The Transmission of Trypanosoma lewisi *by the Rat-flea* (Ceratophyllus fasciatus). (*Preliminary Communication.*)

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(Communicated by Sir Ray Lankester, K.C.B., F.R.S. Received January 7,—Read February 3, 1910.)

Since the spring of 1909 we have been engaged in conducting experiments and observations upon the transmission of *Trypanosoma lewisi* by rat-fleas, and we hope at some future period to publish a full account of the whole work, including the development of the trypanosome in the flea. The experiments here recorded have been selected for special publication because they are complete in themselves and because they appear to us decisive on a point of fundamental importance, namely the method of transmission by the flea.

Nuttall (1908) reviews the subject of the transmission of *T. lewisi* and records experiments of his own on that subject up to December, 1908. Reference will be made to Nuttall's experiments later on, and we will first proceed to describe the experiments that form the subject of the present communication under the following headings:—

I. The object of the experiments.
II. Account of the experiments (general and detailed).
III. Conclusions to be drawn from the results of the experiments.

I. *The Object of the Experiments* is chiefly to make clear the *method* of transmission; subdivided it may be stated thus:—first and incidentally, to confirm conclusions that fleas are capable of transferring *T. lewisi* from infected to "clean" (*i.e.* non-infected) healthy rats, but secondly and principally, to determine the mode of infection, whether "direct" or "cyclical," and thirdly, when the cyclical method was indicated by the progress of the experiments, to confirm results and to ascertain further facts connected with the cyclical method.

In order to avoid confusion and misconception, it may be well to make clear what we mean by the terms "direct" and "cyclical" in this connection. The Editor of the Bulletin issued from the Sleeping Sickness Bureau (1909) writes:—"There are two methods of transmission of trypanosomes generally recognised: (*a*) that which is mechanical, dependent on physical conditions alone, and (*b*) that which occurs after a 'cycle of development,' an expression which implies the conjugation of two individuals. The work of Novy and MacNeal

has shown that a third method (*c*) is possible, partly mechanical and partly biological, resulting from the late multiplication of the parasites in the intestine and their subsequent introduction into the body of the bitten animal." It may be pointed out, first that the mechanical element is present equally in (*a*), (*b*), and (*c*), and secondly that the question of "the conjugation of two individuals" seems to us of quite secondary importance in this connection. There are non-sexual cycles of development as well as sexual; in the malarial parasite, for instance, there is a non-sexual cycle, which takes place in the human body, and a sexual cycle, which takes place in the mosquito. At the present time the belief that a sexual cycle in the development of trypanosomes takes place in the invertebrate host is largely an assumption, based on the analogy of the malarial parasite, and in need of objective proof.

We desire to approach the subject in an unbiased manner, and we would recognise, for the present at least, only two methods of transmission, of fundamental scientific and practical importance, which we shall term the "direct" and "cyclical" methods respectively. In the direct method the invertebrate acts merely as a suitable instrument in the transmission. Experimentally the chances that the invertebrate will convey the infection to a susceptible animal by the direct method are greatest immediately after it has contaminated its proboscis by feeding on an infected one, and these chances gradually diminish and cease altogether within a comparatively limited period of time. And experimentally the invertebrate is found to have exhausted its power to infect in the process of cleaning its proboscis at the feed which follows next after that on the infected animal. In the cyclical method, on the other hand, the invertebrate is more than a suitable instrument in the transmission: it acts as a host in which the parasite establishes itself and maintains the existence of its species. Infection by the cyclical method can take place only after the parasite has established itself, and it can then continue to take place so long as the parasite maintains its existence in the invertebrate. Experimentally it is found that an invertebrate of the right species, after having fed on an infected animal, may feed many times on susceptible animals without conveying the infection, and may then, without having fed again on an infected animal, become infective; and, further, that once infective, it may remain infective for an indefinite period of time—possibly for the rest of its life. Of such an invertebrate we may say that it is a true host, and that the parasite it transmits passes through a cycle of development within it, meaning by the word cycle a series of changes and generations which follow one another in more or less definite order in the development and multiplication of the parasite, and leaving it an open

question whether or not a sexual process takes place in the course of the development.

Experiments A and B were arranged so as to eliminate possible infection other than by fleas, and to separate "direct" from "cyclical" infection, so that if infections did take place, it would be clear whether they were the result of the direct method, the cyclical method, or both. When it had been ascertained in the course of Experiments A and B that infections did take place, but not by the direct method, these experiments were prolonged in order to determine if fleas, once infective, retain the infection so as to infect a succession of healthy, clean rats. This is also a clear issue in Experiments C and D, which were arranged with a view to determine further by direct observation, and within narrow limits, (1) the length of the incubation-period in the flea, that is to say, the length of time required for the parasite to establish itself in the flea and render it infective, and (2) the length of the multiplication-period in the rat, that is to say, the length of time from the actual inoculation of the parasite into the rat until the trypanosomes cease to multiply in the rat's blood. As is well known, when a rat is first infected with *T. lewisi*, the parasite multiplies in the blood very rapidly. After a certain length of time the multiplication ceases entirely and rather suddenly. When rats are infected artificially in the laboratory by the ordinary method of intra-peritoneal inoculation of blood from an infected rat, the multiplication-period lasts for some 10 or 12 days after inoculation, as a rule; but it by no means follows that multiplication lasts for the same length of time when the inoculation is performed by fleas. If the length of the multiplication-period is known, and is found to be a constant, it is evident that in any given experiment the time when the infection of the rat took place can be ascertained by simply observing when multiplication of the trypanosome ceases in the blood and then deducting the known period; and further, if the infection is a first one and the time during which the fleas were exposed to infection was short and is known, then the length of the incubation-period in the flea can be calculated also. Experiments D and E were undertaken with the special object of determining the incubation-period in the flea.

II. *Account of the Experiments.*

(1) *General.*—The same arrangements were made for all the experiments, which differ only in details. The fleas used in all cases had been bred in flea-proof cages in the laboratory, the parents being some 50 fleas which were obtained in the early autumn of 1908 from rats trapped in the neighbourhood of the Sutton Broad Laboratory in Norfolk. These fleas multiplied in our cages during last winter and spring to produce literally thousands; we have

no difficulty in obtaining 200 fleas from the breeding cages for an experiment when required. The fleas are kept alive in the cages by being fed on non-infected healthy tame rats.

Although it is not known whether any of the rats on which the original 50 fleas were caught were infected with *T. lewisi* or not, it is quite certain that the stock of fleas in our breeding cages is free from infection, since not one of the many non-infected rats used for feeding them has ever become infected. We are indebted to the Hon. N. C. Rothschild for kindly identifying the genus and species of our fleas as *Ceratophyllus fasciatus*.

In preparing for each of these experiments, the first step was to collect a sufficient number of fleas, usually about 200, from the breeding cages. These fleas were then exposed to infection by being put, together with a heavily-infected rat, into a tin cage specially constructed to facilitate the recovery of the fleas. The infected rat itself was ascertained to be free from fleas before it was put into the tin cage; and the fleas from the breeding cages were left with it in this cage for a known period of time. At the end of this period as many as could be recovered from the infected rat and from the tin cage were used to colonise a freshly prepared cage, constructed on the model used by the Indian Plague Commission for their experiments on rats and fleas.* This cage, which we will call Cage X, was carefully cleaned and disinfected before being used for the experiment, and into it a healthy, non-infected rat (Rat X 1) was now introduced, together with the fleas collected from the tin cage already mentioned. Three or four days later, or when under the special conditions a sufficient time had elapsed to ensure that any flea that survived must have fed on Rat X 1, this rat was removed from Cage X, and was carefully cleaned from fleas by the aid of chloroform-vapour, after which it was put into a fresh cage by itself. The fleas removed from Rat X 1, after they had recovered from the chloroform-vapour, were put back into Cage X. Another healthy, non-infected rat (Rat X 2) was then put into Cage X, and so on with Rat X 3, X 4, etc. (each treated similarly to Rat X 1). Each of these rats, after removal from Cage X, was then examined daily or every other day subsequently for trypanosomes in its blood.

(2) *Detailed Account.*

Experiment A.

4/10/09.—200 fleas taken from the breeding cage were put into the special tin cage together with a heavily-infected tame rat showing many trypanosomes in its blood.

8/10/09.—44 fleas recovered from the above-mentioned cage were put into a freshly-prepared flea-proof cage (Cage A), together with a clean, healthy rat (Rat A 1).

* See 'Journal of Hygiene,' vol. 6, 1906, p. 435, pl. 4.

6 other fleas recovered from the tin cage were dissected ; in 5 of them trypanosomes were found, and in one instance multiplying forms were seen in the rectum. (The recta of the others were not specially examined.)

12/10/09.—Rat A 1 was removed from Cage A, was carefully cleaned from fleas by the aid of chloroform-vapour, and was put into a separate cage by itself ; two fleas removed from it were dissected. A healthy, clean tame rat (Rat A 2) was then put into Cage A.

Rat A 1 was examined regularly from 16/10/09 to 13/11/09, but no trypanosomes were found. On 15/11/09 Rat A 1 was found dead ; no trypanosomes were found in its blood.

On 26/10/09 Rat A 2 was found dead ; no trypanosomes were found in its blood.

27/10/09.—A healthy, non-infected tame rat (Rat A 3) was put into Cage A, whence the dead Rat A 2 had been removed the day before.

6/11/09.—Rat A 3 examined, no trypanosomes found.

8/11/09.— „ „ „ „

10/11/09.— „ „ „ „

11/11/09.—Rat A 3 examined, trypanosomes found ; a smear made showed multiplying forms.

13/11/09.—A smear made from the blood of Rat A 3 showed trypanosomes abundant, still multiplying forms.

15/11/09.—A smear of the blood of Rat A 3 showed very few multiplying forms.

16/11/09.—A smear from Rat A 3 showed the trypanosomes all of the adult form ; multiplication ended.

Tabular Summary of Experiment A.

Cage A colonised with 44 fleas that had been exposed to infection from October 4 till October 8.

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
A 1	October 8	October 12	0	—	—
A 2	„ 12	Found dead, Cage A, on October 26 ; no trypanosomes seen in its blood.			
A 3	„ 27	Left in	+	November 11	November 16

Experiment B.

11/10/09.—200 fleas taken from the breeding cage were put into the special tin cage together with a heavily-infected tame rat showing many trypanosomes in its blood (the same rat that was used in Experiment A).

15/10/09.—157 fleas recovered from the above cage were put into a freshly-prepared, flea-proof cage (Cage B), together with a healthy, non-infected tame rat (Rat B 1). Two fleas were dissected, multiplying forms of trypanosomes were found in the rectum of one.

19/10/09.—Rat B 1 was removed from Cage B, was carefully freed from fleas by the aid of chloroform-vapour, and was put into a separate cage by itself. Of 26 fleas removed from Rat B 1, 21 were put back into Cage B, and the other 5 were dissected ; in one of the fleas dissected multiplying forms of trypanosomes were found in the rectum, and in another multiplying forms were found in the

rectum and in one of the Malpighian tubes. A healthy, non-infected tame rat (B 2) was put into Cage B.

Rat B 1 was examined regularly from 23/10/09 till 8/11/09; no trypanosomes were found at any time in its blood.

8/11/09.—Rat B 2 examined, many trypanosomes found in its blood; all adult forms, apparently just past the multiplication-period.

Tabular Summary of Experiment B.

Cage B colonised with 157 fleas that had been exposed to infection from October 11 till October 15.

Rat.	Put in.	Taken out.	Result.
B 1	October 15	October 19	0
B 2	„ 19	Left in	+

Having confirmed conclusions that fleas are capable of transferring *T. lewisi* from infected to clean, healthy rats, and having shown that infection did not take place by the direct method, Experiments A and B were prolonged in order to ascertain whether fleas once infective retain infection so as to be capable of infecting a series of clean, healthy rats. In both cases it was found that fleas once infective do so retain the infection, and can infect a series of rats without themselves being exposed to fresh infection, but as this point is so clearly brought out in Experiment C which follows, the detailed accounts of the prolongations of Experiments A and B have not been added.

Experiment C.

24/11/09.—206 fleas taken from the breeding cage were put into the special tin cage together with a heavily-infected tame rat, showing many trypanosomes in it blood. This rat had been submitted to chloroform-vapour immediately before and carefully searched to ensure that it harboured no fleas.

27/11/09.—160 fleas recovered from the above cage were put into a freshly prepared, flea-proof cage (Cage C), together with a healthy, clean tame rat (Rat C 1). 5 fleas were dissected, and in all trypanosomes were found. In the rectum of 1 flea (♀) large masses of multiplying Crithidia-like forms were found. In the rectum of another flea (♂) multiplying clumps were also found.

30/11/09.—Rat C 1 was removed from Cage C, was carefully freed from fleas by the aid of chloroform-vapour, and was put into a separate flea-proof cage by itself. A healthy, clean tame rat (C 2) was put into Cage C.

Rat C 1 was examined regularly from 1/12/09 until 3/1/10. No trypanosomes were found in its blood at any time.

3/12/09.—Rat C 2 was removed from Cage C, was carefully freed from fleas, and was then put into a separate freshly-prepared flea-proof cage by itself. 11 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 3) was then put into Cage C.

Rat C 2 was examined daily from 4/12/09, and on 10/12/09 trypanosomes

were first seen in its blood. Smears were made daily of the blood until 15/12/09, at which date the multiplication-period came to an end.

6/12/09.—Rat C 3 was removed from Cage C, was carefully freed from fleas, and was put into a fresh cage by itself. 2 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (C 4) was then put into Cage C.

Rat C 3 was examined daily from 6/12/09 until 11/12/09, and again on 13/12/09, when trypanosomes were first seen in its blood. Permanent smears of its blood were made daily from 13/12/09 till 18/12/09, when the multiplication-period ended.

8/12/09.—Rat C 4 was removed from Cage C, was carefully freed from fleas, and was then put into a separate cage by itself. 3 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 5) was then put into Cage C.

Rat C 4 was examined daily from 8/12/09 to 11/12/09, and again on 13/12/09, when trypanosomes were first found in its blood. Permanent smears were made daily of its blood from 13/12/09 till 18/12/09, when the multiplication-period had practically ended.

10/12/09.—Rat C 5 was removed from Cage C, was carefully freed from fleas, and was then put into a fresh cage by itself. 14 fleas recovered were put back into Cage C. A healthy, clean tame rat (Rat C 6) was then put into Cage C.

Rat C 5 was examined regularly from 11/12/09 till 14/12/09, when trypanosomes were first seen in the blood. Permanent smears were made of its blood from 14/12/09 till 21/12/09, when the multiplication-period came to an end.

11/12/09.—Rat C 6 was removed from Cage C, was carefully freed from fleas, and was then put into a separate cage by itself. 20 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 7) was then put into Cage C.

Rat C 6 was examined regularly from 13/12/09 till 16/12/09, when trypanosomes were first found in its blood. Permanent smears of its blood were made from 16/12/09 till 23/12/09, when the multiplication-period came to an end.

13/12/09.—Rat C 7 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself. 16 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 8) was then put into Cage C.

Rat C 7 was examined regularly from 15/12/09 till 17/12/09, when trypanosomes were first seen in its blood. Permanent smears were made of its blood from 17/12/09 till 24/12/09, when the multiplication-period came to an end.

15/12/09.—Rat C 8 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself. 5 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 9) was then put into Cage C.

Rat C 8 was examined regularly from 16/12/09 till 20/12/09, when trypanosomes were first seen in its blood. Permanent smears were made from 20/12/09 till 26/12/09, when the multiplication-period came to an end.

17/12/09.—Rat C 9 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself. 23 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 10) was then put into Cage C.

Rat C 9 was examined regularly from 18/12/09 till 21/12/09, when trypanosomes were first seen in its blood. Permanent smears were made from 21/12/09 till it was found dead on 28/12/09. On this date the multiplication-period had not quite ended, but from appearances would probably have ended on the following day, 29/12/09

20/12/09.—Rat C 10 was removed from Cage C, was carefully freed from fleas, and was then put into a separate cage by itself. 6 fleas recovered from it were put back into Cage C. A healthy, clean rat (Rat C 11) was then put into Cage C.

Rat C 10 was examined regularly from 21/12/09 till 26/12/09, when trypanosomes were first seen in its blood. Permanent smears were then made from 26/12/09 till 30/12/09, when the multiplication-period came to an end.

22/12/09.—Rat C 11 was removed from Cage C, was carefully examined for fleas by aid of chloroform-vapour, but no flea was found on it. It was put into a separate cage by itself. A healthy, clean tame rat (Rat C 12) was then put into Cage C.

Rat C 11 was examined regularly from 23/12/09 till 28/12/09, when trypanosomes were first seen in its blood. Permanent smears were then made from 28/12/09 till 1/1/10, when the multiplication-period came to an end.

24/12/09.—Rat C 12 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself; 9 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 13) was then put into Cage C.

Rat C 12 was examined on 25/12/09 and was found dead on 26/12/09 before trypanosomes had time to appear in its blood.

28/12/09.—Rat C 13 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself; 3 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 14) was then put into Cage C.

Rat C 13 was examined regularly from 29/12/09 till 3/1/10, when trypanosomes were first seen in its blood. Permanent smears were made from 3/1/10 till 8/1/10, when the multiplication-period came to an end.

31/12/09.—Rat C 14 was removed from Cage C, was carefully searched, but no flea found on it, and was put into a separate cage by itself. A healthy, clean tame rat (Rat C 15) was then put into Cage C.

Rat C 14 was examined regularly from 1/1/10 till 22/1/10. No trypanosomes were found in its blood at any time.

(Experiment still proceeding—see below.)

Experiment D.

6/12/09.—254 fleas from the breeding cage were put into the freshly-prepared and cleaned special tin cage.

7/12/09.—A heavily-infected tame rat showing many trypanosomes in its blood, after being carefully searched with chloroform-vapour to ensure that it harboured no fleas, was put into the above-mentioned tin cage at 2 p.m.

8/12/09.—212 fleas were recovered from the above cage and rat (203 from the cage and 9 from the rat) at 12 noon. Of these, 162, including the 9 from the rat, were put into the freshly-prepared flea-proof cage (Cage D), together with a healthy, clean tame rat (Rat D 1), and the remaining 50 (all from the cage) were put into another cage (Cage E), with another healthy, clean tame rat (Rat E 1).

11/12/09.—Rat D 1 was removed from Cage D, was carefully freed from fleas by the aid of chloroform-vapour, and was put into a separate freshly-prepared flea-proof cage by itself. 20 fleas recovered from Rat D 1 were returned to Cage D. A clean, healthy tame rat (D 2) was put into Cage D.

Rat D 1 was examined regularly from 14/12/09 until 22/1/10. No trypanosomes were found in its blood at any time.

13/12/09.—Rat D 2 was removed from Cage D, was carefully freed from fleas, and was then put into a separate freshly-prepared flea-proof cage by itself. 54 fleas recovered from Rat D 2 were returned to Cage D.

Rat D 2 was examined regularly from 16/12/09 until 22/1/10. No trypanosomes were found at any time in its blood.

14/12/09.—A healthy, clean tame rat (Rat D 3) was put into Cage D.

15/12/09.—Rat D 3 was removed from Cage D, was carefully freed from fleas, and was then put into a freshly-prepared flea-proof cage by itself. 36 or 38 fleas recovered from Rat D 3 were returned to Cage D.

Rat D 3 was examined regularly from 17/12/09 until 21/12/09, when trypanosomes were first seen in its blood. Permanent smears were then made of its blood from 21/12/09 till 26/12/09, when it was found dead before the multiplication-period had come to an end.

16/12/09.—A healthy, clean tame rat (D 4) was put into Cage D.

17/12/09.—Rat D 4 was removed from Cage D, was carefully freed from fleas, and was then put into a freshly-prepared cage by itself. 15 fleas were recovered from Rat D 4, but were all accidentally killed.

Rat D 4 was examined regularly from 20/12/09 till 22/1/10. No trypanosomes were found at any time in its blood.

18/12/09.—A healthy, clean tame rat (Rat D 5) was put into Cage D.

20/12/09.—Rat D 5 was removed from Cage D. Though carefully searched by the aid of chloroform-vapour, no fleas were found on it. Rat D 5 was put into a freshly-prepared cage by itself. A healthy, clean tame rat (Rat D 6) was then put into Cage D.

Rat D 5 was examined regularly from 21/12/09 till 22/1/10. No trypanosomes were found at any time in its blood.

22/12/09.—Rat D 6 was removed from Cage D, was carefully freed from fleas, and was then put into a separate freshly-prepared cage by itself. 4 fleas recovered from Rat D 6 were returned to Cage D. A clean, healthy tame rat (Rat D 7) was then put into Cage D.

Rat D 6 was regularly examined from 23/12/09 till 22/1/10. No trypanosomes were found at any time in its blood.

24/12/09.—Rat D 7 was removed from Cage D. Though carefully searched by aid of chloroform-vapour, no fleas were found on it. Rat D 7 was then put into a freshly-prepared cage by itself. A healthy, clean tame rat (Rat D 8) was then put into Cage D.

Rat D 7 was examined on 25/12/09 and was found dead on 26/12/09.

28/12/09.—Rat D 8 was removed from Cage D, was carefully freed from fleas, and was put into a separate cage by itself; one flea recovered from it was returned to Cage D. A healthy, clean tame rat (Rat D 9) was then put into Cage D.

Rat D 8 was regularly examined from 29/12/09 till 3/1/10, when trypanosomes were first seen in its blood. Permanent smears were then made from 3/1/10 till 8/1/10, when the multiplication-period came to an end.

3/1/10.—Rat D 9 was removed from Cage D, was carefully searched for fleas, but no flea was found on it and it was put into a separate cage by itself. A healthy, clean tame rat (Rat D 10) was then put into Cage D.

Rat D 9 was regularly examined from 1/1/10 till 4/1/10, when trypanosomes were first seen in its blood. Permanent smears were then made from 4/1/10 till 10/1/10, when the multiplication-period came to an end.

(Experiment still proceeding—see below.)

Remarks.—It should be noted that on some occasions considerable numbers of fleas were recovered from the experimental rats in Experiment D, *e.g.*, 54 fleas on December 13, 38 fleas on December 15. As these fleas were

in all cases chloroformed in order to get them off the rats, it is possible that some of them may not have recovered from the chloroform when returned to Cage D, but may have succumbed; if so, the number of fleas active in this experiment would have diminished as the experiment proceeded. This and the short time during which some of the experimental rats were exposed may account for the break in the series of infections. Allowing for differences in individual susceptibility, it may be that the chances of any given rat becoming infected increase, up to a certain limit, with each bite of an infective flea. It must be pointed out, further, that the fleas were exposed to infection for only 22 hours. As they had been kept without food for 24 hours previous to this, it is probable that at least the greater number of fleas that survived must have fed on the infected rat. Comparing the results of Experiment D with the almost unbroken series of infections in Experiment C and with the positive results of Experiment A, and allowing for all possible errors, we may conclude that the chances of the flea becoming infective increase, up to a certain limit, with the time during which it is exposed to infection. It may be that only certain individual trypanosomes in the blood are capable of producing a permanent infection in the flea.

From Experiments C and D, it would appear that bred clean fleas that have fed on a rat heavily infected with *T. lewisi*, and showing many trypanosomes in its blood, are not infective until at least six days, or more, have elapsed from the time of their having first fed on infected blood; but that from the seventh day onwards they may retain the infection, as is most clearly seen in Experiment C, so as to be capable of infecting a series of clean, healthy rats, without themselves being exposed to re-infection. It is possible, even probable, that with less heavily infected rats and with varying conditions of temperature, season, etc., the incubation-period in the flea may vary somewhat, and that six days may be about the minimum period required for the completion of the cycle in the flea. In Experiments A and B this period seems to have been exceeded, although these two experiments deal, strictly speaking, with the method of infection without special attention to the length of the incubation-period in the flea. In none of our experiments, neither in those recorded here nor in any others we have performed, has direct infection taken place. In the case of *T. lewisi*, it is very doubtful if infection by the direct method ever does take place in nature, and even when conditions are favourably arranged in experiments, infection by the direct method has not been proved. Nuttall's experiments with fleas, to which reference has been made, were concerned with the *fact* of transmission only, and were not designed in such a way as to allow of any conclusion being drawn as to the *method* of transmission. He records a

positive experiment with *Ceratophyllus fasciatus*, and two positive experiments with *Ctenophthalmus agyrtes*. The fleas were in every case taken from infected wild rats, without any knowledge of when they first had an opportunity of feeding on infected blood. From such experiments it is useless to speculate on the method of infection. Nuttall, in the same paper, records a positive result with the rat-louse *Hæmatopinus spinulosus*, and concludes—"Since three distinct kinds of blood-sucking insects are capable of transmitting *T. lewisi*, it appears doubtful that this flagellate is a parasite of the invertebrate host in the sense claimed by Prowazek and other investigators." From the standpoint of transmission, however, the most important consideration in the general trypanosome problem is the distinction between "direct" and "cyclical" infection, which we laid down and defined above; and from the experiments which form the subject of the present communication, we conclude that the rat-flea *Ceratophyllus fasciatus* transmits *T. lewisi* from infected to non-infected healthy rats by the "cyclical" method, and that transmission by the "direct" method has not taken place. The importance, both from the scientific and the practical standpoint, of this "cyclical" method of infection cannot be over-estimated.

The length of the multiplication-period in the rat is probably less dependent on external conditions, and appears to be fairly constant. From these experiments, 12 days may be taken as the average length of the period from the time of inoculation by the flea until the multiplication ceases of the trypanosomes in the rat's blood. On the other hand, Experiment D defines the incubation-period in the flea within narrow limits: at the most from December 7 to 15, *i.e.* eight days, at the least from December 8 to 14, *i.e.* six days. From some of our other experiments, however, it would appear that in some cases the incubation-period is much longer, and that the cycle takes longer to complete in some fleas, or under some circumstances which are perhaps related to the conditions of the trypanosomes when taken up by the flea.

With regard to the cycle of development which the trypanosomes undergo in the flea, we hope to return to this in a future communication, but in the meantime we may point out from the observations recorded above of fleas dissected, that multiplication probably starts in the rectum of the flea, where masses of *Crithidia*-like forms are seen attached to the walls of the gut between the large rectal glands so-called. That these forms are stages in the development of *T. lewisi* in the flea is supported by the fact that in 50 fleas from the breeding cages dissected and examined, no trace of any flagellates was seen, whereas of 24 fleas from the same source, dissected after they had fed upon rats infected with *T. lewisi*, flagellates were found in 15, in 8 of which *Crithidia*-like forms were found in the rectum.

III. *Conclusions to be drawn from the Experiments.*

(1) The rat-flea *Ceratophyllus fasciatus* can transmit *Trypanosoma lewisi* from infected to non-infected rats.

(2) The transmission takes place by the cyclical method.

(3) Transmission by the direct method has not been proved to occur.

(4) The incubation-period of the flea, that is to say the period occupied by the developmental cycle of the trypanosome, has a minimum length of six or seven days, but may be longer.

(5) The multiplication-period of the trypanosome in the rat has a length of about 12 days.

(6) In the developmental cycle the establishment of the trypanosome in the flea begins with multiplication of *Crithidia*-like forms in the rectum.

Our conclusions with regard to the method of infection by the invertebrate host agree in the main with the results of Kleine's investigations upon the transmission of Sleeping Sickness of man, and Tsetse-fly Disease of animals, by *Glossina palpalis* and *G. morsitans* respectively.

Postscript.—January 24, 1910. *Tabular Summaries of Experiments C, D, and E.*

Experiment C.

Cage C colonised with 160 fleas that had been exposed to infection from November 24 till November 27.

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
C 1	November 27	November 30	0	—	—
C 2	,, 30	December 3	+	December 10	December 15
C 3	December 3	,, 6	+	,, 13	,, 18
C 4	,, 6	,, 8	+	,, 13	,, 19
C 5	,, 8	,, 10	+	,, 14	,, 21
C 6	,, 10	,, 11	+	,, 15	,, 23
C 7	,, 11	,, 13	+	,, 17	,, 24
C 8	,, 13	,, 15	+	,, 20	,, 26
C 9	,, 15	,, 17	+	,, 21	Found dead on December 28
C 10.....	,, 17	,, 20	+	,, 26	December 30
C 11.....	,, 20	,, 22	+	,, 28	January 1
C 12.....	,, 22	,, 24	Found dead on December 26.		
C 13.....	,, 24	,, 28	+	January 3	,, 8
C 14.....	,, 28	,, 31	0	—	—
C 15.....	,, 31	January 3	+	January 8	January 13
C 16.....	January 3	,, 6	+	,, 10	,, 15
C 17.....	,, 6	,, 10	+	,, 12	,, 17
C 18.....	,, 10	,, 13	+	,, 19	,, 24

Experiment D.

Cage D colonised with 162 fleas that had been exposed to infection from 2 p.m. on December 7 to noon on December 8, *i.e.* for a period of 22 hours only.

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
D 1	December 8	December 11	0		
D 2	,, 11	,, 13	0		
D 3	,, 14	,, 15	+	December 21	Rat died 26/12/09 before multiplication ended.
D 4	,, 16	,, 17	0		
D 5	,, 18	,, 20	0		
D 6	,, 20	,, 22	0		
D 7	,, 22	,, 24	0		
D 8	,, 24	,, 28	+	January 3	January 8
D 9	,, 28	January 3	+	,, 4	,, 10
D 10.....	January 3	,, 6	0	—	—
D 11.....	,, 6	,, 10	+	January 13	January 19
D 12.....	,, 10	,, 13	+	,, 17	,, 23

Experiment E.

Cage E colonised with 50 fleas that had been exposed to infection from 2 p.m. on December 7 to noon on December 8, a period of 22 hours. (See account of Experiment D.)

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
E 1	December 8	December 11	0	—	—
E 2	,, 11	left in	+	January 10	January 15

Remarks on Experiment E.—Allowing 12 days for the multiplication-period in the rat, we arrive at January 3 as being the date on which Rat E 2 became infected. The length of the incubation-period in the flea would thus appear to have been 26 or 27 days in this experiment, but this long incubation-period is, in our opinion, capable of a different explanation. We know that fleas once infective retain infection, and as Rat E 2 was left in Cage E, we may suppose that, being comparatively immune to begin with, it withstood infection for a long time, but that at last its resistance was overcome. That some tame rats are resistant in this way is shown by the breaks in Experiment D, and perhaps even more strikingly by the single break (Rat C 14) in the series in Experiment C.

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