LXVII. OBSERVATIONS ON THE MECHANISM OF THE TRANSMISSION OF PLAGUE BY FLEAS.

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(With Plates XXIV—XXVI and 4 Text-figures.)

The literature on the transmission of plague by fleas was reviewed in 1905 in the first series of Reports on Plague Investigation in India (*Journal of Hygiene*, Vol. VI. p. 422), and it is only necessary to epitomise briefly the main facts here. The conclusion, that this insect plays an important *rôle* in the spread of plague, was arrived at on epidemiological grounds by Ogata (1897), Simond (1898), Ashburton Thompson (1903), and Liston (1905). Simond also made a few experiments, which strongly indicated that infection from rat to rat could be brought about by the agency of fleas. Gauthier and Raybaud (1902 and 1903), and Verjbitzki (1904) by more extensive, more varied and better controlled experiments, confirmed Simond's observations, and proved clearly the possibility of transmission by this agency.

The question of flea transmission and its epidemiological importance was extensively studied by the Commission for the Investigation of Plague in India (1906 and 1907). They found that, once control of the experimental conditions had been obtained, transmission from one animal to another could readily be brought about by fleas, and further made the very important observation that only in the presence of fleas did an epizootic amongst rats or guinea-pigs ensue. Close contact with infected animals, including the devouring of infected carcases, was occasionally followed by a case of plague, but no spread occurred.

A number of experiments, in which animals were allowed to remain in animal houses in which epizootics had occurred and in plague-infected native quarters, resulted in infection, but if the simplest measures were taken to prevent the access of fleas to animals, they remained unaffected—showing that the infection lurking in such situations was resident in the flea population.

Simond was of opinion that infection was caused by the rat rubbing flea-faeces containing plague bacilli into recent flea-bites.

Verjbitzki (1904) demonstrated that the puncture in the skin occasioned by the bite of bugs and fleas affords a channel through which plague bacilli can enter, for the application of crushed infected bugs and fleas and their faeces, as well as other plague material to the situation recently bitten, was found to infect an animal; more than one puncture was requisite to obtain infection.

The Commission for the Investigation of Plague in India (1907), 2nd Plague Report, discusses the following possible methods by which the flea may transmit plague:

1. By the animals eating the infected fleas.
2. By the proboscis of the flea mechanically conveying the bacilli from the infected to the healthy animal.
3. By the salivary glands of the flea becoming infected, the bacilli being then inoculated along with the saliva.
4. By a regurgitation of the stomach contents through the oesophagus and pharynx, the bacilli being then injected with the saliva, or on the pricker, or being rubbed into the wounds made by the pricker.
5. By a retention of infected blood in the pharynx or about the mouth-parts of the flea, the bacilli multiplying there and then being inoculated into the animal in the same manner as in hypothesis No. 4.
6. By the bacilli contained in the faeces being deposited on the skin, and then being either injected by the pricker or rubbed into wounds made by the pricker.

Methods 1 to 3 are set aside on what seem to us satisfactory grounds, No evidence could be found for 4 or 5. It was, however, shown that infection could be brought about by smearing recent flea-bites with septicaemic blood or a virulent culture of plague, and the conclusion is arrived at that the possibility of infection by the rat rubbing flea-faeces into recent flea-bites is demonstrated, but on the question whether this is the usual method of infection, the Commission did not feel justified in expressing an opinion.

The Commission dissected many hundreds of fleas and searched for the presence of plague bacilli in their salivary glands and body cavities, but on no occasion, either by microscopic examination or by culture, were they able to find plague bacilli outside the alimentary canal of the insects. We may say at once that our own observations on this point coincide with those of the Commission, and it appears certain that transmission is not occasioned through infection of the salivary glands of the insect transmitter, as in the case of malaria and sleeping sickness.

The probability of an infection taking place by the inoculation of infected flea-faeces into flea-bites has been questioned by Cranston Walker (1911). This observer experimented upon himself and others—using tuberculin, vaccine lymph and a virulent culture of *Staphylococcus albus*. Having found that the application of each of these materials, either before or after the skin was punctured with a fine needle, ·37 mm. in diameter, gave upwards of 90 % successful inoculations, he repeated the experiment with the puncture made in each case by one flea. The diameter of the proboscis of this insect is about ·02 mm. Only with the tuberculin did the flea puncture succeed in producing an inoculation, and then only in a small minority of cases. Walker's observations show that, although one hole ·02 mm. in diameter does not usually admit of sufficient tuberculin, vaccine lymph or staphylococci to occasion infection, a hole 20 times as large does. To produce infection the number of plague bacilli necessary to be inoculated is, however, probably much less than in the case of staphylococci. As shown by Barber (1912) *one* may suffice. Moreover, under natural conditions, whether in rat or man, scratching is not uncommon after flea-bites, whereas this was avoided in Walker's experiments. We do not think these observations very seriously militate against the view that infection of plague may be brought about by rubbing flea-faeces containing plague bacilli into recent flea-bites, with the probable assistance of scratching.

We have repeated the experiments of the Commission on this point, applying to the bitten area (*a*) the surface of the spleen of a rat recently dead from plague; (*b*) a strong emulsion of plague bacilli from the stomachs of fleas which had been nourished on animals with septicaemia. The stomachs used were full of plague culture (see p. 435 below).

The experiments were performed as follows:

A number of rats were carefully shaved over a part of the abdomen a few square cms. in area. Three days later 20 normal fleas were given the opportunity to feed upon the rats. The fleas were in a test-tube, the mouth of which was covered with gauze, and the mouth of the tube was applied to the shaved area for about one minute. The majority appeared to feed, but as many of the insects retire to the lip of the test-tube for the purpose, they could not all be seen. This manœuvre on their part results in a ring of punctures round the circumference of the test-tube. Immediately afterwards, in Series A, the cut surface of a spleen was gently applied to the same area and, in Series B, the

emulsion was dabbed over it with a pledget of cotton-wool. The rats were immediately returned to separate cages.

A third control series, Series C, contained 13 rats, which were treated in the same manner as those of A, with the omission of the flea-punctures.

Most of the rats promptly licked off the spleen-pulp or emulsion which had been applied.

Series A consisted of ten rats, of which nine died of plague in under 3 days.

Series B consisted of 23 rats, of which five died of plague.

Series C consisted of 13 rats, none of these contracted the disease.

Four out of the nine rats of Series A which contracted the disease displayed phlectenules—in two cases arranged in a ring corresponding to the situation of the punctures. All showed buboes either in the axillae or groin.

The experiments show a striking difference in the incidence of infection. This might be accounted for either by variation in the number of bacilli deposited or in their virulence. We incline to the latter interpretation, as we endeavoured to arrange that the amount of bacilli applied should be of the same order. We have not actually determined the relative virulence of the two kinds of organisms, as this could only be done by experiments on a long series of animals. One significant difference in the properties of the two strains of organisms was, however, observed. When mixed with a drop of our own blood, and incubated for 15 minutes between a slide and cover glass, as in the original method devised by Leishman for the quantitative estimation of phagocytosis, the bacilli direct from the spleen were not taken up by the phagocytes, whereas those from the flea's stomach were freely ingested.

The experiments were performed as follows: to obtain bacilli from the spleen a piece of the organ was minced up with ·85 % NaCl solution and centrifuged at a low speed until the tissue cells and blood corpuscles were deposited. After centrifugation the plague bacilli were partly in suspension and partly in a loose deposit on the surface of the cells. This loose deposit was dispersed into the medium by gently shaking, without disturbing the cells, etc., and pipetted off. The emulsion was centrifuged at a high speed, the supernatant fluid, which now consisted of bacilli with an occasional cell, removed, and the deposit emulsified in fresh saline. The emulsion consisted only of single bacilli. The number of bacilli per c.c. were determined by counting in a Thoma Zeiss cell,

using dark-ground illumination. The emulsions were diluted so as to contain approximately 1000 million bacilli per c.c.

To obtain an emulsion of the bacilli from the flea's stomach the contents of 30–40 strongly infected stomachs (see p. 435 below) were rubbed up with saline by means of a short stumpy brush made of cotton wool, centrifuged at high speed, and the supernatant fluid removed. The deposit was re-emulsified in the same manner and centrifuged at low speed to get rid of the aggregates. The emulsion was not good and still contained many clumps of bacilli, but from the nature of the material this could not be overcome. The bacillary content of the emulsion was counted in the way mentioned above. The emulsion was subsequently diluted so as to afford about 100 million per c.c. A stronger emulsion could not be made, as the amount of material was inadequate.

One volume of each of these emulsions was mixed with one volume of the blood of one or other of us, and a suitably sized drop placed upon a glass slide, covered and incubated for 15 minutes at 37° C. in a saturated atmosphere. The coverslip was slid off the slide, and as soon as dry both films were fixed in alcohol and stained with Leishman's stain.

The experiment with both varieties of bacillus was made on three occasions; on each occasion the appearance presented on examination of the two sets of films was strikingly different. Whereas the majority of the polymorphonuclear leucocytes had taken up bacilli derived from the fleas' stomachs, the average number per leucocyte varying from three to five in the different experiments, we found none of the bacilli taken directly from the spleen inside the cells.

These observations in conjunction with the lesser infectivity of the bacilli from the fleas' stomachs lead us to conclude that by growth in the stomach of the insect, a race of diminished virulence had bred out, which had lost the resistance to phagocytosis possessed by the original blood-strain. In an adjoining paper in this *Journal* by St John Brooks (1913) the author shows that this property of blood-strains of plague is rapidly lost by a few generations on broth agar. The blood in the stomach of the flea is soon attacked by the digestive ferments, so that it is likely that the characteristics of the medium are soon lost and that the bacilli are really nourished on the hydration products of protein. The digestion is an alkaline one.

Observations showing that infection may occur during the act of sucking.

There is no doubt plague may be inoculated by the dejecta from infected fleas. In the case of a flea-infested rat, the hair, especially in those areas where the animal cannot dislodge them, such as the back of the neck and root of the tail, is often sprinkled with the dried excrement from the parasites, and the animal is frequently to be seen scratching itself. Nevertheless, we have long felt dubious as to whether this was the only, or even the principal method by which infection is conveyed. In comparison with the masses in the fleas' stomachs, the faeces do not as a rule contain many bacilli, and soon dry up. We have also given the reasons which lead us to believe that bacilli which have grown in the stomach of the insect are not of a high degree of virulence. Infection by this means must leave much to chance.

We therefore set out to ascertain whether or not the flea could infect during the act of sucking. The Commission for Investigation of Plague in India frequently conveyed plague to guinea-pigs by feeding infected fleas through fine muslin gauze. The same was done by Swellengrebel (1913) in Java. This suggests that infection at the time of feeding may not be an unusual occurrence, but the Indian observers were not satisfied that by this means inoculation by faeces was excluded, for the insects defaecated on the muslin at the time of the experiment.

METHODS OF EXPERIMENT.

In our experiments, we fed under supervision on rats infected fleas which had been deprived of food for from 24 to 28 hours. Each flea was watched with a hand-lens during the act of sucking and removed to a test-tube directly it had completed its meal. Such starved fleas rarely pass faeces at the time of feeding, and, in the very few instances when this occurred, the dejectum was removed by the corner of a piece of blotting-paper and strong lysol immediately applied to the spot. Under the conditions of our experiments inoculation of possibly infected faeces was excluded. The fleas belonged to the species *Ceratophyllus fasciatus* and *Xenopsylla cheopis*.

Our first care was to obtain a supply of the fleas well infected with plague. White rats, although quite susceptible to plague, usually die before a high degree of septicaemia has developed. As, to obtain a satisfactory supply of infected fleas, numerous microbes in the blood of

the host are necessary, our first attempts were not very successful. Subsequently, this difficulty was overcome by feeding our fleas on mice.

Mice are not so susceptible to plague as rats, but the degree of septicaemia intervening before the death of the animal is often extraordinary. In one case we counted 2·7 bacilli for every corpuscle (Plate XXIV, fig. 1) and the number of organisms frequently equalled that of the blood corpuscles. Neither rat-flea feeds upon mice with the same readiness as upon rats, but as no other item in the *menu* was provided, they fed well enough for the majority of our insects to become infected.

The fleas were kept in a glass cage with several inches of sawdust, and containing two separate compartments screened off by coarse meshed wire gauze for the mice. The cages were similar to those employed by the Indian Commission for their transmission experiments (1906), and are shown in Plate XXIV, fig. 2. The cages were supported above a shallow tray containing lysol, which extended six inches beyond their margin. At first, the mice were inoculated with plague, and replaced by fresh inoculated mice every two days as they died. As time went on it was not found necessary to supply inoculated mice, as normal mice became infected from the fleas with regularity. The fleas bred in the cages and the system became automatic—the fleas infecting the mice, and these in turn infecting other fleas.

To collect a supply of fleas for an experiment some of the sawdust in a cage was decanted into a wide bowl, commonly known as a "chef-bowl," 17 inches in diameter, with smooth white surface and nearly vertical sides of 9 inches. As the fleas emerge from the sawdust on to the white surface they are readily seen and swept into test-tubes by a paint brush. A small loose fragment of cotton-wool was added for the insect to perch in.

The rats were carefully shaved three days previous to use, so as to avoid any minute abrasions. In order to immobilise them at the time of feeding we employed the method used by Chick and Martin (1911). The rats were gently bandaged with soft gauze bandage, with all four legs in the extended position, but leaving the abdomen exposed. When this is done, the animal seems quite comfortable and remains quiet when laid on its back; although it could quite easily disengage itself, it does not seem to know how to begin. A white rat so secured will lie supine for an hour or more. Each animal was placed upon a pad of cotton-wool, about 18 inches square. Any flea which jumped landed in the cotton-wool and became entangled.

To feed the fleas, the test-tube was inverted over the shaved area,

and, as soon as the insect had settled down to feed, the test-tube was removed and it was watched with a hand-lens. If the fleas are allowed to reach the skin of the rat through a minute and loose fragment of cotton-wool, or to burrow in cotton-wool before being placed on the shaved area, they will feed more readily. The wool possibly suggests fur to them and they feel more at home. Before each flea had filled its stomach it was removed by the leg with a fine pair of forceps and transferred to its test-tube, but in Series III, IV and V fleas were usually removed by entangling in cotton-wool to avoid any injury to their limbs. In the experiments of Series I and II they were subsequently dissected, and a film made of the contents of their stomachs to ascertain whether they were infected with plague bacilli.

Our cages were stocked with a supply of fleas in January of this year, and evidence of the fleas being infected and capable of transmitting plague to the uninoculated mice was forthcoming nine days later.

Series I.

Our first experiments were made with fleas of the species *Ceratophyllus fasciatus*, before the population was strongly infected. A number of fleas, varying from 7 to 27, were separately fed for one minute upon a rat. 25 % of the fleas were afterwards found to contain plague bacilli in their stomachs.

None of the animals contracted plague. The protocols of the experiment are given in Table I below:

Table I. *No. of rats in the series*, 10.

Min. No. of fleas fed on 1 rat ...	7
Max. ,, ,, ,, ...	27
Mean ,, ,, ,, ...	16
Min. No. infected fleas fed on 1 rat ...	2
Max. ,, ,, ,, ...	7
Mean ,, ,, ,, ...	4

Mortality from Plague, 0.

Series II.

The second series of experiments was made a week later when the insects were more heavily infected. *Ceratophyllus fasciatus* was the species of flea used, and the experiments were conducted in a similar way to those of the previous series. The proportion of infected fleas

was, however, much greater, 60% of those used being subsequently found to contain plague bacilli. The degree of infection of the individual insects was also higher. The details are summarised in

TABLE II. *No. of rats in the series*, 10.

Min. No. of fleas fed on 1 rat	15
Max. ,, ,, ,,	26
Mean ,, ,, ,,	20
Min. No. infected fleas fed on 1 rat	5
Max. ,, ,, ,,	25
Mean ,, ,, ,,	12·5
Mortality from Plague, 2.	

These experiments show that infection may be conveyed during the act of feeding, but that it by no means occurs every time a flea with plague bacilli in its stomach feeds on a susceptible animal.

SERIES III.

The third series of experiments was made with what we designate "fleas certified as plague infected." A number of fleas were segregated and the faeces deposited examined daily for plague bacilli, and only those passing bacilli were used for the experiment. Twenty "certified" fleas were given the opportunity to feed on each rat on two successive days. Most of the insects were of the species *Ceratophyllus fasciatus*, but some *Xenopsylla cheopis* were included.

Thirteen experiments were made; nine of the rats died of plague.

The proportion of infections is thus seen to rise with the number of opportunities (here 40) for infected fleas to feed upon the animal.

In the course of our experiments we made the observation that, whereas certain of our fleas sucked energetically and persistently, no blood entered their stomachs, but the oesophagus became unusually distinct. Usually, during feeding, the latter can only just be seen with a hand-lens as a fine red streak in the younger and more transparent fleas. The insects showing abnormality in this respect were, on removal to their tube, specially marked. On dissecting them a curious condition was discovered. Their proventriculi were blocked with what proved to be a solid culture of plague, and the oesophagi were more or less distended with fresh-clotted blood. (See Text-figs. 3 and 4, page 436, and Plates XXV and XXVI.)

It occurred to us that fleas whose proventriculi were obstructed with plague-culture were likely to be responsible for the conveyance of infection, so we next turned our attention to those insects which presented this interesting pathological condition. At the same time we made a study of the condition and how it is brought about, but this will best be dealt with at a later stage of our paper.

Experiments with fleas suffering from obstruction in the proventriculus.

Two methods of diagnosing the existence of obstruction were open to us. (1) By allowing a number of fleas to feed under supervision upon a rat, selecting those which could not satisfy their thirst and therefore when disturbed during the act of sucking would immediately start again at a fresh situation. (2) By examining under the microscope the fleas lying on their sides in a drop of water. Under these circumstances the obstruction could be actually seen owing to the dark brown colour of the alkaline haematin adsorbed due to the growth of plague bacilli. (See Figs. 2, 3 and 4, page 436.)

Having selected "obstructed" fleas from our supply of infected insects (they were already identified by a number) one or two were fed upon each of a series of rats, on one, or two, or sometimes three days in succession. Series IV gives the details of the experiments with fleas of the species *Xenopsylla cheopis*, and Series V those with the species *Ceratophyllus fasciatus*. As may be seen from the protocols below, every rat became infected with the former and one in six with the latter.

Although we have gained the impression throughout our experiments that infection is more easily produced by *Xenopsylla cheopis*, these figures alone are too small to warrant this conclusion. Under the conditions of our experiments obstruction of the proventriculus by plague culture certainly occurred more readily in the case of fleas of the species *Xenopsylla cheopis* than with those of *Ceratophyllus fasciatus*. The former is also a more persistent feeder if starved. While individuals of the species *Xenopsylla cheopis* would frequently renew their attempts six, eight or more times if disturbed, those of the latter species usually became restless and tried to get away after three or four attempts. In one or two cases, however, individual fleas of the species *Ceratophyllus fasciatus* were almost as persistent as those of *Xenopsylla cheopis*.

Series IV.

Experiments with specimens of Xenopsylla cheopis *having a blocked proventriculus.*

The figures 69 etc. in the tables below refer to the identification number of the flea. Where more than one number occurs in the same column it signifies that two fleas were fed upon the animal.

No. of rat	Days on which fleas fed and rats died						
	1	2	3	4	5	6	7
(1)	69	—	68 and 70	—	Died of pest	—	—
(2)	67	67	103 and 93	—	—	—	Died of pest
(3)	67	67	103 and 93	—	—	—	Died of pest
(4)	103 and 93	—	—	—	—	Died of pest	—

Series V.

Experiments with specimens of Ceratophyllus fasciatus *having a blocked proventriculus.*

No. of rat	Days on which fleas fed and rats died							
	1	2	3	4	5	6	7	
(5)	64	—	—	—	—	—	—	lived
(6)	64	—	—	Died of pest	—	—	—	—
(7)	64	—	—	—	—	—	—	lived
(8)	94	—	94	—	—	—	—	lived
(9)	94	—	94	—	—	—	—	lived
(10)	94	—	94	—	—	—	—	lived

We finally varied the experimental conditions and allowed one or two "obstructed" fleas to attempt to feed once upon a succession of rats on the same day, the rats being thereafter segregated for observation.

Series VI. Two specimens of *Xenopsylla cheopis*, Nos. 95 and 103, were given two minutes each upon the shaved abdomen of eight rats in succession. Three contracted plague.

Series VII. On the same day as Series VI *Xenopsylla cheopis*, No. 95, was given the same opportunities on a further four rats. None contracted plague.

Series VIII. Two of three *Xenopsylla cheopis*, Nos. 105, 109 and 120, were allowed to feed upon nine rats in succession. Three died of plague.

Series IX. Two of three *Ceratophyllus fasciatus*, Nos. 99, 111 and 113, were given the opportunity to feed upon nine rats in succession. Three contracted plague.

The above experiments show that, given a flea in this pathological condition, the probability that it will convey infection is high. In considering the risk it must be borne in mind that in experiments VI to IX the fleas only had the chance of making one puncture and a time limit was imposed. Loose in the fur of an animal the insect would make a number of punctures in its efforts to satisfy its thirst.

The development of the plague bacillus in the alimentary canal of the flea and the pathological condition thereby brought about.

The large number of fleas which we dissected in order to ascertain the presence of infection, furnished us with considerable material to study this subject, and the interesting pathological condition which came to light stimulated us to prosecute this enquiry with diligence.

The mouth-parts and alimentary canal of the insect have been described in detail, and figured in a paper by the Commission which appeared in this *Journal* in 1906. For the appreciation of the curious pathological condition we have discovered a short description of the organs of alimentation will suffice. The diagram, Text-fig. 1 below, shows the general arrangement of the digestive system of the insect.

The mouth is situated at the attachment of the appendages, the apposition of which forms the piercing organ and sucking tube. From the mouth the pharynx passes upwards to the pump, the muscles actuating which are attached to the exo-skeleton along the curvature of the head. By the coordinated contraction of these muscles from before backwards, and the elastic recoil of the chitinous walls of the pump, the blood is sucked up the tube formed by the piercing organs and propelled backwards along the narrow oesophagus through the proventriculus into the stomach. The proventriculus is provided internally with a series of hair-like cells, broad at the base, fine at the free extremity and covered with chitin. These are arranged radially in seven rows, one above the other (see Plate XXVI, figs. 1 and 2), and curve posteriorly, their points touching and projecting into the stomach. The encircling bands of muscle contract the proventriculus until the tooth-like cells meet, and these circles of long-curved epithelial cells form an efficient valve between the proventriculus and stomach. Normally this valve is competent, and the energetic peristaltic contractions of the stomach, which take place during digestion, do not drive any blood back into the oesophagus. External to these tooth-like cells is a basement membrane, and outside this a series of bands of large striated muscle cells arranged circularly.

When watching the act of sucking, the proventriculus appears as a pulsating red globe, but it is not easy to determine whether the muscles of this organ actively participate or whether the appearance is merely due to the intermittent expansion of the organ as the blood is propelled into it by the pharyngeal pump.

The stomach is nearly as long as the abdomen, and its capacity depends upon its state of distension. It is lined internally with a single layer of epithelial cells of irregular shape and full of granules. In the distended stomach they are much flattened out. Outside this are two layers of muscles, the internal circularly and the external longitudinally arranged. Where the stomach joins the intestine four long malpighian tubes arise. Posterior to the stomach is a thin walled intestine of about the same length as the former, terminating in a much wider rectum with its six rectal glands.

Naturally we have not been able to follow the development of the bacilli in the alimentary canal of any one flea fed on septicaemic blood, and the following description is built upon the numerous observations we have made of insects in different stages of the infection. On examining the contents of the stomach of a flea a day or two after it has fed upon infected blood, clusters of minute brown specks darker in colour and firmer in consistency than the rest of the contents are visible with a magnification of 16 diameters (see the Text-fig. 1, p. 436). These, on examination with an immersion lens after staining, are seen to consist of plague bacilli (see Plate XXIV, figs. 3 and 4).

Later the stomach and proventriculus show definite jelly-like masses of a brown colour. These masses are possessed of considerable cohesion, and are with difficulty teased out so as to make a film suitable for microscopical examination. Plate XXIV, fig. 4, is a typical representation of the edge of such a mass, which is seen to be a piece of solid bacterial culture. The growth of such a culture-mass increases and, owing to its brown colour from adsorbed haematin, is very obvious on dissecting the stomach, and may often be quite readily seen when the entire flea is cleared and examined by transmitted light under a magnification of 20 diameters. The Text-figs. 1 to 4 p. 436, have been drawn from such preparations and represent stages in the development of the condition. The plague-culture grows in the proventriculus as well as in the stomach, and in Fig. 3 it is shown filling the whole of the stomach and proventriculus. Owing to its gelatinous consistency it not infrequently leads to incompetence and even complete blocking of the proventricular valve, as shown in figs. 1—6 Plate XXV, and fig. 2 Plate XXVI.

What precisely happens is seen on referring to Plate XXVI, figs. 1 and 2, which represent camera lucida drawings under a higher magnification of transverse and longitudinal sections of proventriculi in this pathological condition. Fig. 1 is from an early stage of infection. Between the tooth-like epithelial cells numerous bacilli may be seen. Later their

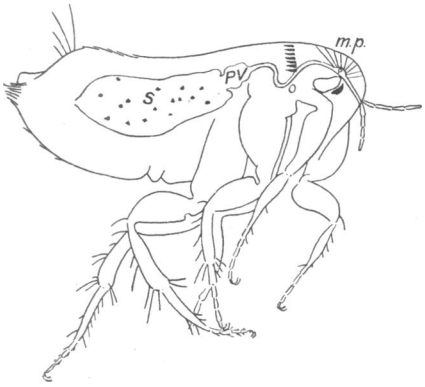


Fig. 1.

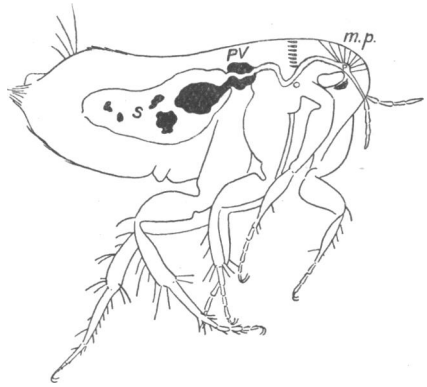


Fig. 2.

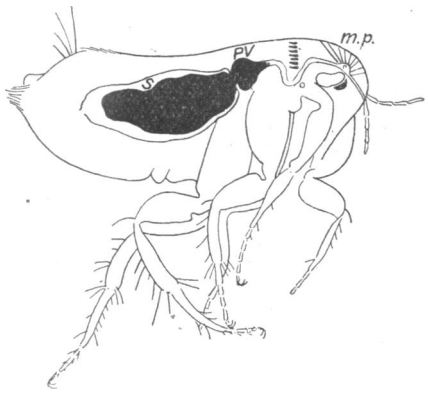


Fig. 3.

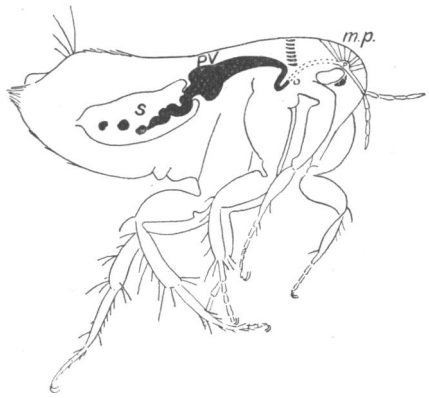


Fig. 4.

multiplication occurs to such an extent that these cells are widely separated and the proventriculus distended with culture. At the same time its lumen is obliterated, as is well seen in fig. 2 which represents a longitudinal section of an advanced condition. In this case the plug of culture extends into the oesophagus, and is capped by a clot of fresh blood which the insect had taken in just prior to the time the preparation was made.

Although, with the proventriculus obstructed in this manner, fresh blood cannot find its way into the stomach, this does not prevent the insect sucking, as the pump which aspirates blood up the sucking-tube and propels it into the stomach is situated in the pharynx. On the contrary, the flea suffers from thirst and is persistent in its efforts to satisfy this appetite, but only succeeds in distending the oesophagus. The blood in the distended oesophagus may flow out again on cessation of the sucking act, and we have seen drops of blood escape from the mouth-parts of "blocked" fleas when the insect withdrew its proboscis. Generally, however, sufficient time has elapsed for clotting to occur, and some blood remains in the oesophagus. Figs. 1 to 6 Plate XXV exhibit in diagrammatic form types of the appearances presented on dissecting out the alimentary canal. The plague-culture is sepia-brown in colour and easily distinguishable. The fresh blood which has recently been taken in is red.

The significance of these observations is obvious in view of our experiments, showing that fleas which are in this abnormal condition are particularly liable to transmit plague. In such fleas the oesophagus is infected with plague and fresh blood introduced becomes contaminated. Given the opportunity, the insects suck blood again and again, and if the pharyngeal pump ceases for a moment, some of the blood will by the elastic recoil of the oesophageal wall be driven back into the wound and carry with it plague bacilli.

The obstruction to the alimentary canal does not necessarily occasion the death of the insect, and, if kept at a cool temperature and in a moist atmosphere, the insects live for many days in this condition. We kept our specially selected fleas in a cool room at 10° C. In course of time the culture of plague obstructing the proventriculus undergoes autolysis and the passage is re-established. The obstructive process may, however, recur.

We have made experiments in which infected fleas were kept at different temperatures to learn whether they ever became free from bacilli. These fleas lived as long as 50 days at from 10° C. to 15° C. and 23 days at 27° C., and died infected.

Our results may be briefly summarised as follows:

Under conditions precluding the possibility of infection by dejecta it was found that two species of rat-fleas, *Xenopsylla cheopis* and

Ceratophyllus fasciatus, fed upon septicaemic blood, can transmit plague during the act of sucking, and that certain individuals suffering from a temporary obstruction at the entrance to the stomach were responsible for most of the infections obtained, and probably for all.

In a proportion of infected fleas the development of the bacilli was found to take place to such an extent as to occlude the alimentary canal at the entrance to the stomach. The culture of pest appears to start in the intercellular recesses of the proventriculus, and grows so abundantly as to choke this organ and extend into the oesophagus. Fleas in this condition are not prevented from sucking blood as the pump is in the pharynx, but they only succeed in distending an already contaminated oesophagus, and, on the cessation of the pumping act, some of the blood is forced back into the wound. Such fleas are persistent in their endeavours to feed, and this renders them particularly dangerous. Fleas suffering from obstruction do not necessarily perish, and in course of some days the culture obliterating the lumen of the proventriculus may autolyse and the passage again become pervious. They are, however, incapable for the time being of imbibing fresh fluid, and are, therefore, in danger of drying up if the temperature is high and the degree of saturation of the atmosphere low. Although, as far as our observations go, they withstand desiccation quite as well as normal fleas which are not fed, their length of life must be short directly hot, dry weather sets in, and we are led to wonder whether this fact may not, to some extent, explain why in India epidemic plague is confined to the cooler and moister seasons, and particularly why in Northern and Central India the epidemics are abruptly terminated on the onset of the hot dry weather.

In conclusion we desire to express our indebtedness to our assistant, Mr D. J. Russell, who helped us in many of the experiments, and to Miss M. Rhodes, who made the drawings and diagrams which illustrate this paper.

BIBLIOGRAPHY.

BARBER, M. A. (1912). The infection of guinea-pigs, etc., with doses of plague bacilli ranging from one upwards. *Philippine Journal of Science*, Vol. 7, p. 251.

CHICK and MARTIN (1911). The fleas common on rats in different parts of the world and the readiness with which they bite man. *Journal of Hygiene*, Vol. XI, No. 1.

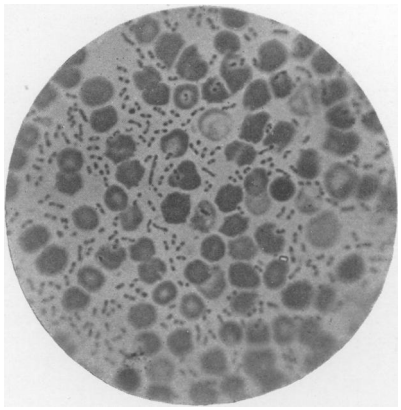
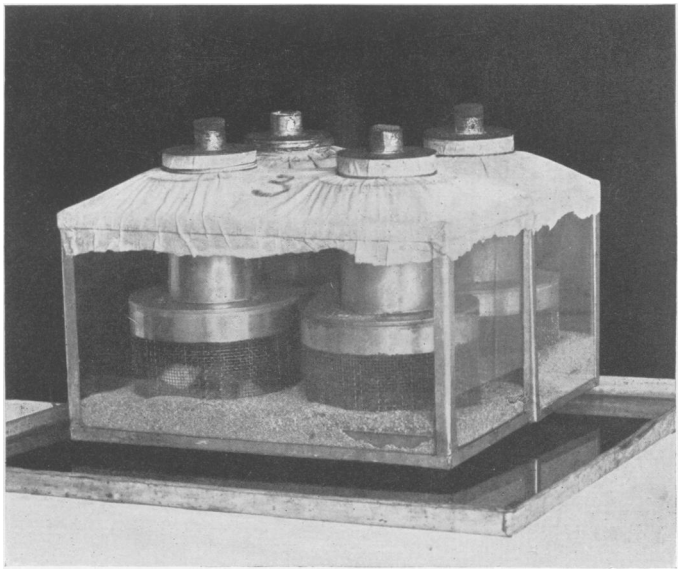


Fig. 1.



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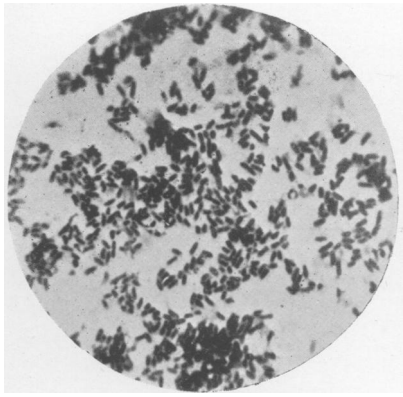
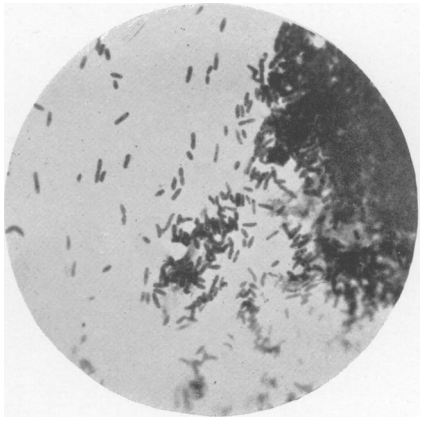


Fig. 3.



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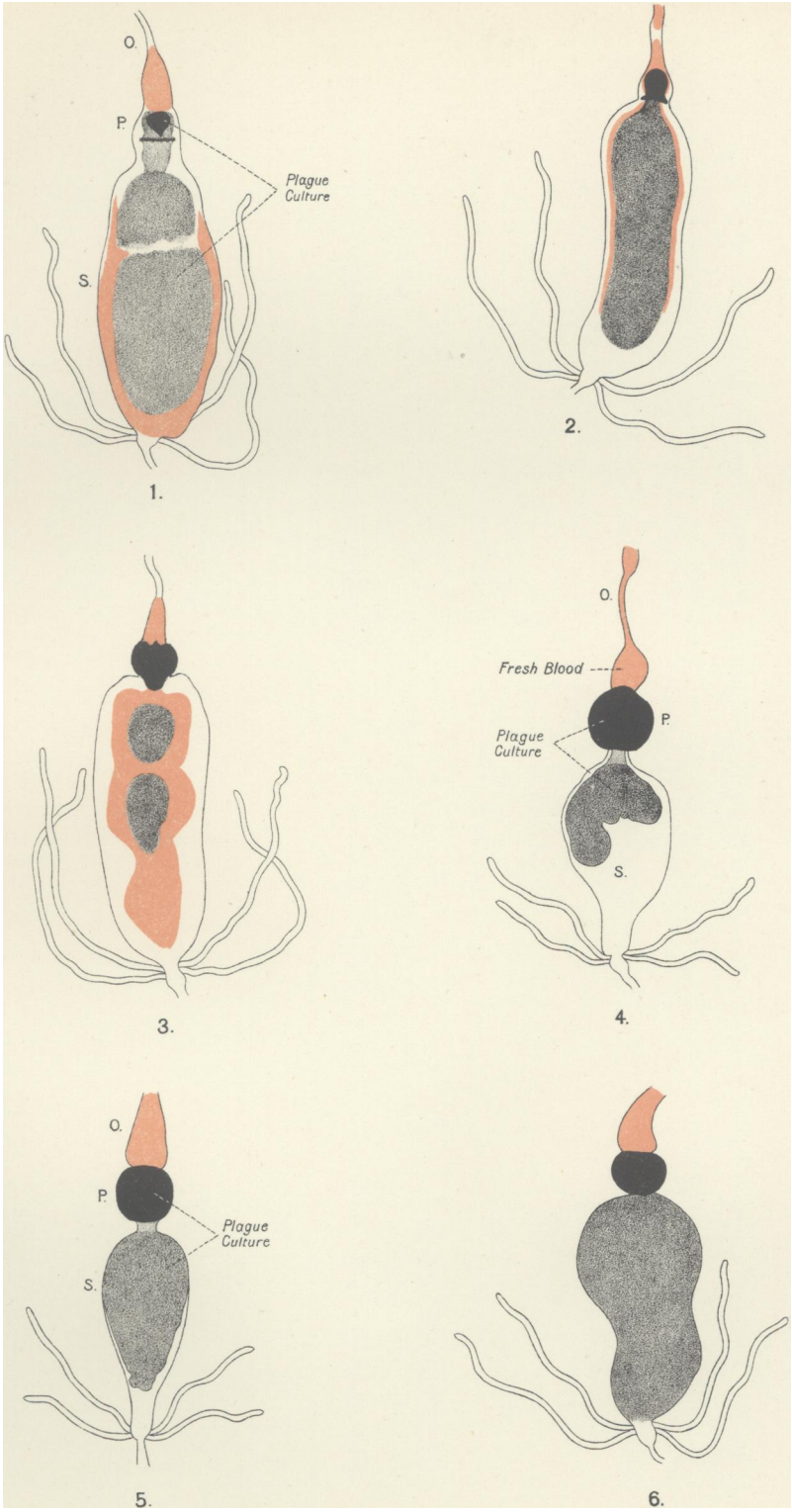
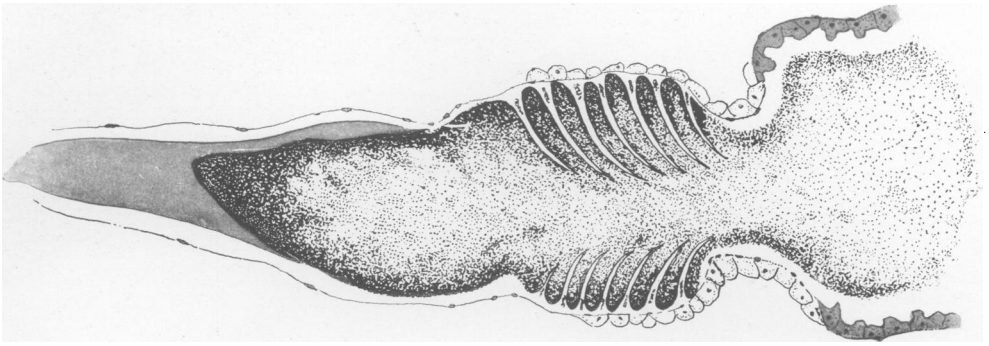


Cambridge University Press.



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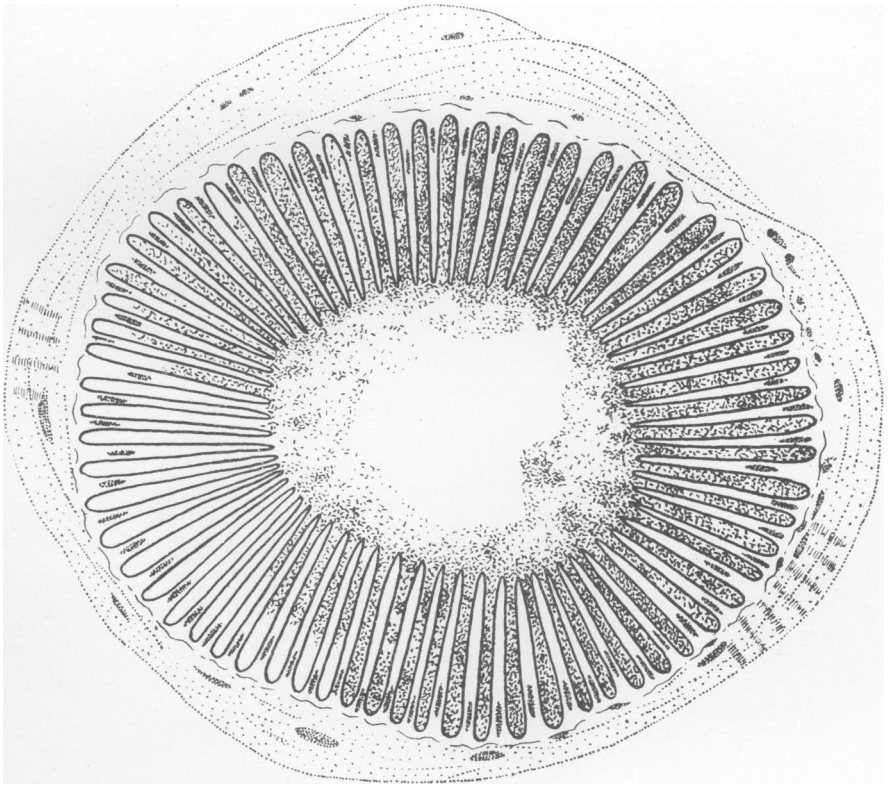

Fig. 1.

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GAUTHIER and RAYBAUD (1902). Sur le rôle des parasites du rat dans la transmission de la peste. *Compt. Rend. Heb. de la Soc. de Biol.* Vol. LIV, p. 1497.

—— (1903). Recherches expérimentales sur le rôle des parasites du rat dans la transmission de la peste. *Revue d'Hygiène*, Vol. XXV, p. 426.

LISTON, W. G. (1905). Plague, rats and fleas. *Journal Bombay Nat. Hist. Soc.* Vol. XVI, p. 253, also published as a separate pamphlet (enlarged by Thacker, Spink & Co., Calcutta, 1905).

OGATA, M. (1897). Ueber die Pestepidemie in Formosa. *Centralb. f. Bakteriol.* Vol. XXI, p. 774.

Reports on Plague Investigations in India (1906). *Journal of Hygiene*, Vol. VI, p. 435.

—— (1907), *Journal of Hygiene*, Vol. VII, p. 415.

SIMOND (1898). La propagation de la Peste. *Ann. de l'Inst. Pasteur*, Vol. XII, p. 625.

SWELLENGREBEL (1913). Record of observations on the bionomics of fleas and rats and on other subjects, bearing on the Epidemiology of Plague in Eastern Java. *Mededeelingen van den Burgerlijken Geneeskundigen Dienst in Nederlandsch-Indie.*

THOMPSON, A. (1903). *Report on a second Outbreak of Plague at Sydney*, 1902.

VERJBITZKI, D. T. (1904). The part played by insects in the epidemiology of plague. *Journal of Hygiene*, Vol. VIII, p. 162.

WALKER, CRANSTON (1911). Upon the inoculation of *materies morbi* through the human skin by flea-bites. *Journal of Hygiene*, Vol. XI, p. 290.

DESCRIPTION OF PLATES XXIV—XXVI.

Plate XXIV. Fig. 1. Type of plague septicaemia in the mice upon which the fleas were fed. ×500.

Fig. 2. Cages supported over a trough of lysol in which the fleas were bred and became infected.

Figs. 3 and 4. Smears of contents of the stomachs of infected fleas showing plague bacilli. ×1000.

Plate XXV. Figs. 1—6. Types of the appearances seen on dissecting out the stomachs of "obstructed fleas." Plague culture shown dark grey to black. Fresh blood distending the oesophagus red.

Plate XXVI. Fig. 1. Transverse section of proventriculus of *C. fasciatus* in early stage of infection; showing striated muscle fibres circularly disposed; tooth-like epithelial cells covered with chitin and plague bacilli growing between cells commencing to block lumen.

Fig. 2. Longitudinal section of oesophagus, proventriculus and portion of stomach of *C. fasciatus* in late stage of infection. Fresh blood recently imbibed by the insect is seen on top of a cap of plague culture which projects into oesophagus.

(For detailed description see text p. 434.)