V. ON THE EFFECT UPON VIRULENCE OF PASSAGE OF *B. PESTIS* THROUGH RATS BY CUTANEOUS INOCULATION WITHOUT INTERMEDIATE CULTURE.

In the preceding paper we have put forward the result of some experiments in which *B. pestis* was passed through a series of 26 rats by means of the subcutaneous method and without the intervention of any culture on artificial media. From this series of experiments it appeared that the virulence of the organism was not affected by passage through the ordinary rats of Bombay.

In continuation of these observations we have now to record further experiments in which *B. pestis* was passed through a series of 26 rats by means of the cutaneous method, again without the intervention of any culture on artificial media. The following was the method adopted:

The rats used for all the passages were the ordinary Bombay rats, either *Mus decumanus* or *Mus rattus*: no distinction was made between these two species. The bacillus in the first instance was obtained from the spleen of a rat which had been found dead of plague in the streets of Bombay. The post-mortem appearances of this rat were typical of plague, while the spleen was crowded with plague bacilli. For the first and every subsequent passage the spleen was used. The organ was placed in a sterile watch-glass and cut up into small pieces and the pulp pressed out with a knife. A small patch on the upper part of the abdomen of a rat was shaved dry and lightly scarified with a sharp knife. The splenic juice was then firmly rubbed into the scarifications with the back of the knife or the blunt blades of a pair of forceps. A post-mortem examination was made of every rat which died and the appearances noted. Further, a microscopical examination was made of smears prepared from the buboes, if present, from the spleen and from the heart's blood. Only those spleens which showed plague bacilli microscopically in large quantity were used for the next passage.

In the first few passages only a few rats, numbering from 6 to 16, were used in each instance. It was soon found, however, as we shall

see, that a considerable percentage of the Bombay rats were immune to this method of infection and that consequently, in order to avoid any possible interruption in the series, it was necessary to inoculate at each passage from 20 to 40 or 50 rats. The same spleen was not used for every rat of any one passage; in some instances the spleens of three or four rats were employed.

Table I gives in detail the results of these passages.

TABLE I.

Passage	No. of rats inoculated	No. which died of intercurrent disease	No. of rats which died of plague. Day of death							No. of rats alive after 14—21 days
			2nd	3rd	4th	5th	6th	7th	8th—14th	
I.	6	3		3						0
II.	10	2		1	2					5
III.	14	1	2	1	3	1		1		5
IV.	16	2		1	5	1	2			5
V.	22	6		1	1		1			13
VI.	20	4		3	4	1	1			7
VII.	30	7	1	8	3	1	1			9
VIII.	30	14	2	4	1					9
IX.	30	8		2	2	2	1			15
X.	50	8		8	3	2		1	1	27
XI.	30	8		1	3	1	1	1	1	14
XII.	30	5		3	2	1	1	1		17
XIII.	30	3		2	2	1	1	2		19
XIV.	30	2		8	1	3	1		1	14
XV.	45	9	2	6	4	1	2		1	20
XVI.	41	5		1	2	2	1			30
XVII.	20	4	3	3	2		1			7
XVIII.	40	2		1	3		2		1	31
XIX.	20	0		5	2	1				12
XX.	30	2		9	4	2	2	1		10
XXI.	35	2		2	3	1		1	1	25
XXII.	20	1	1	5	4	3	2			4
XXIII.	20	4	2	1		1	1			11
XXIV.	25	3		1	2	2	1		1	15
XXV.	20	4		3	3					10
XXVI.	35	10		6	10	1		1		7
Total	699	119	13	89	71	28	22	9	7	341
					Total	239				

On consideration of this table it is at once apparent that a large percentage of Bombay rats are immune to plague inoculated by this method. Thus, deducting 119 rats, viz. those which died of some intercurrent disease, we have 580 rats which came under observation during

Table II.

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
Total no. of rats inoculated	No. which died of intercurrent disease	No. which were observed throughout	Total no. which died of plague	Percentage which died of plague	No. which died of plague on the 2nd—4th day	Percentage which died of plague on the 2nd—4th day	Total no. which were alive after 14 days	Percentage immune
699	119	580	239	41·2	173	72·4	341	58·8

The percentages in columns V and IX are calculated on the total number of rats which were observed throughout, namely 580, and in column VII on the total plague mortality, namely 239.

Table III.

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
Passages	Total no. of rats inoculated	No. which died of intercurrent disease	No. which were observed throughout	Total no. which died of plague	Percentage which died of plague	No. which died of plague on 2nd—4th day	Percentage which died of plague on 2nd—4th day	Total no. which were alive after 14 days	Percentage immune
I—VII.	118	25	93	49	52·7	39	79·6	44	47·3
XXII—XXVI.	120	22	98	51	52·0	38	74·5	47	48

The percentages in columns V and IX are calculated on the total number of rats which were observed throughout, namely column III; and in column VII on the total plague mortality, namely column IV.

the whole period. Of this number 239 died of plague, while 341 were alive and well after 14 days, that is to say, that 59 °/₀ were immune to the cutaneous method of inoculation. Table II contains a summary of these figures, as well as showing the number, and percentage on the total number, which died of plague of those rats which died on the 2nd to the 4th day after inoculation.

We have next to consider whether the virulence of the organism was affected in any way by these passages.

Table I shows that it was as easy to kill rats at the end of the passages as at the beginning. But, in order to compare a considerable number of rats at the beginning of the passages with a similar number at the end, we have constructed Table III. We have taken approximately the first 100 and the last 100 rats, which were observed throughout, and have calculated in each instance, first, the percentage which died of plague; secondly, the percentage which died of plague on the 2nd to the 4th day; and thirdly, the percentage which were immune to this method of injection. A glance at this table will show how closely the figures correspond, allowing us to draw the conclusion that neither a diminution nor an exaltation of virulence had taken place during the passages.

From the epidemiological standpoint these observations are of considerable importance. They show that the Bombay rat is a difficult animal to infect with plague by this method, and that the plague bacillus retains its virulence in passing from rat to rat.