OBSERVATIONS RELATING TO THE STRUCTURE AND GEOGRAPHICAL DISTRIBUTION OF CERTAIN TRYPANOSOMES.

By G. F. PETRIE, M.D.,

Assistant-Bacteriologist, Serum Department, Lister Institute of Preventive Medicine.

The following notes on trypanosomes which I have observed in certain mammals, birds, and goldfish obtained in the neighbourhood of Elstree in Hertfordshire appear worthy of publication, for the reason that our knowledge regarding the geographical distribution of trypanosomes is far from complete. No description has as yet been given of the trypanosomes found in bats by various observers, and a species encountered by me in the mole is, I believe, new.

I. Mammals.

Trypanosomes of Bats.

Laveran and Mesnil (1904, p. 104) state that trypanosomes have been observed by Dionisi in *Miniopterus schreibersii*, in other bats by Testi (1902) and Sambon (1904) in Italy, and by Durham (1900) in Parà; Donovan in Madras found trypanosomes in *Pteropus medius*. Hitherto no account has been published of the structure of bat trypanosomes[1].

Of eight bats caught here during the summer (June—July, 1904) (*Pipistrellus pipistrellus*) and examined shortly after death three showed trypanosomes in their blood.

[1] During the preparation of this paper for publication an account of trypanosomes occurring in bats in North Africa has been given by Ed. and Ét. Sergent (1905). They describe two forms, a small and a large trypanosome, the latter occurring rarely, and having been seen only in the fresh state. The small forms were found in 10 *Vespertilio kuhli* (Natterer) out of 26 examined, and in 7 *Myotis murinus* (Schreber) out of 35 examined. The large trypanosome was found in 2 *V. kuhli* (26 examined). The observers have named the small form *Trypanosoma nicolleorum* and the large one *T. vespertilionis*.

Numerous trypanosomes were found in the blood of one of the infected bats (a young female); the organisms disappeared from a hanging-drop preparation which was kept at 36° C. overnight. In the second bat a few trypanosomes were seen in the peripheral blood. The animal was etherised and the heart-blood examined but none could then be seen. In the third bat the trypanosomes were abundant in the blood. The skin of this bat was infested with fleas. Careful examination revealed no other external parasites on the skin.

A little of the blood from the last bat was inoculated intraperitoneally into a young white rat but an infection did not follow. Cultivation of the trypanosomes was attempted on rabbit-blood agar, kept at room temperature. At the end of 8 days numerous parasites were seen and one small clump of 2 or 3 members was suggestive of commencing multiplication. Unfortunately the medium became contaminated by a mould and the organisms soon disappeared.

It may be added that a specimen of a larger species of bat (*Pterygistes noctula*) showed no trypanosomes in its blood.

Structure of the Bat Trypanosome.

In a fresh preparation the movements of this trypanosome are very active, and seem to indicate a greater contortion than in the case of the rat trypanosome. It appears, moreover, to be considerably smaller than *Trypanosoma lewisi*. Preparations stained by Leishman's method show that it is in reality a small organism. Measurements were difficult on account of the almost invariably contorted position of the parasite in stained preparations. The length of one, which was in an extended position, was 16 μ (including the flagellum); the flagellum measured 8 μ. The breadth, including the membrane, near the macronucleus, was 1·5 μ.

In stained specimens the appearance of this trypanosome is quite characteristic, the large majority forming a circle or oval on account of the apposition of the anterior and posterior ends. Another distinctive feature lies in the close approximation of the macronucleus to the anterior end. The posterior extremity is pointed. The macro- and micro-nucleus stain deeply, while the protoplasm of the body of the parasite stains uniformly pale-blue—no granules being observable. The general appearance of the stained organism is such as to render its differentiation from the trypanosomes hitherto described a very easy matter.

Trypanosome of the Rabbit.

In a previous paper (1904) I described certain features of a trypanosome found accidentally in the blood of three tame rabbits, the parasite resembling very closely that of the rat. Microscopically it is impossible to distinguish them. Another similarity is found in the fact that the rabbit trypanosome is able to live for at least a month outside the body if kept at a comparatively low temperature.

Further observations have shown that tame rabbits are rarely infected; thus, 230 rabbits have been examined without discovering a single infected animal. Forty wild rabbits have, however, yielded 4 positive results. It may, therefore, be fairly assumed that the wild variety is more frequently infected than the tame, exactly as in the case of the rat. To ensure that the parasites should not be overlooked, in eleven instances 10 c.c. of blood were defibrinated and centrifugalized, but the results were again negative.

So far I have not been successful in transmitting the infection from the wild variety to tame rabbits by inoculation; and attempts to infect white rats have also been unsuccessful.

A culture in rabbit-blood agar of trypanosomes from one of the wild rabbits has recently been obtained. After being kept for 16 days at room temperature a hanging-drop preparation was made and showed dividing forms, though not in great numbers. The largest number seen in any one field under a $\frac{1}{12}$ oil immersion lens was 12, arranged in groups of 5, 4 and 3 members; whereas in the original blood many fields had to be examined before one was observed. The forms seen were similar to those in cultures of *Trypanosoma lewisi*.

Trypanosoma lewisi.

Trypanosomes occur in wild rats in this locality with considerable frequency. No exact data are available but the proportion infected may be stated roughly as 30 %. In a lot of 6 rats captured in one place in the middle of December 1 full-grown and 4 young rats were found to be infected. The blood of 2 of the young rats contained numerous dividing forms, including rosettes consisting of small trypanosomes—one group being composed of 10 individuals. It may be noted that this stage is very rarely observed in wild rats; the only observers of it hitherto being Sivori and Lecler. In specimens stained by Leishman's method numerous forms arising from division were seen

similar to those observed in an artificially produced infection. Special attention was paid to the character of the rosettes in stained preparations in view of the recently published observations of MacNeal (1904) on the multiplication forms. MacNeal contends that the grouping by Laveran and Mesnil of the methods of division cannot be accepted. The latter observers classify the dividing forms into two groups, the 1st including those arising from unequal longitudinal division, the 2nd consisting of forms in which there is "a simultaneous division into several coequal daughter-cells, no mother-cell being distinguishable." The forms which I have observed in the specimens of the natural infection undoubtedly support the views held by MacNeal. In every rosette the flagellum of the parent cell can be easily distinguished, while on the other hand on no occasion have rosettes been seen which resemble the figures given by Laveran and Mesnil. The anomalous forms referred to by MacNeal, in which a long, slender extension of the posterior extremity occurs, were also noted. The significance of this does not appear to be known.

Mole (*Talpa europaea*).

The blood of a mole was examined a few hours after death. There were a very few trypanosomes present, which appeared to be similar in size and form to the rat trypanosome. The blood was pipetted into rabbit-blood agar, and kept at room temperature. 24 hours later one trypanosome with extremely active movements was seen in a hanging drop. Cultivation proved unsuccessful owing to contamination. Films of the blood were stained, but stained organisms could not be found. 3 moles captured in the month of January were examined with negative results.

Recently 5 other moles have been found to be infected, *i.e.* 6 altogether out of 20 examined (30 %). The parasites in every case were present in such small numbers that a satisfactory stained preparation has not yet been obtained. A white rat was inoculated intraperitoneally with the heart-blood of one of the infected animals, but no infection followed.

Field-Vole (*Microtus* [*Arvicola*] *agrestis*).

12 of these have been examined with negative results.

II. Birds.

Trypanosomes of Birds.

It may be recalled that trypanosomes in birds have been found by Danilewsky (1885–1889) in Russia, by Dutton and Todd (1903) in the Gambia, by Hanna and Donovan (1903) in India, and by Ed. and Ét. Sergent (1904) in Algeria. Laveran and Mesnil (1904, p. 353) describe them as occurring in the owl (*Syrnium aluco*) in France, but it is interesting to note that they record their failure to observe them in a great number of other birds in that country. Levaditi, however, has discovered a trypanosome in the blood of *Padda orizyvora* obtained in Paris. An account of the structure of this organism is given by Laveran and Mesnil, and inoculation experiments have been carried out quite recently by Thiroux (1904) in the Pasteur Institute. Schaudinn's (1904) conclusions on the relationship between trypanosomes and endoglobular parasites were based on observations of the blood parasites of *Athene noctua* in Rovigno.

The examination of the blood of birds in this district proved negative with regard to the occurrence of trypanosomes; on the other hand, these parasites were found in several cases in the bone-marrow. The birds were mostly obtained in the months of June, July and September, and preparations of the blood and marrow were made within an hour or two of death. The following Table gives the results in a succinct form.

Thus out of 67 birds examined 11 had trypanosomes in the bone-marrow (16·4 %). In those cases in which they were present a careful search was necessary in order to detect them, and, indeed, in stained preparations it was almost impossible to find a specimen. The blood was in every case examined for trypanosomes and intra-corpuscular parasites but on no occasion were these observed.

The trypanosomes found in the marrow showed active movements when examined in normal salt solution; movements from place to place did not occur to any extent. In a fresh specimen from a blackbird several refractive granules were seen in the middle of the body of the parasite. A trypanosome found in a swallow appeared to be smaller than one observed at the same time from a blackbird.

The discovery of a *Spirochaete* in the blood of a martin and a trypanosome in its bone-marrow is interesting in the light of Schaudinn's researches. In support of his views regarding the relationship between

Bird	Number examined	Date	Result
House-Martin; *Chelidon urbica* (Linn.)	1	June	Spirochaetes in blood; a few trypanosomes in bone-marrow.
Song-Thrush: *Turdus musicus* (Linn.)	6	June, July, Sept., Nov., Dec.	3 examined in June and July had trypanosomes in marrow. 1 (28. 9) negative. 1 (22. 11) blood negative; one trypanosome seen in fresh preparation of marrow. 1 (19. 12) blood negative; one trypanosome seen in fresh preparation of marrow.
Blackbird; *Merula merula* (Linn.)	6	July, Sept., Nov., Dec.	1 (15. 9) blood negative; one trypanosome seen in preparation of marrow. 1 (25. 11. 4) blood negative; fair number of trypanosomes in marrow. Fresh preparations of liver, spleen, ovary, kidney, all negative. 1 in July; blood and marrow negative. 1 (17. 11) ,, ,, ,, ,, 1 (26. 11) ,, ,, ,, ,, 1 (12. 12) ,, ,, ,, ,,
Swallow; *Hirundo rustica* (Linn.)	11	June, July, Sept.	1 (15. 9) a very few trypanosomes in marrow. 5 in June—July; blood and marrow negative. 3 (14. 9) ,, ,, ,, ,, 2 (22. 9) ,, ,, ,, ,,
Chaffinch; *Fringilla coelebs*	1	26. 11. 4	Blood negative; a few trypanosomes in marrow.
Yellowhammer; *Emberiza citrinella*	1	16. 11. 4	,, ,, ,, ,, ,, ,,
House-Sparrow; *Passer domesticus* (Linn.)	27	June, July, Sept., Nov.	4 in June—July; blood and marrow negative. 2 (15. 9) ,, ,, ,, ,, 16 (21. 9) ,, ,, ,, ,, 1 (9. 11) ,, ,, ,, ,, 4 (16. 11) ,, ,, ,, ,,
Starling; *Sturnus vulgaris* (Linn.)	8	June, July, Sept., Nov., Dec.	5 in June, July, Sept.; blood and marrow negative. 1 (28. 11) ,, ,, ,, ,, 1 (12. 12) ,, ,, ,, ,, 1 (19. 12) ,, ,, ,, ,,
Crow; *Corvus frugilegus* (Linn.)	4	June, July, Sept.	3, blood and bone-marrow negative. 1, ,, ,, ,, ,, ,,
Jackdaw; *Coloeus monedula* (Linn.)	2	June, July	,, ,, ,, ,, ,,

these two organisms he mentions a similar instance, viz., the *Spirochaete* and *Trypanosome* found by Theiler (1903) in ordinary red-water and Rhodesian red-water fever (African Coast Fever). The spirochaete was present in considerable numbers in the martin's blood when examined immediately after death. In the fresh specimen the organism appeared to have several spiral twists (4 or 5), but in the stained preparation not more than two. In a film of the blood stained by Leishman's method the protoplasm has a pale-blue tint with no chromatin staining. The average length is 6·7 μ, breadth 1·5 μ. The spirochaetes were not present in the bone-marrow and there were no intra-corpuscular parasites.

III. Fishes.

Trypanosoma danilewskyi.

19 goldfish were examined, chiefly in the early months of the year. Trypanosomes were found in all, though in such small numbers that prolonged examination of the fresh specimen was usually necessary.

In a hanging-drop preparation the movements were exceedingly active and very contorted. The protoplasm had a granular appearance. With Leishman's stain the protoplasm of the body has a pale-blue colour, and contains small unstained spaces and chromatin granules. The macronucleus is situated near the middle of the body and stains more feebly than the micronucleus. As regards measurements the length of one was 38·4 μ, of another 32 μ, and of a third 48 μ: the breadth varied from 2 to 3 μ.

The blood of an infected fish was removed aseptically from the heart and pipetted into the condensation water of rabbit-blood agar. The tubes were then kept at room temperature. In 10 days a considerable number of trypanosomes were seen in a hanging-drop preparation. In shape they were curiously tadpole-like, contrasting with the slender form of the fresh specimen. Their movements were much less active than those in a fresh blood preparation. On the 11th day a group of 4 members was seen, and on the 12th day one with 5 individuals. On the 13th day the trypanosomes were much fewer. Sub-cultures were made but did not grow, though a considerable amount of inoculating material was added to the culture tubes. The shape of the trypanosomes, the feeble motility of their flagella, and the fact that they did not give rise to a sub-culture point to their being degeneration

forms, similar to those observed in cultures of *Trypanosoma lewisi* and *Trypanosoma brucei*.

Small leeches were occasionally found attached to the scales of the fish during the summer months, and as they were the only external parasite seen it is not unlikely (as has already been suggested) that the infection is conveyed by them.

The principal point of interest elicited by these observations lies in the fact that in a limited area so many animals of widely varying species should harbour trypanosomes. The following Table shows this clearly:

I. MAMMALS.

1. Bats:—(*a*) *Pipistrellus pipistrellus*, 8 examined, 3 positive.
   (*b*) *Pterygistes noctula*, 1 examined, negative.
2. Rats:—proportion of infected animals estimated at 30 %.
3. Wild Rabbits:—40 examined, 4 positive—(10 %).
4. Field-Vole (*Microtus agrestis*):—12 examined, all negative.
5. Mole (*Talpa europaea*):—20 examined, 6 positive—(30 %).

II. BIRDS.

67 examined, 11 positive—(16·4 %).
Trypanosomes were found in 6 out of 10 species.

III. FISHES.

19 Goldfish examined, all positive.

EXPLANATION OF PLATE VIII.

Fig. 1. *a—g*: Trypanosome of the bat—showing frequent contortion of the body of the parasite.

Fig. 2. Multiplication forms of *Trypanosoma lewisi* occurring in blood of naturally infected wild rat; the flagellum of the parent-cell is easily distinguishable.

Fig. 3. Spirochaete observed in blood of the house-martin; trypanosomes were present in the bone-marrow.

Fig. 4. Trypanosome of the rabbit—showing a close resemblance to *Trypanosoma lewisi*.

Fig. 5. Trypanosome found in blood of goldfish (*Tryp. danilewskyi*).

Fig. 6. Sketch of fresh preparation of trypanosome found in the bone-marrow of the blackbird.

In conclusion, the more noteworthy facts recorded above may be summarised as follows:

1. The trypanosome of the bat seems to be sharply defined in

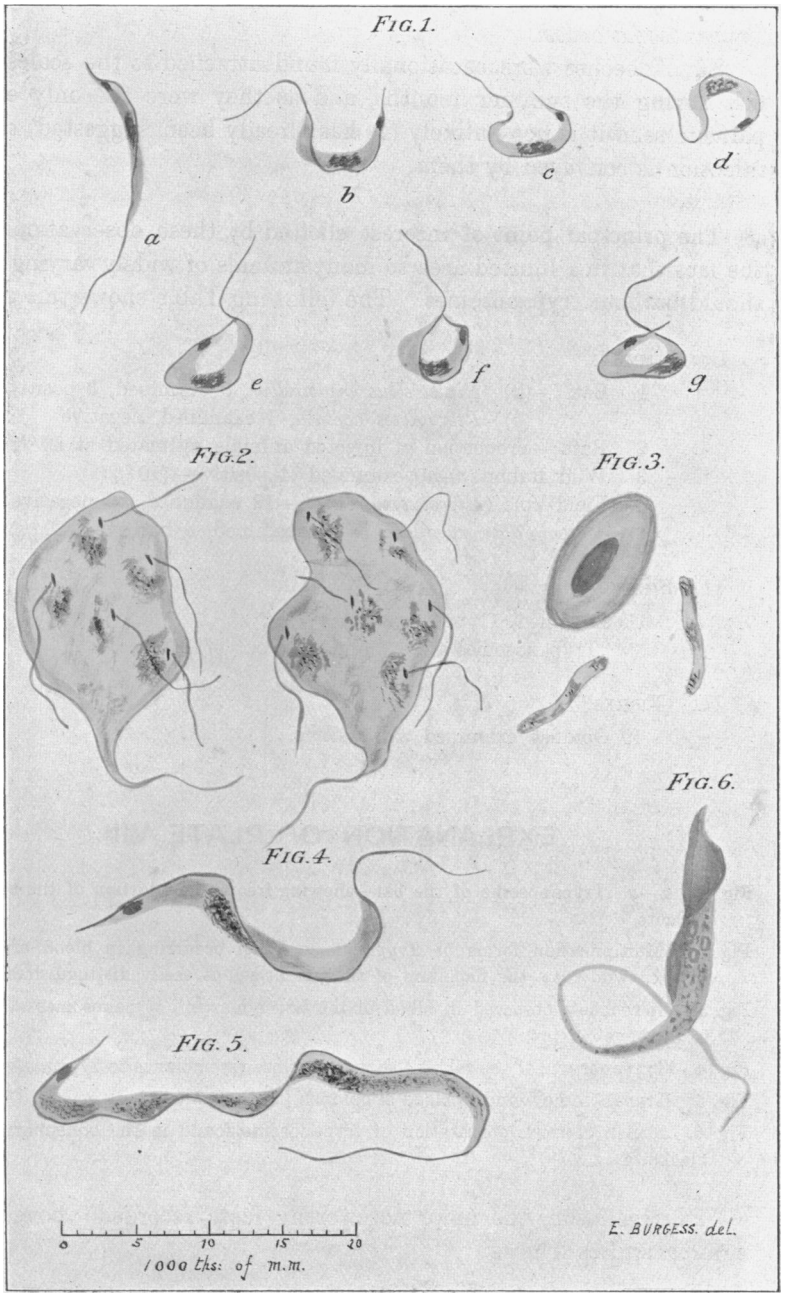
FIG. 1.
a
b
c
d
e
f
g
FIG. 2.
FIG. 3.
FIG. 6.
FIG. 4.
FIG. 5.
0 5 10 15 20
1000 ths: of m.m.
E. BURGESS. del.

several respects from trypanosomes hitherto described; chiefly from its small size and from its characteristic looped appearance in the stained specimen.

2. The wild rabbit, as in the case of the rat, is more frequently infected than the tame variety. Cultivation of the trypanosome for one generation at least presents no difficulty.

3. Blood-films from 2 wild rats with a natural infection of *Trypanosoma lewisi* in the stage of multiplication have been examined. The forms observed support the contention of Wasielewski and Senn and MacNeal that the mode of multiplication can be traced in every instance to an unequal longitudinal division.

4. A new species of trypanosome has been found in the blood of the mole.

5. Trypanosomes have been observed not infrequently in this locality in the bone-marrow of certain common birds but not in their blood.

6. No intra-corpuscular parasites have been observed in any of the birds examined.

7. In the case of one bird (*Chelidon urbica*, Linn.) a spirochaete in the blood was associated with a trypanosome in the bone-marrow.

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