

UPON THE INOCULATION OF MATERIA MORBI
THROUGH THE HUMAN SKIN BY FLEA-BITES.

BY CRANSTON WALKER, B.Sc., M.B., CH.B.

Walter Myers Research Student of the University of Birmingham.

Introductory. It has long been supposed that fleas may play a part in the transmission of disease, but no evidence which would clearly incriminate fleas has been forthcoming until the last few years. It has been clearly established within the last fifteen years that other blood-sucking parasites may convey disease, that mosquitoes propagate malaria, yellow fever, filariasis; that certain brachicerous flies propagate trypanosome diseases; that ticks transmit Rocky Mountain fever, African relapsing fever, piroplasmosis in cattle and other animals; and that bed-bugs convey relapsing fever.

Fleas, like the other blood-sucking parasites above enumerated, frequently leave and change their hosts, and not uncommonly pass from animals to man, and *vice versa*. Hence it appears desirable to enquire into the possibilities of fleas as disease-transmitters.

Papers on the transmission of *Trypanosoma lewisi* by rat-fleas have been recently published by Nuttall (1908), by Minchin and Thomson (1910), and by Strickland and Swellengrebel (1908–1910).

The conclusion that fleas were an essential factor in the spreading of plague was drawn by Simond in 1898; the same conclusion was reached by Hankin (1898), and by Liston. Ashburton Thompson (1902), from a careful examination of the epidemiological evidence afforded by outbreaks at Sidney, also concluded that plague was transmitted by fleas. Verjbitski (1904) in Russia performed numerous experiments demonstrating the ability of fleas to transmit plague among rats. Since 1906 the Advisory Committee for Plague Investigation in India appointed by the Royal Society, the Lister Institute, and the Secretary

of State for India, has published a mass of observational and experimental evidence to the effect that plague is conveyed both to rats and to man almost exclusively by flea-bites, other—possible—modes of infection being very rare. That fleas may transmit plague to other animals has also been shown by the Advisory Committee (1906–1908), and by McCoy (1910) in America¹.

Direct experiments with fleas on man have not been recorded.

Some enquiries related to the possibility of disease transmission by fleas were begun by the writer; the following experiments to determine the ability of fleas to inoculate *materia morbi* lying upon the skin form a fairly complete series in themselves.

Method of experiment. The fleas were kept in small glass tubes; they were fed on an average once a day; in this way they can be kept alive for several weeks. The experimental bites were mostly made on the flexor aspect of the arm but some were made on the front of the thigh and some on the calf. The inoculable material was spread on a small area of skin until the latter was moist; one of the flea-tubes, with open end, was then inverted over the spot. The fleas bit through the film of material. Occasional clean bites were made for comparison. In other experiments the fleas were allowed to bite clean skin, and the disease material was immediately afterwards rubbed over the site. At first each presumed bite was confirmed by examination of the site with a low power of the microscope, but the writer shortly became able to recognise a bite with certainty by the attitude and behaviour of the flea, apart from the appearance of the skin. The fleas generally bit within a minute or two of being applied to the skin, so that the film was still moist; in a few instances of unusual delay the film had dried. Control experiments through wet and dried films however gave uniformly positive results.

The materia morbi. Three kinds of material were used, (a) Tuberculin, (b) Vaccine Lymph, and (c) an Emulsion of a *Staphylococcus albus*.

(a) The *Tuberculin* used was that prepared by Allen and Hanbury for von Pirquet's diagnostic cutaneous reaction. It consists essentially of the concentrated filtrate from a broth culture of human tubercle bacilli. In from twelve to twenty-four hours after it has been inoculated

¹ If we add that *Cysticerci* of Cat, Dog and Rat Tapeworms have been found in fleas, we have summarized about all that is at present known of fleas in relation to disease. Sir Jonathan Hutchinson (1909) suspects that fleas may occasionally convey Syphilis, but the evidence is exceedingly slight.

into the skin of a susceptible person a papule measuring 2–3 mm. in diameter appears at the site of inoculation. It is vivid red in colour, and is surrounded by a bright pink areola about 1 mm. wide. The patient is also conscious of some soreness. A few hours later one or more vesicles may appear. The reaction is generally at its height about the end of the second or third day, after which it slowly subsides, being usually recognisable up to three weeks later; it often gives rise to a slight pigmentation which fades even more slowly; simple needle-pricks of a similar depth, made as controls, disappear completely in about two days.

(b) *Vaccinia* was selected as being an actual disease of kindred nature with the acute specific fevers, which is very highly inoculable and which may be imparted with safety. Its behaviour is exceedingly regular; and positive results, which are quite characteristic, follow with great certainty except in highly immune persons. It is therefore eminently suitable for experiments of this kind; its chief drawback is that a positive result renders the subject useless for further experiment for a considerable time. Ordinary government glycerinated lymph was used.

(c) From a small receding pustule on the neck of the writer was cultivated a Gram-positive *Staphylococcus*, which grew on agar in dirty-white, moist, fairly robust colonies of a rather gluey consistence. From about 36-hour agar growths emulsions were made in normal saline, of 2,000,000 per cubic millimetre. When these emulsions were pricked into the skin, a deep red papule formed in about 24 hours; it was surrounded by a zone of inflammation about a centimetre across. In about 36 hours the centre became pustular. At the end of each series of experiments the emulsions were recultured and gave robust growths in each case. These experiments were only performed on the writer.

The fleas. The fleas were all obtained in a general hospital. About 80 were used in all; of which about 35 were male, and about 45 female. Of twenty-eight which were examined with a view to identification 27 conformed to the description of Jordan and Rothschild (1908) for *Pulex irritans*, and one to that of *Ceratophyllus fasciatus*.

The persons. There are great differences between individuals in the local reaction with which they respond to flea-bites. Of the three persons of the experiments, L. K., H. W., and C. W. (the writer), the two latter exhibit the minimum of reaction, consisting of a pale pink areola about three millimetres in diameter, which fades in a few hours, and is unaccompanied by sensation. L. K. is at the other end of the

scale, and a bite is followed after a few minutes by a bright red flush several centimetres across, in the centre of which a raised white wheal about a centimetre wide appears after a few minutes; there is a sensation of extreme irritation. The flush is a day or two in fading.

The controls. Control experiments were repeatedly made, with ordinary precautions against sepsis, by laying a film of the material on the skin as usual and then pricking through it with the finest sewing needle made, the diameter of the shaft of which was 0·37 mm.

The proboscis of *Pulex irritans* measures 0·025 to 0·03 mm. at its greatest diameter, and 0·015 mm. at 0·02 mm. from the point. The needle, though a very fine one, is thus, speaking quite roughly, more than twelve times the size of the flea's proboscis.

The pricks were made with a single sharp movement; their depth was exceedingly slight, so that on many occasions the writer doubted whether the needle-point had entered the epidermis at all. Nevertheless of 48 such pricks only one failed to give a decided positive reaction.

THE EXPERIMENTS.

A. *With Tuberculin.*

(a) The fleas biting through films of Tuberculin.

1. C. W. 80 bites were made. 75 gave completely negative results, one was doubtful, and four were followed by positive reactions.

The four positive results all occurred together, and were from a series of five bites made just above the patella.

60 bites were on the arm and wrist, 29 were on the leg. 39 were by male fleas, and 41 by females.

Controls: 19 needle-pricks through Tuberculin films, 18 were positive, one doubtful (probably the needle did not penetrate).

Two pricks were made through films which had been dry for six hours, both were positive.

2. H. W. 12 bites. 10 negative, 2 positive.

Controls: 5, all positive.

3. L. K. 2 bites, both negative.

Controls: 3, all positive.

Summary.

94 bites were made, 87 gave no inoculation, one was doubtful, and 6 were followed by inoculation.

68 bites were on the arm and wrist, 26 on the leg. 45 were bites by male fleas, 49 by female.

Of the 6 inoculating bites, 4 were by males and 2 by females.

26 controls were made, 25 were positive, one was doubtful.

Inoculation by Flea-bites

(b) Tuberculin rubbed over recent flea-bites.

1. C. W. 25 experiments, all negative.
Controls, Tuberculin rubbed over needle-pricks.
3 experiments, all positive.
2. H. W. 3 experiments, all negative.

B. *With Vaccinia.*

(a) The fleas biting through films of Vaccine Lymph.

1. C. W. 13 bites, all negative.
No controls were made, as the subject is required for further experiment. C. W. has been vaccinated once in infancy (two "very poor" marks), and is therefore presumably not immune to vaccinia.
2. H. W. 13 bites, all negative.
Controls, 3 needle-pricks were made through a vaccine film, each gave a small but characteristic vaccine pustule. H. W. has been vaccinated twice, once in infancy and once about seventeen years ago.
3. L. K. 11 bites, all negative.
Controls, 2 needle-pricks were made through a vaccine film, in five days small bright papules formed with slight areolae. The papules were visible three weeks later, but no vesication had occurred. Clean needle-pricks left no mark after two days. Two further pricks were made through vaccine films, no reaction followed. The controls are therefore taken as positive. L. K. has been vaccinated three times, the last about seven years ago.

Summary. 45 bites, none inoculated.

25 bites were on the arm, 20 on the leg. 19 were by male fleas, 26 by female.

5 controls, all positive. The fact that positive controls were obtained confirms the observation that the flea-bites had failed to inoculate.

(b) Vaccine Lymph rubbed over recent flea-bites.

1. C. W. 4 experiments, all negative.
2. H. W. 5 experiments, all negative.

C. *With Emulsions of Staphylococcus albus.*

(a) The fleas biting through films of the emulsion.

C. W. 56 bites, all negative.
Controls, 15, all positive. Of these four were made through dried films.
15 bites were on the arm, 5 on the leg. 23 were by male, 33 by female fleas.

(b) The emulsion rubbed over recent bites.

C. W. 9 experiments, all negative.
Controls, 2, both positive.

General Summary. Fleas biting through films of inoculable materials, 196 experiments, 188 negative, 1 doubtful, 6 positive. No positive results with Vaccine Lymph and Staphylococcus Emulsion. Controls, 47. 45 positive, 1 doubtful.

Inoculable Materials rubbed over recent flea-bites, 46 experiments, all negative. Controls, 8, all positive.

Discussion of result. The circumstances of the experiments appear to be the most favourable for "mechanical" flea-bite inoculation, and yet inoculation only followed in about 3 per cent. of cases, and only with Tuberculin. In these few cases also it is not improbable that the Tuberculin may have penetrated some small patch of unusually deep desquamation, or some small abrasion, as invisible as many of the control needle-pricks were. It was not possible to say whether the reaction started from the precise point of penetration by the flea or not. Tuberculin when mixed with ointment is inoculable through the unbroken skin, as in Moro's diagnostic method.

The writer regards these six positive results as not necessarily due to the flea-bites. Without, however, pressing this opinion, it is obvious from the experiments that, with highly inoculable materials, and under apparently favourable circumstances, *Pulex irritans* only rarely effects inoculation when biting.

It may be suggested that as the amount of material, if any, introduced by a flea's proboscis must be exceedingly minute it may be too small to produce any recognisable effect. In order to test the possibilities of minute doses, experiments were made on C. W. with Tuberculin. Two methods suggested themselves, (1) to dilute the Tuberculin, using the same pricker, (2) to use a smaller pricker. There are difficulties in the way of the latter method in that if a pricker much smaller than the smallest commercial needle is used it is liable to break

EXPERIMENTS WITH MINUTE TUBERCULIN DOSES.

(1) Inoculations with diluted Tuberculin, needle as before.

| | |
|--------------------------------------|---------------------------------------------------------------------------------------------------|
| Dilutions up to 1 in 10. | Reactions differing little if any from those with full strength. |
| Dilutions from 1 in 10 to 1 in 50. | Reaction slightly less than with full strength. |
| Dilutions from 1 in 50 to 1 in 100. | Reactions characteristic but smaller and diminishing with the dilution. |
| Dilutions from 1 in 100 to 1 in 150. | Pricks still easily recognisable from clean controls, especially after the lapse of a day or two. |

- (2) Full strength Tuberculin pricked through by nettle-hairs.

The nettle hairs are fairly regular cones 1 to 1.3 mm. long; their diameter is 0.085 mm. at the base, 0.06 mm. in the middle, and 0.014 mm. just below the terminal knob, which is broken off as the hair enters the skin; at 0.02 mm. behind the knob the diameter is 0.018 mm. Thus, to make a rough comparison between the proboscis of *Pulex irritans* and the nettle hair, the latter is about three times as long, it is about the same diameter near the point, but farther back it becomes 2-3 times as thick.

The skin-reactions from nettle-stings and those from Tuberculin can run their courses concurrently, without apparently influencing each other.

With "slight" nettle-stings, *i.e.* less than about ten hairs penetrating within two or three mm., no trace is left after the lapse of a day. With "severe" stings, more than about twenty hairs piercing in close proximity and producing confluent wheals, there are often several small pink papules on the following day which are not at once distinguishable from small Tuberculin reactions. Upon development however a distinction appears, as the nettle-sting papules fade completely in about twenty-four hours more, while the Tuberculin papules steadily increase in size and characteristics for several days.

The experiments showed that when single hairs, obtained by cutting out a piece of leaf bearing a solitary hair, were pricked through a Tuberculin film there was no wheal, but a Tuberculin reaction followed, which, though small, passed through the usual stages in the usual times.

When two or three or more hairs were passed through in close proximity so as to give a confluent wheal, a correspondingly greater reaction followed.

(*Note.* These two sets of experiments were carried out at an interval of some months after the others, but the appearance of the usual controls did not suggest that there had been any great change in the susceptibility of the subject to Tuberculin.)

upon, or within, the epidermis; the latter event is liable to cause a spot of inflammation which obscures the issue, but in its absence there may be complete uncertainty as to whether the pricker has penetrated at all. An opportunity is offered however by the hairs of the stinging-nettle, which while very minute announce their penetration to sensation and by the shortly subsequent appearance of the well-known skin reaction.

These experiments show that a recognisable reaction is obtainable with an amount of Tuberculin which is exceedingly minute, and which is comparable to the amount which might be carried into the skin by a flea's proboscis. The conclusion is, therefore, that the failure to obtain inoculation with flea-bites is not attributable solely to the smallness of the piercing-organ.

Bearing on plague. The foregoing experiments derive interest from their bearing on the transmission of plague by fleas. It has been

shown by the Indian Commission (1907, p. 405) that fleas which have fed upon plague animals are liable to deposit faeces containing abundant virulent plague bacilli upon the skin of their host¹. Simond (1898) had suggested that plague was transmitted by fleas subsequently biting through such faeces, or by the faeces being rubbed into the bite holes. The Indian Committee examined experimentally a number of presumably possible modes of transmission by fleas, and failed to find evidence in favour of any but the above two. (1907, pp. 415 *et seq.* The reader is also referred to this place for a discussion on the mechanism of transmission.) Successful inoculations were obtained with guinea-pigs by allowing fleas to bite them through films of plague culture, and also by smearing cultures over recent bites. Verjbitski (1908, p. 183) also performed the latter experiment, with variations, upon rats, obtaining positive results in about 50 per cent. of cases. Neither mention whether precautions were taken against the animals scratching themselves.

In applying the here recorded experiments to the question of plague transmission it is necessary to refer to differences in each of the factors of the two sets of experiments.

(a) While the writer, as shown on p. 292, used *Pulex irritans* almost entirely, the Indian Committee used for their similar experiments *Xenopsylla cheopis* (Rothschild)². The differences between the two fleas in vital anatomy are so slight that they are not likely to affect the result. According to Jordan and Rothschild (1908, p. 17) the piercing organ of *X. cheopis* is somewhat longer and slenderer than that of *P. irritans*, but it is not obvious in which direction this might tend to act. Also, in the recorded work upon the general fact that fleas are concerned in plague transmission, no distinction between different species of fleas has been indicated, beyond their affinities for different hosts and the consequences deducible therefrom.

(b) Instead of *B. pestis* in various media, the writer used the above mentioned three materials. It still may be suggested that the flea's proboscis may carry in a quantity of material which is so slight as to be below the limit of effectiveness of the writer's materials, but

¹ The statement that defaecation frequently occurs during the act of biting has been questioned. The writer observed it 51 times in 241 bites of *Pulex irritans*. It appears to occur more frequently the shorter the intervals between the feeds; the intervals averaged about a day in this case. The proportion of defaecating females to males was about 2.5 to 1.

² Verjbitski worked with *Typhlopsylla musculi*, *Pulex irritans*, *P. canis*, *P. felis*. The flea used in the precise experiments here referred to does not appear to have been recorded.

which nevertheless may be sufficient to originate fatal plague. This however is very improbable, for, while the minuteness of effective Tuberculin doses has been indicated above, the most virulent cultures of *B. pestis* which Verjbitski was able to obtain required 0.001 cubic millimeters for a minimum lethal dose for rats. Now the volume of the whole proboscis of *Pulex irritans* is only about 0.00018 cu. mm., and the amount of material which it is likely to carry in with it when piercing the skin will be smaller still, *i.e.* very much smaller than Verjbitski's minimum lethal dose. The Indian Commission also (1907, pp. 346-8) showed with guinea-pigs that the smaller the dose of plague bacilli the less the effect produced. With very small doses no result was discovered.

(c) The plague experiments have been conducted on rats and guinea-pigs, the others on man. It is possible that differences exist between man and the experimental animals in this respect, but if this be so the conclusions from the experimental work on plague must be modified on being applied to man.

A further experimental difference exists in that with the writer the bites were nearly all single (six were double), whereas in the plague experiments they were mostly multiple. The Indian Commission (*loc. cit.*) succeeded only once in conveying infection to rats by a *single* flea-bite. Verjbitski failed to inoculate fatally with a single bite in four trials by smearing plague cultures over recent bites. He concluded that at least two bite-holes were necessary for a fatal inoculation in a rat, but he obtained fatal inoculations in guinea-pigs by smearing the cultures over one bug-bite (1908, pp. 183 and 197). The ability of one inoculation to influence another obviously depends on the overlapping of their spheres of influence. If the important action is confined to the immediate neighbourhood of the place of introduction, as is most probably the case with Tuberculin and Staphylococci, two inoculations will not affect each other, unless they occur very close together. Hence it is improbable that with the writer's materials the appearances, or lack of appearances, following a single bite will be altered by multiple bites, unless the latter are closely set. However, it is intended to carry out further experiments along these lines.

Although in no way affecting the conclusion that fleas are responsible for plague conveyance, the above recorded experiments are at variance with the view which at present meets with most acceptance regarding the mechanism by which fleas transmit the disease. It appears that yet further enquiry is needed.

Acknowledgements. The writer is under obligation to several correspondents for their ready courtesies. He also gladly acknowledges the assistance of Sister Lindsay, and of many members, too numerous for individual mention, of the Nursing Staff of Queen's Hospital, Birmingham.

He is specially indebted to his sister, Hilda Walker, and to Dr Lina Kurz for their willingness to assist in and be the subjects of a series of somewhat unpleasant experiments.

SUMMARY.

1. When fleas bit through films of Tuberculin, Vaccine Lymph, and Staphylococcus Emulsion lying upon the human skin, no inoculation was observed in 188 instances out of 195. In six of the experiments with Tuberculin, inoculation followed, which possibly was not due to the flea-bites. Controls gave positive results.

2. When the same materials were rubbed over recent flea-bites, no inoculations followed. Controls were positive.

3. A very minute dose of Tuberculin, such as might be conveyed by a flea's proboscis, will produce a recognisable reaction in a susceptible person.

4. The bearing of the results on the question of plague transmission is discussed.

REFERENCES.

- ADVISORY COMMITTEE *Reports on Plague investigations in India.* 1906, *Journal of Hygiene*, vi. 421-536. 1907, *Journal of Hygiene*, vii. 333-476. 1908, *Journal of Hygiene*, viii. 162-308.
- GALLI-VALERIO, B. (1900). Les puces des rats et des souris jouent-elles un rôle important dans la transmission de la peste bubonique a l'homme. *Centralbl. f. Bakteriol.* xxvii. 1-4.
- (1900). Quelques observations sur la morphologie du *Bacterium pestis* et sur la transmission de la peste bubonique par les puces des rats et des souris. *Ibid.* xxviii. 842-845. (These two papers disputed Simond's suggestions regarding flea-transmission.)
- HANKIN, E. H. (1898). La propagation de la peste. *Ann. de l'Inst. Pasteur*, xii. 705-762.
- HUTCHINSON, JONATHAN (1909). *Syphilis*, p. 35.
- JORDAN, K., and ROTHSCILD, N. C. (1908). A Revision of the Non-combed Eyed Syphonaptera. *Parasitology*, i. 1-100.
- McCoy, G. W. (1910). Fleas as Plague-Carriers between Rats and Ground Squirrels. *Public Health Reports*, Washington, U.S.A., xxv. 659. Also former Reports.

- MANTEUFFEL (1909). Studien über die Trypanosomiasis der Ratten, &c. *Arb. a. d. Kaiserl. Gesundheitsamte*, xxxiii. Heft 1. Nachtrag, pp. 79–83.
- MINCHIN, E. A., and THOMPSON, J. D. (1910). The Transmission of *Trypanosoma lewisi* by the rat flea (*Ceratophyllus fasciatus*). *Proc. Royal Society B.*, lxxxii. 273.
- NUTTALL, G. H. F. (1899). The Rôle of Insects, Arachnids, &c., as the carriers of bacterial and parasitic Diseases. *Johns Hopkins Hospital Reports*, viii. 1–155.
- (1897). Zur Aufklärung der Rolle, welche stechende Insekten bei der Verbreitung von Infektionskrankheiten spielen. *Centralbl. f. Bakteriol.* xxii. 87.
- (1908). The Transmission of *Trypanosoma lewisi* by Fleas and Lice. *Parasitology*, i. 296.
- PATTON and STRICKLAND (1908). A critical review of the relation of blood-sucking invertebrates to the life-cycles of the trypanosomes of vertebrates. *Parasitology*, i. 256–301.
- SIMOND, P. L. (1898). La Propagation de la Peste. *Ann. de l'Inst. Pasteur*, xii. 429.
- (1910). "Peste." *Traité d'Hygiène*, xvii. 429.
- STRICKLAND (1909). On the supposed development of *Trypanosoma lewisi* in lice and fleas, &c. *Parasitology*, ii. 81–90.
- STRICKLAND and SWELLENGREBEL (1910). Notes on *Trypanosoma lewisi* and its relation to certain Arthropoda. *Parasitology*, iii. 436–454.
- SWELLENGREBEL and STRICKLAND (1910). The development of *Trypanosoma lewisi* outside the vertebrate host. *Parasitology*, iii. 360–389.
- THOMPSON, ASHBURTON (1901). A contribution to the Etiology of Plague. *Journ. of Hygiene*, i. 153–167. On the Epidemiology of Plague. *Ibid.* iv. 552. Also *Reports on Plague at Sydney, 1900–1908*. Gov. Printer.
- VERJBITSKI, D. T. (1904, St Petersburg). The part played by Insects in the Epidemiology of Plague. (Translation.) *Journal of Hygiene*, 1908, viii. 162–208.
- The Rat and its Relation to the Public Health, 1911*, by various Authors, prepared by direction of the Surgeon-General, Washington, U.S.A.