected rat to a healthy rat confined in close proximity, but in such a way as to prevent contact with the body or excreta of the sick rat.

Method. In the figure (Plate IV) two wire cages are seen standing in a glass box. The cages rise above the level of the top of the box, the intervening space being bridged over by a fine variety of muslin (tulle) which is impervious to fleas. Each cage stands in a tin tray which collects the urine. After the first series of experiments, both trays were filled with either dry earth or sand. This was found to be necessary in order to provide dryness and shelter for the fleas, which without some such cover rapidly died out. Each cage is provided with a lid through which the rats are introduced and food and water given to them. The lids when put on are also covered with fine muslin.

Both rats are therefore protected against invasion from outside the whole apparatus by particles larger than such as are capable of passing through fine muslin; they cannot come into contact with the bodies, faeces or urine of each other.

Into one cage (X) is placed a rat inoculated with plague<sup>1</sup>, together with 10 to 20 fleas obtained from Bombay rats<sup>2</sup>. More fleas were in some experiments added subsequently. As soon as the inoculated rat was found dead, a healthy rat was placed in the other cage (Y). The dead rat was left in the X cage till 8 to 12 hours later when it was removed and examined for septicaemia. If no plague bacilli were found in the blood microscopically, the experiment was abandoned.

<sup>1</sup> The cultures of plague employed in these experiments were agar cultures obtained from the spleen of guinea-pigs that had been used to test, by the cutaneous rubbing method, the identity of plague-like organisms found in Bombay rats dead of supposed plague.

 $^2$  Over 99 per cent. of the fleas captured off rats in Bombay have been found to be *P. cheopis.* 

If the healthy rat died, a careful post-mortem examination was made, the position of the buboes noted and smears from the bubo, spleen and heart's blood stained and examined. Further, cultures were made from the liver and heart's blood, and the resulting growths tested both culturally and on animals, a number of rats being inoculated with each strain. All rats which had not died in three weeks from the day of being first placed in the Y cages were killed with chloroform and examined, so as to make certain that they were not suffering from a subacute or chronic form of plague. In these cases also, if there was any doubt in the matter, cultures were taken and animal tests employed.

## SERIES I.

In this series wild Bombay rats were used in both X and Y cages. The experiments were made in October and November, *i.e.* before the commencement of epizootic or epidemic plague in Bombay. In all cases (eleven in number) the healthy rat in the Y cage did not contract plague. The expedient of filling the trays beneath the cages with dry earth had not at this time been devised; the tins consequently soon became wet, and the fleas rapidly died out. Experiments have shown that a considerable percentage of Bombay rats are immune to small doses of *B. pestis*<sup>1</sup>: the successful results of Series III show that the unsuitability of the cages for fleas rather than immunity of the animal employed was the probable explanation of the failure of these early experiments. To exclude the factor of varying susceptibility, however, non-immune rats were used in the next series.

# SERIES II.

In this series of 16 completed experiments, the inoculated rat was a Bombay rat, while the healthy rat was always a white rat imported from England and kept isolated in the laboratory. No case of plague had occurred in these rats.

In 11 out of 16 experiments (69 p. c.) the healthy rat contracted plague. The full details of the positive results are given below: no explanation of the five negative experiments can be suggested.

<sup>1</sup> See below, p. 506.

# Reports on Plague Investigations in India 437

A diagrammatic representation of the eleven successful experiments which needs little explanation is given in each instance. The daily course of events in the two cages is designated by a series of signs, the two cages themselves being represented by two circles (X and Y). The signs used are:  $\bigcirc$  = rat dead;  $\oplus$  = rat living;  $\infty$  = septicaemia in rat; F fleas (thus F 10, means 10 fleas introduced).

Experiment 1.

		Х	Y								
F	10	$\odot$	0							Nov.	23
		$( \cdot )$	0			•			•	,,	<b>24</b>
$\mathbf{F}$	10	0	$\odot$				•			,,	<b>25</b>
	$\infty$	0	$(\bullet)$	С	)	$\odot$				,,	<b>2</b> 6
		Ο	$\odot$							"	27
		0	$\odot$							,,	28
		Ο	0	Six	fleas	found				,,	<b>29</b>

The above experiment reads: On Nov. 23 an inoculated rat was put into cage X with ten fleas, cage Y remaining empty. On Nov. 24 this rat remained well, but was found dead on the morning of the 25th. Later in the day 10 more fleas were added and at the same time a live rat was put into cage Y. On the morning of the 26th the conditions remained the same. Later in the day the dead rat was removed from the X cage and found to have died of septicaemic plague. On the 27th the Y rat was well and remained well till the 29th when it died.

*P.-M. of Y rat.* November 29th; died 1 p.m.; 6 fleas found. General subcutaneous congestion; no buboes; pleural effusion and haemorrhagic patches throughout both lungs, the apex of the left lung being solid; spleen slightly enlarged and liver slightly congested. Smears of the spleen and heart's blood contained abundant plague-like bacilli. Cultures made from the spleen, heart's blood and liver gave pure growths of *B. pestis*<sup>1</sup>. The spleen was inoculated cutaneously by rubbing into three guinea-pigs, 1238, 1239, and 1240; all of these died of plague on Dec. 4th and 5th.

The 6 fleas were fed on a fresh guinea-pig which remained healthy; they were then dissected, and the stomach of one was found to be full of organisms microscopically like plague bacilli.

<sup>1</sup> The following growth-characteristics were taken as indicating true plague :

- 1. Appearance on agar slope, and staining reactions of same.
- 2. Appearance on salt agar,-involution forms.
- 3. Appearance in oil broth,-stalactite formation.

Journ. of Hyg. vi

Exp	perime	nt 2.										
		X	Y									
$\mathbf{F}$	10	÷	0	•					•		Nov.	23
$\mathbf{F}$	10	$\oplus$	Ō			•				•	,,	24
		Ð	0		•						,,	25
		Ð	Ο	The	first	rat no	t ha	ving	died o	on	,,	26
$\mathbf{F}$	10	$( \div )$	Ο	$^{\mathrm{th}}$	e 27t	h Nov	., it v	was r	eplace	ed	,,	27
	$\infty$	0	$\odot$	$\mathbf{th}$	at day	y by a	nothe	er ino	culate	ed	,,	<b>28</b>
		0	$\oplus$	ra	t.					•	,,	29
		0	$\oplus$		•	•	•	•		•	"	30
		0	$\oplus$	•						•	Dec.	1
		0	0	Two	fleas	found				•	,,	<b>2</b>

*P.-M.* Submaxillary bubo; congested and enlarged spleen; microscopically smears of the bubo, spleen and heart's blood contained numerous plague-like bacilli. Cultures made from the spleen, liver and heart's blood gave pure growths of *B. pestis.* The spleen was rubbed into guinea-pigs 1242 and 1243, which died on Dec. 12 and Dec. 6 respectively of typical plague.

Dissection revealed the presence of bacilli microscopically like plague in the stomach of one of the fleas.

Experiment 5.

	Х	Y									
F 10	Ð	0		•		•				Nov.	23
	$\odot$	0		•		•		•		,,	24
	$\odot$	0	$\mathbf{Th}$	e firs	t rat	not h	aving	g died	on	,,	25
	Ð	0	1	the 2	$27  \mathrm{th}$	Nov.,	on t	that	day	,,	<b>26</b>
	$\odot$	0	ŧ	anoth	er ind	oculat	ed rat	was	put	,,	27
F 10	$\odot$	Ο	j	into t	he X	cage	•		•	,,	27
	$\odot$	0	•		•		•	•	•	"	<b>28</b>
P.M. much	0	÷			•	•			•	,,	<b>29</b>
decom-	0	$( \cdot )$	•		•		•		•	"	30
posed.	Ο	$( \mathbf{f} )$					•		•	Dec	. 1
∞ ?	Ο	÷		•					•	,,	<b>2</b>
	Ο	•		•						,,	3
	Ο	$\odot$	$\mathbf{III}$				•			· ,,	4
	Ο	$(\mathbf{r})$	Ill	•	•					"	<b>5</b>
	Ο	$\odot$	Ill	•		•	•			,,	6
	Ο	$( \mathbf{+} )$	$\mathbf{I}$			•				,,	7
	Ο	0	12	fleas	found	d.		•		,,	8

438

*P.-M.* Right inguinal bubo; right axillary gland enlarged; pelvic bubo on each side; spleen congested; liver congested and mottled; microscopically smears of the liver, spleen and heart's blood were seen to contain numerous plague-like bacilli.

Cultures made from spleen, liver and heart's blood gave pure growths of *B. pestis.* Three fleas were dissected but nothing was found: nine fleas were transferred to cage 31 with a fresh white rat; this rat was alive and well on Dec. 26th when the experiment was abandoned.

Experiment 6.

	X	Y									
F	10 <b>(</b> )	Ο					-			Nov.	23
F	$10 \infty$	$( \mathbf{f} )$								"	<b>24</b>
	0	Ð						•	•	,,	25
	0	Ð			•				•	"	<b>26</b>
	0	Ð								"	<b>27</b>
	0	$\odot$				•	•	•	•	"	<b>28</b>
	0	0	РМ.	no p	lague	e; a	nother	whit	te	,,	29
	Ο	Ð	$\mathbf{rat}$	$\mathbf{put}$	in	•	•			,,	30
	0	Ð				•		•	•	Dec	. 1
	0	Ð			•		•	•	•	,,	2
	0	Ð	•			•	•	•		"	3
	0	÷	•			•	•	•		,,	4
	0	$\odot$	Ill	•	•		•	•	•	"	<b>5</b>
	0	0	Three	fleas	foun	d	•			"	6

*P.-M.* General subcutaneous congestion; right axillary and right inguinal buboes; liver congested and granular; lungs slightly congested.

Microscopically smears of the buboes contained abundant plaguelike bacilli while the smears of the spleen, liver and heart's blood were seen to have only a few. The spleen was rubbed into guinea-pig 1258, the inguinal buboes into guinea-pig 1259, and the axillary bubo into guinea-pig 1260. 1258 and 1260 died on Dec. 13th of typical plague; 1259 died on Dec. 12th of typical plague.

Cultures made from the spleen, liver and heart's blood gave pure growths of *B. pestis.* 

The stomach of one flea was found full of bacilli microscopically like plague.

28 - 2

This experiment is remarkable as showing that the "infection" passed over one rat, killing another 12 days afterwards.

Experiment 14.

		$\mathbf{X}$	Υ									
F	10	$\odot$	Ο	•				•			Nov.	24
		€	0	•		•					"	<b>25</b>
		Ð	0	•				•			,,	<b>26</b>
$\mathbf{F}$	<b>10</b>	$\odot$	0	On	Nov.	27	$\mathbf{the}$	$\mathbf{first}$	$\mathbf{rat}$	$\mathbf{not}$	,,	27
		$( \mathbf{ \cdot } )$	0	ha	ving o	died,	a fr	esh in	ocula	ated	,,	<b>28</b>
$\mathbf{F}$	10	$( \cdot )$	Ο	ra	t was	put	$\mathbf{in}$	to re	place	e it.	,,	<b>29</b>
	$\infty$	0	$   \mathbf{ \mathbf{ \bullet}} $	O	n Nov	29	the s	second	l rat	not	,,	30
		0	$( \mathbf{ \cdot } )$	ha	ving	died	, it	was	repla	aced	Dec	. 1
		Ō	$( \bullet )$	by	r a th	ird i	nocu	lated	rat	•	,,	<b>2</b>
		0	$( \cdot )$								,,	3
		Ō	$(\bullet)$								"	4
		Ο	<b>(+)</b>								"	5
		Ο	$( \mathbf{+} )$	•	•			•			**	6
		Ο	0	Five	fleas	foun	d.	•			,,	7

**P.-M.** Slight subcutaneous congestion; left inguinal and left submaxillary buboes; right axillary gland enlarged; spleen congested and enlarged; liver congested.

Only a few bacteria were found microscopically in the blood.

Cultures made from spleen, liver and heart's blood gave a pure growth of *B. pestis.* The spleen was rubbed into guinea-pigs 1262 and 1263; 1262 died on Dec. 15 of typical plague; 1263 was killed Dec. 26th when no plague germs could be recovered by culture. Subcultures were injected into 18 rats, Nos. 1414 to 1431; of these 13 died of typical plague by the fifth day, while the others died from other causes.

Four out of the five fleas caught had very large numbers of organisms microscopically indistinguishable from plague in their stomachs.

Experiment 17.

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	Х	Y									
	Ð	0		•	•		•			Nov.	28
F 10	€	0	On	Nov.	$29 \mathrm{th}$	$\mathbf{the}$	Х	rat	was	,,	<b>29</b>
	Ð	0	re	eplace	d by a	secor	ıd iı	nocul	ated	,,	30
∞	0	(+)	ra	nt.	•			•		Dec.	1
	Ο	•	٠		•		•	•		,,	<b>2</b>

Experiment 17 (cont.).

Х	Υ									
0	÷								Dec.	3
0	(+)	Ill					•		,,	4
0	+	Ill	•	•		•			,,	5
0	$( \cdot )$	Well							"	6
0	$( \mathbf{f} )$			•					,,	7
0	$( \cdot )$		•	•		•	•		,,	8
0	€	•	•		•	•	•		,,	9
0	€	•	•		•		•	•	,,	10
0	$( \cdot )$	Very	ill;	killed;	14	fleas	found		"	11

*P.-M.* No typical bubo, but submaxillary glands enlarged; lungs solid. Blood and splenic smears were full of bacilli microscopically similar to *B. pestis.* 

By an oversight no cultures were made. One flea was dissected; in its stomach were found bacilli like those of plague. The remaining fleas were transferred to a fresh rat in cage 33. This rat was seen to be ill on the 18th Dec., but on being killed was found not to be plague-infected.

Experiment 20.

		Х	Y									
F	10	Ŧ	Ο								Nov.	30
		$( \mathbf{f} )$	0			•		•		•	Dec.	1
	$\infty$	$\odot$	$( \cdot )$	•		•	•		•		,,	<b>2</b>
		Ο	÷	•			•	•	•	•	"	3
		Ο	$\odot$						•	•	,,	4
		Ο	÷	•		•	•	•		•	,,	$\mathbf{\tilde{5}}$
		Ο	•		•	•	•		•		,,	6
		Ο	$\odot$	Ver	y ill;	kille	d; tw	o flea	ıs fou	nd	,,	7

*P.-M.* Submaxillary bubo; this contained caseous material which was full of plague-like bacilli and involution forms. Splenic and blood smears contained a few bacteria like plague.

Cultures made from the spleen, liver and heart's blood gave pure growths of *B. pestis*. Subcultures were inoculated into 15 rats; 13 of these died of typical plague. The two fleas were dissected; in the stomach of one abundant plague-like bacilli were found.

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Expe	eriment 21								
-	$\mathbf{X}$	Υ							
F 20	Ð	0			•			Nov.	30
F 20	∞ ⊚	$( \mathbf{+} )$						Dec.	1
	Ο	Ŧ						17	<b>2</b>
	0	Ð						"	3
	0	÷						,,	4
	0	Ð						,,	<b>5</b>
	0	Ŧ						,,	6
	Õ	Đ						,,	7
	Õ	Ð			•			,,	8
	Õ	Ð						. ,,	9
	õ	(+)						,,	10
	ŏ	Ŧ						,,	11
	õ	õ	22	fleas	found			"	12

*P.-M.* Right submaxillary and left axillary buboes; left inguinal gland enlarged and indurated; spleen congested; liver mottled. Smears of bubo, spleen, liver and heart's blood contained abundant plague-like bacilli.

Cultures made from spleen, liver and heart's blood gave pure growths of *B. pestis.* The liver cultures were inoculated into 10 rats, eight of which died of typical plague.

Two fleas were dissected; in the stomach of both of these swarms of plague-like bacilli were found. Twenty fleas were put into cage 34 with a fresh white rat. This rat died of typical plague on Dec. 15th (vide infra).

Experiment 22.

	X	Y									
F 20	$( \cdot )$	0		•	•		•			Nov.	30
F 20	∞ ⊚	$\odot$						•		Dec.	1
	0	Ð								,,	2
	0	$\odot$			•		•			"	3
	Ó	Ð		•						,,	4
	0									"	<b>5</b>
	0	÷								,,	6
	Ō	$( \cdot )$		•			•			"	7
	0	Ð								,,	8
	Ō	Ð				•	•		•	,,	9
	Ō	Ð					•			,,	10
	ō	0	$\operatorname{Eight}$	fleas	foun	d		•		۰,	11

442

*P.-M.* Left submaxillary bubo; left inguinal gland congested and enlarged; spleen congested; liver mottled; pleural effusion; upper lobe of right lung solid. Smears of bubo, spleen and heart's blood contained numerous plague-like bacilli.

Cultures made from spleen, liver and heart's blood gave pure growths of B. pestis. Subcultures were inoculated into 10 rats; 9 of these died of typical plague.

The eight fleas were transferred to a fresh white rat, cage 32, which subsequently died of plague (vide infra).

Experiment 26.

		Х	Y							
F	<b>20</b>	$\odot$	0						Dec.	4
	×	$\odot$	$( \cdot )$						,,	5
		Ο	$( \mathbf{ + } )$				•		,,	6
		Ο	$\odot$		•		•		,,	7
		0	(  )				•	•	,,	8
		Ō	$(\bullet)$						,,	9
		0	0	One	flea	found			"	10

*P.-M.* Right inguinal bubo; secondary pelvic bubo; spleen congested; liver granular; slight congestion of the bases of both lungs. Smears of bubo, spleen, liver and heart's blood contained numerous plague-like bacilli.

Cultures made from the spleen, liver and heart's blood gave pure growths of *B. pestis.* Subcultures were inoculated into 10 rats, 9 of which died of typical plague.

Experiment 29.

	Х	Y									
F 20	$\odot$	Ο					•	•		Dec.	4
	$\odot$	Ο		•		•				,,	5
$\infty$	0	Ð					•		•	,,	6
	0	Ð		•						"	<b>7</b>
	Ο	Ð								,,	8
	Ο	$( \cdot )$						•	•	,,	9
	0	$( \cdot )$				•	•		•	,,	10
	0	$( \mathbf{+} )$			. •		-			,,	11
	0	$\odot$	•			•	•	•		,,	12
	0	€				•	•			,,	13
	0	$\odot$	•	•		•	•			,	14
	0	$( \cdot )$	•	•	•	•	•	•		,,	15
	0	0	No	fleas	$\mathbf{found}$					.,	16

*P.-M.* General subcutaneous congestion; right submaxillary bubo; spleen congested; liver congested and mottled; lungs congested; abundant pleural effusion. Numerous plague-like bacilli were found in smears of the spleen, liver, bubo and heart's blood.

Cultures made from liver, spleen and heart's blood gave pure growths of B. pestis. Subcultures were inoculated into 10 rats, 8 of which died of typical plague.

## SERIES III.

Having found in the manner described above that, under conditions suitable for fleas, positive results could be obtained when there was no danger of the receptive rat being an immune animal, **a** further series of experiments was instituted in which Bombay rats were used in both X and Y cages, and in which, therefore, some of the receptive animals were probably immune to small doses of plague. On the other hand it is clear that results obtained in this way may be more naturally transferred to the epizootics of India.

The rats which were employed throughout were supplied to us by the Health Department of the Municipality of Bombay. They were caught in the gullies and houses in the city and sent daily to the Laboratory. Before being used they were segregated for some time so as to ensure that they were not already plague-infected at the commencement of the experiment. As most of the traps were set in the houses, the rats which we received belonged as a rule to the species *Mus rattus*, the common house rat of Bombay, a few only belonging to the species *Mus decumanus*.

Seventy-six experiments of this series were begun but, either because the original inoculated rat did not develop a septicaemia or because the second rat died of some intercurrent disease before three weeks were over, twenty-six of these experiments had to be abandoned. We have, therefore, records of 50 such experiments which were observed throughout.

The following table shows the results obtained:

#### TABLE I.

Rats observed throughout			Rats in Y cages which developed plague			
M. rattus	. rattus M. decumanus Total		M. rattus	M. decumanus	Total	
35	15	50	13=37 p.c.	6 = 40 p.c.	19=38 p.c.	

In Table II are set out the details of the 19 successful experiments:

No. of rat in series	Species	No. of fleas used	death expos	and first sure to ites	Situation of bubo	Cultural tests	Animal tests	Remarks
2	$\mathbf{R}$	40	11	days	nil	positive	positive	
8	D	20	7	"	left sub- maxillary	,,	,,	
13	R	20	<b>5</b>	"	right sub- maxillary	"	,,	
16	$\mathbf{R}$	<b>20</b>	8	,,	nil	,,	,,	
18	D	20	6	,,	right & left submaxillary	,,	••	
26	D	20	<b>2</b>	"	left sub- maxillary	,,	,,	
<b>27</b>	$\mathbf{R}$	<b>20</b>	10	,,	left cervical	,,	,,	
30	$\mathbf{R}$	20	7	,,	right inguinal	,,	,,	
<b>32</b>	$\mathbf{R}$	20	<b>20</b>	,,	left inguinal	,,	,,	
34	$\mathbf{R}$	20	9	,,	left sub- maxillary	,,	,,	
36	$\mathbf{R}$	20	14	"	nil	,,	,,	
<b>47</b>	$\mathbf{R}$	20	11	,,	nil	,,	,,	
50	D	30	5	"	right sub- maxillary	,,	,,	
52	$\mathbf{R}$	30	22	,,	right inguinal abscess	,,	,,	Was killed on 22nd day &
61	D	30	6	,,	left axillary	,,	,,	found plague
68	$\mathbf{R}$	30	4	,,	nil	,,	,,	infected
69	$\mathbf{R}$	30	5	,,	right sub- maxillary	,,	,,	
72	D	30	8	,,	right inguinal	••	,,	
76	$\mathbf{R}$	30	10	,,	nil	,,	,,	

#### TABLE II.

Interval hotw

Assuming that 40 per cent. of Bombay rats are immune to the cutaneous inoculation of small doses of *B. pestis*<sup>1</sup>, the percentage of positive results with susceptible animals is increased to 61 p.c., which is not materially different from that (69 p.c.) found in the series (II) where non-immune rats were placed in the Y cages.

Summary of Section A (Series I, II, III).

Conditions not un to life of fle	favourable eas	Immunity of rats which were exposed to contagion	No. and p.c. of rats exposed which contracted plague		
Series I	no	partly immune	0 = 0 p.c.		
Series II	yes	not immune	11=69 p.c.		
Series III	yes	partly immune	19=38 p.c.		

On 30 occasions therefore has a healthy rat contracted plague in sequence to living in the neighbourhood of a plague-infected rat

<sup>1</sup> See papers IV and V, below, pp. 496 and 502.

under circumstances which prevented the healthy rat coming in contact with either the body or excreta of the plague-infected rat. In all cases a fairly abundant supply of fleas was present; these could pass freely between the two rats, and, except for "aerial contagia," formed the only apparent means of communication between the animals. The presumption is that plague was transferred from the sick to the healthy rat by the agency of the fleas.

To exclude aerial infection, attempts were made to conduct a similar series of experiments in the absence of fleas. This was, however, found to be impracticable as we could not with the greatest care ensure that the animals were entirely free from fleas. Experiments were, therefore, instituted in which the fleas themselves were taken from a sick rat and placed on a healthy animal.

# Section B. Transference of plague from rat to rat by transference of fleas.

Bombay rats were inoculated with a virulent culture of *B. pestis*, placed separately in flea-proof cages and supplied with rat fleas. In the event of any of these rats dying of plague and if their blood contained any bacilli on microscopical examination, the fleas were caught and transferred to a fresh flea-proof cage in which was placed a healthy rat. The flea supply for each healthy rat was always obtained from two or more septicaemic rats. As in the first series of experiments a careful post-mortem examination was made of all uninoculated rats which died. The position of the buboes was noted; smears from the bubo, spleen and heart's blood were stained and examined; and cultures were made from the liver and heart's blood. The resulting growths were tested both as regards their cultural and animal reactions.

All rats which had not died after three weeks were killed with chloroform and examined.

#### SERIES IV.

In this series fleas collected from the rats dead of plague were transferred to healthy English white rats. In 8 out of 13 (61 p.c.) completed experiments, the rats which received the fleas died of plague, while in five instances they remained healthy. Details of the eight positive experiments follow.

Experiment 32. Dec. 11th, white rat received eight fleas from Experiment 22 (Series II, p. 442 above). Dec. 14th, rat died.

*P.-M.* Subcutaneous congestion; left axillary bubo; right submaxillary and right axillary glands enlarged and indurated; spleen congested; lungs slightly congested.

Microscopically a few plague-like bacilli were found in the bubo smear. Smears made from the spleen, liver and heart's blood contained numerous plague-like bacilli. Cultures made from heart's blood, spleen and liver gave pure growths of *B. pestis*.

Subcultures from the spleen were inoculated into five rats; all of these died of typical plague. Three fleas caught on the rat after death were dissected. In the stomachs of all of these a great number of plague-like bacilli were found.

*Experiment* 34. Dec. 12th, white rat received 20 fleas from Experiment 21 above (p. 442). Dec. 15th, rat died.

*P.-M.* Marked subcutaneous congestion; submaxillary bubo; left inguinal bubo; spleen congested; liver congested and mottled; lungs congested and haemorrhagic; some pleural effusion.

Abundant plague-like bacilli were found in smears made from the buboes and a few in the smears of spleen, liver and heart's blood. Cultures made from heart's blood, spleen and liver gave pure growths of *B. pestis.* 

Subcultures from the heart's blood were inoculated into five rats, all of which died of typical plague.

Experiment 41. Dec. 16th, white rat received 20 fleas from three septicaemic Bombay rats. Dec. 23rd, rat died.

P.-M. Slight subcutaneous congestion; left submaxillary gland enlarged and hard; spleen congested; liver mottled. Smears from the bubo and spleen showed a few plague-like bacilli; none were found in the smears of heart's blood. Agar slopes were inoculated from the spleen and heart's blood and *B. pestis* isolated in pure culture. Subcultures were inoculated into 15 rats, 13 of which died of typical plague.

*Experiment* 44. Dec. 21st, white rat received 24 fleas from septicaemic Bombay rats. Dec. 28th, found dead.

**P.-M.** Some emaciation: slight subcutaneous congestion; right and left submaxillary buboes; spleen congested; liver mottled; some pleural effusion. Smears from the bubo, spleen and heart's blood contained numerous plague-like bacilli. Cultures made from the spleen and heart's blood were so badly contaminated that no further tests could be applied. *Experiment* 45. Dec. 21st, white rat received 20 fleas from septicaemic Bombay rats. Dec. 31st, rat found dead.

P.-M. With the exception of a slightly granular liver there were no signs of plague.

Microscopically smears of spleen and heart's blood showed no bacilli. Cultures were made from the liver, spleen and heart's blood. The tubes inoculated from the liver and heart's blood remained sterile; a few colonies were obtained on the tubes inseminated from the spleen. These on testing gave the typical cultural reactions of B. pestis. Subcultures were inoculated into five rats, four of which died of typical plague.

*Experiment* 46. Dec. 23rd, white rat received 11 fleas from septicaemic Bombay rats. Dec. 29th, found dead.

P.-M. Slight subcutaneous congestion; no buboes; spleen congested; haemorrhages in the liver. A few plague-like bacilli were found microscopically in smears made from the spleen and heart's blood. Cultures were made from the heart's blood and spleen: *B. pestis* was isolated from these in pure culture.

Subcultures were inoculated into eight rats; two of these died of typical plague, the others dying within twenty-four hours from other causes.

*Experiment* 47. Dec. 27th, white rat received 16 fleas from septicaemic Bombay rats. Jan. 4th, found dead.

*P.-M.* Subcutaneous congestion; right submaxillary bubo; spleen congested; liver congested and faintly granular. Microscopically abundant plague-like bacilli were found in smears from the bubo and spleen, a few only in those from heart's blood.

Cultures made from heart's blood and spleen gave pure growths of *B. pestis.* Subcultures were inoculated into 11 rats, 10 of which died of typical plague.

*Experiment* 48. Dec. 28th, white rat received seven fleas from septicaemic Bombay rats. Jan. 7th, found dead.

P.-M. Subcutaneous congestion; right axillary and right inguinal buboes, secondary right pelvic bubo; spleen congested and faintly granular. Microscopically abundant plague-like bacilli were found in smears from the buboes and spleen, a few only in those from heart's blood. Pure cultures of *B. pestis* were isolated from spleen and heart's blood; subcultures were inoculated into eight rats, all of which died of typical plague.

#### SERIES V.

In these experiments fleas were transferred from Bombay rats dead of septicaemic plague to healthy Bombay rats (M. rattus and M. decumanus). Twenty-five experiments were brought to a conclusion: the results are summarized in Table III, and the details of the thirteen positive experiments given in Table IV.

### TABLE III.

Rats observed throughout			Rats which contracted plague			
M. rattus	M. decumanus	Total	M. rattus	M. decumanus	Total	
19	6	<b>25</b>	9=47 p.c.	4=66 p.c.	13=52 p.c.	

If an allowance is made here as in Series III (p. 445) for 40 per cent. of the Bombay rats being immune to plague, the percentage of positive results is raised to 87 p.c.

No. of rat in series	Species	No. of fleas used	Interv death expos bi	al betw. and first sure to ites	Situation of buboes	Cultural tests	Animal tests	Remarks
1	D	<b>24</b>	8 d	lays	nil	positive	positiv	e
4	D	<b>26</b>	4	,,	right submaxillary	,,	,,	
5	$\mathbf{R}$	29	5	,,	nil	,,	,,	
6	$\mathbf{R}$	<b>24</b>	7	,,	double inguinal & right axillary	,,	,,	
11	$\mathbf{R}$	37	9	,,	right axillary	,,	,,	
16	D	39	6	,,	right submaxillary	,,	,,	
17	D	40	<b>22</b>	,,	right pelvic	all con- taminated	a "	Was killed on 22nd day &
18	$\mathbf{R}$	47	4	,,	right submaxil- lary & r. inguinal	positive	,,	found plague infected
19	$\mathbf{R}$	38	15	,,	nil	,,	· ,,	
20	$\mathbf{R}$	<b>49</b>	4	,,	right submaxillary	,,	,,	
22	R	32	5	,,	right inguinal	all con- taminated	a "	
23	$\mathbf{R}$	44	9	,,	double submaxil- lary & r. axillary	positive	,,	
24	R	28	6	,,	nil	,,	,,	

## TABLE IV.

## Summary of Section B.

In twenty-one experiments out of thirty-eight (55 p.c.) healthy rats living in flea-proof cages have contracted plague in sequence to receiving fleas collected from rats dead or dying of septicaemic plague in another cage. The possibility of the rat flea (*P. cheopis*) carrying plague from one rat to another is therefore demonstrated directly.