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

The following experiment was undertaken with a view to testing the statement previously made by Hankin, Yersin and Walton, that "passage" of the plague bacillus through a series of rats without intervening culture on artificial media is a matter of great difficulty. This result is of epidemiological importance, for it has been taken to indicate that *B. pestis* rapidly loses virulence in the bodies of rats, and that for the maintenance of the epizootic some intermediate medium or host is a necessity. Hankin has suggested that the bacillus may regain virulence by growth in the soil or the body of an insect.

Hankin (1898) described how he failed to carry on a virulent strain of *B. pestis* by direct passage through more than three rats in succession; these were presumably rats caught in Bombay. He also mentions that he was informed by Yersin that the latter had encountered the same difficulty. Both Yersin and Hankin found no difficulty however in the case of mice. Walton (1899) performed similar experiments on Bombay rats and obtained precisely the same results; he never succeeded in killing more than three rats in series. He attributed his failure to a diminution of the virulence of the bacilli by passage, though it is of interest to note in this connection, as a possible explanation of his results, his observation as to the frequent immunity of the Bombay rat.

Otto (1902, 1904) performed an extensive series of experiments to test this point and also the statement of Yersin, Calmette (1895) and Borrel, that by continued passage through one species of animal the virulence was diminished for other species. Otto found no particular difficulty in passing the infection directly from rat to rat, nor did he discover any falling off in virulence either for rats or other laboratory animals. He found, however, that in the case of white rats a virulent culture after numerous passages caused death acutely although the

bacilli tended to become localised in the glands corresponding to the site of injection and that death often occurred before a septicaemia supervened. This localisation of the bacilli is due, in his opinion, to an increase of their toxicity as the result of the progressive passages.

The data of the experiment performed by us are set forth in tabular form below. With regard to the method adopted throughout, a brief description will suffice.

The original culture used was a virulent one, isolated 11 days previously from the blood of an hospital patient. 0·5 c.c. of a broth culture was inoculated subcutaneously into a rat which died in three days. The spleen of this rat was excised, emulsified in sterile broth, and a portion of the turbid fluid, obtained after the larger particles had sedimented, was at once injected subcutaneously into each of three rats. This procedure was repeated in successive series, and as a rule, when a selection was possible on account of two or more of the rats dying about the same time, the spleen of the animal showing bacilli most plentifully on microscopical examination was chosen as the inoculation material for the next passage. On certain occasions, namely, in the 9th, 11th, 14th, 18th, 20th, 21st and 22nd passages, six rats instead of three were inoculated with the spleen emulsion of one of the rats of the preceding series. This naturally increased the chances of recovering the bacilli in sufficient numbers to ensure the continuation of the passages.

It was found possible in this way to carry on the series as far as the 26th passage, involving 109 separate inoculations without having recourse on any occasion to cultures isolated from the rats.

In the 2nd passage only one rat was inoculated. In 18 of the series three rats were used, and as already pointed out, in seven instances six rats were inoculated with the same material. It may be mentioned that the rats were selected for inoculation without reference to species; they were all wild Bombay rats.

Although no exact data are available for determining any diminution or increase of virulence in the bacillus after its final passage, the following observations may be recorded as bearing upon the point. The 26 passages occupied in all 89 days, giving an average duration for each of 3·4 days. If those rats only be considered which furnished the material for inoculation of the series next in order, it will be found that the average duration of life of the first 13 is 3·1 days while that of the next 13 is 2·7 days, a difference which is not sufficiently striking to suggest a marked variation in virulence of the bacillus. A similar

calculation has been made in the case of 21 rats abstracted from the first 13 passages, in which plague bacilli were demonstrated after death, and similarly for 18 rats included in the last 13 passages. The average duration of life of the first series is exactly three days, and that of the second series as nearly as possible three days. Taking all these facts in conjunction it may be stated that no evidence is forthcoming either of an increase or of a diminution of virulence of the bacillus during the entire experiment.

Another point of interest may be adverted to, namely, that a certain amount of evidence for the wide variation in susceptibility of the rats used may be drawn from the condition of the spleen and heart-blood of the animals forming the direct series, having regard to the bacterial content of their organs after death. It will be seen by reference to the table that in one or two instances, although numerous bacilli were present in the spleen used for inoculation, yet the spleen of the next rat showed very few bacilli on microscopical examination. On the other hand the reverse occasionally happened, *i.e.* the inoculation of spleen material containing comparatively few bacilli caused the death of the rat of the next series with numerous bacilli in that organ. Fairly numerous *B. pestis* were noted to be present in the heart-blood of the rats of the 15th, 17th and 26th passages.

Additional proof of the varying resistance of the rats is met with in the circumstance that in certain cases, *e.g.* Rats 13, 45 and 46, death occurred a long time after the inoculation, extending even to two months or longer, and that in these rats no signs of plague were detected after death.

It is worthy of remark that in several of the rats, notably in Nos. 9, 32, 42 and 44, a granular condition of the liver was observed strongly suggestive of the focal necroses commonly seen in guinea-pigs dead of plague. Death in these cases supervened in less than one week after the inoculation, and in the case of rat 44, in as short a time as four days. The condition resembles that found in naturally infected rats, in so far as it is generally associated with the presence of a comparatively small number of *B. pestis* in the organs.

Direct passage through Rats subcutaneously.

Serial No.	No. of passage	Test book No.	Date of inoculation	Test	Death in days	Remarks
1	I	14 a	22. vi. 05	Spleen emuls. Rat 9	2	P.-M. nothing marked; *B. pestis* in spleen
2	I	13 a	,,	,, ,,	3	
3	I	15 a	,,	,, ,,	36 hours	P.-M. nothing marked; *B. pestis* in spleen
4	II	18 b	24. vi. 05	,, Rat 14 a	5	P.-M. spleen much enlarged; *B. pestis* abundant in spleen and heart-blood
5	III	21 c	29. vi. 05	,, Rat 18 b	36 hours	P.-M. considerable number of *B. pestis* in spleen
6	III	22 c	,,	,, ,,	2	P.-M. not many *B. pestis* in spleen
7	III	23 c	,,	,, ,,	3	P.-M. spleen not much enlarged; numerous *B. pestis* in spleen
8	IV	29 d	30. vi. 05	,, Rat 21 c	4	P.-M. spleen full of *B. pestis*
9	IV	30 d	,,	,, ,,	5	P.-M. nodules in liver and spleen
10	IV	31 d	,,	,, ,,	4	
11	V	36 e	4. vii. 05	,, Rat 29 d	2	No marked pathological changes to naked eye; spleen smear showed numerous *B. pestis*
12	V	35 e	,,	,, ,,	2	P.-M. no naked-eye changes; spleen contained numerous *B. pestis*
13	V	37 e	,,	,, ,,	23	P.-M. no sign of plague macro- or microscopically
14	VI	43 f	7. vii. 05	,, Rat 36 e	3	P.-M. spleen enlarged; lungs normal; very few *B. pestis* in spleen or liver
15	VI	41 f	,,	,, Rat 35 e		
16	VI	42 f	,,	,, Rat 36 e	3	
17	VII	47 g	10. vii. 05	,, Rat 43 f	2	P.-M. numerous *B. pestis* in spleen—a few in heart-blood
18	VII	48 g	,,	,, ,,	4	
19	VII	49 g	,,	,, ,,	4	
20	VIII	54 h	13. vii. 05	,, Rat 47 g	2	
21	VIII	55 h	,,	,, ,,	36 hours	
22	VIII	56 h	,,	,, ,,	2	
23	IX	65 i	16. vii. 05	,, Rat 54 h	3	Numerous *B. pestis* in spleen
24	IX	63 i	,,	,, ,,		
25	IX	64 i	,,	,, ,,	3	Very few *B. pestis* in spleen
26	X	71 k	20. vii. 05	,, Rat 65 i	4	,, ,, ,,
27	X	72 k	,,	,, ,,	2	Many *B. pestis* seen in spleen smear
28	X	73 k	,,	,, ,,	2	,, ,, ,,
29	XI	81 l	25. vii. 05	,, Rat 71 k	4	No *B. pestis* seen in spleen smear
30	XI	80 l	,,	,, ,,		
31	XI	82 l	,,	,, ,,	36 hours	One or two suspicious germs in spleen smear
32	XII	86 m	30. vii. 05	,, Rat 81 l	5	Not many *B. pestis* in organs; liver granular
33	XII	87 m	,,	,, ,,	2	No *B. pestis* seen in spleen smear
34	XII	88 m	,,	,, ,,	5	A few bipolar organisms, with many putrefactive bacilli in spleen smear
35	XIII	109 n	4. viii. 05	Liver emuls. Rat 86 m	3	Swarms of *B. pestis* in spleen
36	XIII	107 n	,,	,, ,,	4	Numerous *B. pestis* in spleen
37	XIII	108 n	,,	,, ,,		
38	XIV	111 o	7. viii. 05	Spleen and liver emuls. Rat 109 n	2	,, ,, ,,
39	XIV	112 o	,,	,, ,,		
40	XIV	113 o	,,	,, ,,	2	No *B. pestis* seen in spleen smear
41	XV	117 p	9. viii. 05	Spleen emuls. Rat 110 o	2	Numerous *B. pestis* in spleen; fair number of *B. pestis* in heart-blood

Serial No.	No. of passage	Test book No.	Date of inoculation	Test	Death in days	Remarks
42	XV	118 p	9. viii. 05	Spleen emuls. Rat 110 o	6	P.-M. spleen finely granular; liver fatty; a few minute haemorrhages in kidney; *B. pestis* seen in bubo but not in heart-blood nor spleen
43	XV	119 p	,,	,, ,,		
44	**XVI**	**124 q**	12. viii. 05	,, Rat 117 p	4	P.-M. inguinal bubo; spleen finely granular with many *B. pestis* but many other organisms; no *B. pestis* in heart-blood
45	XVI	123 q	,,	,, ,,	Died 16. x. 05	P.-M. not plague
46	XVI	125 q	,,	,, ,,	Died 25. x. 05	,, ,,
47	**XVII**	**126 r**	16. viii. 05	,, Rat 124 q	2	Numerous *B. pestis* in spleen: fair septicaemia
48	XVII	127 r	,,	,, ,,	36 hours	Fairly numerous *B. pestis* in spleen; very few if any *B. pestis* in heart-blood, but fairly numerous trypanosomes
49	XVII	128 r	,,	,, ,,	2	No *B. pestis* in spleen
50	**XVIII**	**133 s**	19. viii. 05	,, Rat 126 r	36 hours	Fairly numerous *B. pestis* in spleen and in heart-blood
51	XVIII	132 s	,,	,, ,,	2	
52	XVIII	134 s	,,	,, ,,	4	Swarms of *B. pestis* in spleen; fairly numerous *B. pestis* in heart-blood
53	**XIX**	**136 t**	21. viii. 05	,, Rat 133 s	6	P.-M. plague
54	XIX	135 t	,,	,, ,,	2	A few *B. pestis* in spleen
55	XIX	137 t	,,	,, ,,		
56	**XX**	**145 u**	27. viii. 05	,, Rat 136 t	2	Numerous *B. pestis* in spleen
57	XX	144 u	,,	,, ,,	2	Considerable number of *B. pestis* in spleen; few in heart-blood
58	XX	146 u	,,	,, ,,	3	
59	**XXI**	**149 v**	29. viii. 05	,, Rat 145 u	2	A few *B. pestis*-like organisms in spleen; none in heart-blood
60	XXI	147 v	,,	,, ,,	2	
61	XXI	148 v	,,	,, ,,	4	Fairly numerous *B. pestis* in spleen; a few in heart-blood
62	**XXII**	**156 w**	1. ix. 05	,, Rat 149 v	3	Spleen crowded with *B. pestis*
63	XXII	157 w	,,	,, ,,		
64	XXII	158 w	,,	,, ,,		
65	**XXIII**	**163 x**	5. ix. 05	,, Rat 156 w	2	Fairly numerous *B. pestis* in spleen smear; no *B. pestis* in heart-blood
66	XXIII	162 x	,,	,, ,,	2	No *B. pestis* in spleen smear and not in heart-blood
67	XXIII	164 x	,,	,, ,,		
68	**XXIV**	**169 y**	7. ix. 05	Spleen and liver emuls. Rat 163 x	5	Very few *B. pestis* in spleen smear
69	XXIV	168 y	,,	,, ,,	Died 25. ix. 05	
70	XXIV	170 y	,,	,, ,,		
71	**XXV**	**172 z**	13. ix. 05	Spleen emuls. Rat 169 y	2	A few *B. pestis* in spleen smear
72	XXV	171 z	,,	,, ,,	2	No *B. pestis* in spleen smear
73	XXV	173 z	,,	,, ,,		
74	**XXVI**	**174 A**	16. ix. 05	,, Rat 172 z	2	Fairly numerous *B. pestis* in spleen smear
75	XXVI	175 A	,,	,, ,,		Fairly numerous *B. pestis* in heart-blood
76	XXVI	176 A	,,	,, ,,	2	No *B. pestis* in spleen smear, not in heart-blood
77	XXVII	177 B	19. ix. 05	,, Rat 174 A		
78	XXVII	178 B	,,	,, ,,	Died 6. x. 05	No *B. pestis* in spleen or heart-blood but had open bubo in right groin
79	XXVII	179 B	,,	,, ,,	3	No *B. pestis* seen in spleen smear or heart-blood

Note. The rats indicated in heavy type are those from which the passages were continued.

Summary and Conclusions.

1. Twenty-six passages from rat to rat have been effected without recourse to artificial media. The time taken was 89 days.

2. Our results furnish no evidence that the virulence of the bacillus used for the passages undergoes any alteration.

3. The experiment gives evidence of the varying susceptibility to plague of the Bombay rat.

REFERENCES.

OTTO, R. (1902), *Zeitschr. f. Hygiene*, vol. XLI. p. 384.
KOLLE, HETSCH and OTTO (1904), *Zeitschr. f. Hygiene*, vol. XLVIII. pp. 431, 432.
HANKIN, E. H. (1898), *Annales de l'Inst. Past.* vol. XII. p. 746.
—— (1899), Minutes of evidence, Indian Plague Commission, vol. II. p. 22.
WALTON, H. J. (1900), Minutes of evidence, Indian Plague Commission, vol. III. p. 337.
YERSIN, CALMETTE and BORREL (1895), *Annales de l'Inst. Past.* vol. IX. p. 589.