XXX. THE MECHANISM BY MEANS OF WHICH THE FLEA CLEARS ITSELF OF PLAGUE BACILLI.

FLEAS that have fed on septicaemic blood are capable of conveying infection to fresh animals on which they feed. This capability is associated with the presence of plague bacilli in the stomach, intestine and faeces.

If such infected fleas be kept in captivity, it is found that after a time they are no longer capable of conveying infection; at the same time on dissection no bacilli are found in them.

The question arises, what is the nature of the mechanism by means of which infected fleas rid themselves of bacilli?

We shall show that:

1. A clearing process exists.
2. The clearing process is more active at 90° F. than at lower temperatures.
3. That it is probably of a phagocytic nature.

1. *A clearing process exists.*

The existence of a clearing process was suggested by the fact that after feeding on the same septicaemic blood, the number of bacilli in the stomachs of a batch of fleas is very variable. A few hours after feeding all degrees of infection are found to exist, varying from crowded masses to such small infections, that it is only by continued search that any bacilli are recognisable. Further, in about 50 % of the fleas examined, no bacilli are discoverable by the microscope. Slight variations might be expected to occur but without the aid of some process of clearing it is difficult to account for the often observed fact of some fleas being free from bacilli and others being crowded to excess.

If a number of fleas be fed upon a septicaemic rat and subsequently kept under observation being meanwhile nourished upon healthy animals, the proportion found to be infected steadily diminishes day

https://doi.org/10.1017/S0022172400003302 Published online by Cambridge University Press

by day (see Table I). At the same time a considerable mortality occurs so that the decrease in infected fleas might be due to those harbouring plague bacilli dying more rapidly than those uninfected.

TABLE I. *Showing the percentage of fleas found infected on successive days after an infecting meal.*

Day	Percentage infected	Day	Percentage infected
2	38	6	15
3	37	7	8
4	53	8	16
5	21		

To ascertain whether such was the case, experiments were made with infected and healthy fleas and the mortality compared. The results are given in Table II from which it is evident that there is no greater mortality amongst infected fleas than usually occurs amongst healthy fleas under the same conditions, viz. about 10 % per day.

TABLE II. *Showing the death-rate of fleas.*

On each day the percentage alive is shown.

Days old	1	2	3	4	5	6	7	8	9	10	11	12
Infected	100	78	60	50	34	30	26	16				
Healthy at 75° F.	100	75	65	58	48	38	27	21				
Healthy at 90° F.	100	72	56	42	44	39	34	28	21	19	11	10

It has been already pointed out that the plague bacilli in infected fleas are always confined to the alimentary tract, and from the experiments just mentioned it appears that the existence of numerous plague organisms in the stomach and gut of fleas does not materially affect the health of the insects.

2. *The clearing process is more active at* 90° F. *than at lower temperatures.*

In Table II above it is shown that bacilli can be recognised in the stomachs of infected fleas up to at least the eighth day after the infecting meal. The recognition was a microscopical one. Using the same means, but maintaining the fleas at a temperature of 90° F., no bacilli were recognisable after the second day.

Thus:

Day	Percentage infected
2	8
3	0
4	0
5	0

It will be noted here that the proportion found infected on the second day is 8 as compared with 38 at the lower temperature.

When we employ the more delicate test of inoculation (of faeces) into animals for recognising the presence of plague bacilli the same fact becomes evident, but the time is longer.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
90° F.	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–
75—80° F.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Day	16	17	18	19	20	21	22	23	24	25	26	27	28	29
90° F.	–	–	–	•	•	•	•	•	•	•	•	•	•	•
75—80° F.	+	+	–	–	+	+	–	–	–	–	–	–	–	–

In this series of experiments the fleas were infected at the lower temperature, and in the one case maintained at that temperature during the subsequent course of the experiments and in the other removed to the higher temperature immediately after infection. In another series, the infection was carried out at the higher temperature and the fleas subsequently maintained at that temperature:—

Day	1	2	3	4	5	6	7	8	9	10
	+	+	+	–	–	–	–	–	•	•

It is evident that not only is infection less likely to take place at the higher temperature, but that if it does occur, the period during which it lasts is still further curtailed.

A process by means of which the flea rids itself of bacilli, therefore, exists—What is its nature? *A priori* a purely physical clearing process must exist, *i.e.* as the flea continues to feed hour by hour and day by day, and as the bacilli continue to multiply, the number of bacilli present at any time will be dependent on the relative activities of these two processes. If the multiplication can keep pace with the washing out of the intestine by successive meals of fresh blood, then the bacilli will be found in considerable number, and *vice versa*. But in addition to this method another method exists as is evident from the results of experiments detailed above.

3. *This process is probably a phagocytic one.*

The bacilli multiply in the blood of the rat which is taken into the stomach but they are at the same time subjected to all the influences which blood is capable of exerting on bacteria. These influences will be most active immediately after a meal and gradually lessen as the

blood becomes altered by the digestive processes. First in importance amongst such influences we place phagocytosis.

If a well infected flea be fed on fresh blood and after the lapse of some fifteen minutes be killed and a film of the stomach contents stained with Leishman's stain the polynuclear cells are seen to be engorged with plague bacilli.

The effect of increasing the temperature at which the fleas were kept upon the rate at which the insects cleared themselves of plague bacilli is seen on reference to the experiments detailed on p. 262 above. These experiments show that a rise of temperature of about 8 degrees centigrade (75° F. to 90° F.) doubles the rate at which the bacilli disappear, *i.e.* 2·3 times per 10°. Phagocytosis has long been known to be increased by a rise of temperature up to 40° C., and recent observations on the temperature coefficient of phagocytosis by Ledingham have shown that for a rise of 10° C. the velocity is increased about 2·2 times. Ledingham has further shown that temperature operates principally upon the opsonization and only slightly if at all upon the actual process of uptake of bacilli (*Proc. Roy. Soc.* read Feb. 27, 1908).

The process of clearing is also influenced by other conditions which are known to affect phagocytosis.

The presence of bacilli in the stomach of the flea, and the length of time they persist therein, is largely influenced according as the bacilli are subjected to the action of fresh blood immediately after the infecting meal or not; so that the extent of infection is dependent on whether it feeds on healthy blood immediately after its infecting meal, or whether it is starved for a while.

The first series of experiments consisted in infecting fleas, and in one case allowing them to feed on healthy animals immediately after infection and in the other immediately after infection starving the fleas for six hours. In each case the faeces of the fleas were injected into guinea-pigs each day to ascertain the presence or absence of bacilli. The result of this series of experiments is shown in Table III, which shows that twice as many starved fleas had bacilli in their faeces as the fed fleas on the same day.

In the second series of experiments infected fleas were fed for the same time, in one case on animals immunised against plague by large doses of living bacilli and in the other case on animals susceptible to the disease. After feeding for 24 hours on these animals the fleas were transferred to guinea-pigs, which are very susceptible to plague. This experiment was repeated eleven times and from the table (IV) it will

TABLE III. *Table showing the presence (+) or absence (−) of plague bacilli in the faeces of infected fleas on various days after infection for two classes of fleas.*

1. Fleas starved for 6 hours after infection. Total 31 deaths.

	Days						
Exp.	1	2	3	4	5	6	7
1	+	+	+	+	+		
2	−	+	+	−	+	+	+
3	+	−	−	+	+	−	−
4	+	+	+	+	+	+	+
5	−	+	+	−	−	−	−
6	+	+	+	−	−	−	−
7	+	+	+	+	+	−	
8	−	−	+	−	−	−	−
9	−	−	−	−	−	−	−

2. Fleas fed immediately after infection. Total 15 deaths.

1	−	−	−	−			
2	−	−	−	−	−	−	−
3	+	−	+	−	−	−	−
4	+	−	−	−	−	−	+
5	+	+	+	−	−	−	−
6	−	+	+	+	−	−	+
7	−	−	−	−	−	+	+
8	+	+	−	−	−	−	−
9	−	−	−	−	−	−	−

TABLE IV. *Table showing effect of feeding infected fleas, in one case on susceptible animals and in the other on immune animals for* 24 *hours, on the power of the flea to transmit the disease.*

Experiment A.	Fleas fed on susceptible animals	Experiment B.	Fleas fed on immune animals
1	+	1	+
2	+	2	−
3	−	3	−
4	+	4	+
5	+	5	−
6	+	6	−
7	−	7	−
8	+	8	+
9	+	9	+
10	+	10	−
11	−	11	−

+ means that the fleas subsequently transmitted plague to a guinea-pig.

− means that the fleas failed to subsequently transmit plague to a guinea-pig.

In all cases the fleas were left on the guinea-pigs and the guinea-pigs observed for a month.

https://doi.org/10.1017/S0022172400003302 Published online by Cambridge University Press

be seen that the fleas which had been fed on immunised animals were just half as capable of infecting the guinea-pigs as were the fleas that had fed on the non-immunised susceptible animals.

It appears therefore that a flea starved after the ingestion of septicaemic blood offers the greatest chances of conveying infection, that these chances are diminished if the insect has in the meantime taken a meal of normal blood, and that a meal of the blood of an immunised animal still further diminishes the infectivity of these insects. A probable explanation is afforded by the discovery that the plague bacilli remaining in the stomach are readily ingested by the leucocytes taken in with the second meal and such phagocytosis might be expected to be more pronounced in the case of immune blood.

Should this hypothesis on further investigation prove correct then it follows that the presence of immune rats in epizootic times will tend to limit the advance of the disease; and it will do this in two ways—not only will immune rats not be attacked by plague, but by serving as a source of immune blood to fleas already infected will diminish their power of transmitting the disease to susceptible animals—rats or man. Whether such a process operates in bringing an epidemic to an end we have no means of judging, but from other evidence we believe that the proportion of immune rats does rise at the end of an epizootic, as indeed it might have been supposed to do.