BLOOD PARASITES OF THE MOLE,—INCLUDING A NEW FORM OF INTRA-CORPUSCULAR PARASITE.

(Plate X and One Diagram.)

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THE material on which these notes are founded was obtained from moles captured in the neighbourhood of the Lister Institute at Elstree in Hertfordshire from the middle of May to the beginning of July, 1906¹. In all 14 moles were examined.

1. *Trypanosomes*. Laveran and Mesnil (1904, p. 98, footnote) state that in 1845 trypanosomes, then described as vermicules, had been seen by Gros in the blood of moles and that they had not been seen since. Petrie (iv. 1905) observed a species of trypanosome in the mole which he believed to be new. This trypanosome he found in the blood of 6 out of 20 moles examined: but "the parasites," he says, "in every case were present in such small numbers that a satisfactory stained preparation has not yet been obtained." In the absence of stained preparations the morphological characters of the trypanosome found in the blood of the mole have, so far as I know, not hitherto been described. For the purpose of comparison with other trypanosomes the following average measurements are given and they are likewise represented in the accompanying diagram (p. 575).

From posterior extremity of body to centre of centrosome	9·3 μ
From centre of centrosome to posterior border of nucleus	4·6 μ
Nucleus	2·0 μ
Anterior border of nucleus to anterior end of body	6·5 μ
Free flagellum	5·2 μ
Greatest depth of body	about 3·5 μ

¹ Specimens were demonstrated before the Inaugural Meeting of the Pathological Society of Great Britain and Ireland in July, 1906.

Only adult forms have been observed and they are fairly uniform in size and shape. The most striking feature, perhaps, is the long, pointed, proboscis-like posterior extremity. Under certain circumstances it can apparently be partially retracted as shown in Plate X, Fig. 6. From its staining reaction it is evidently a prolongation of the body-protoplasm and not a posterior flagellum. The nucleus is small relative to the centrosome and lies close to the ventral border. Though it and the centrosome are usually sharply defined, both are occasionally obscured by groups of chromatin particles varying in size and irregular in shape as shown in Fig. 5. In Fig. 7 there is the appearance of a vacuole in front of the nucleus.

Diagram 1.

In the living condition this trypanosome is actively progressive, and though it has been seen to move short distances with its posterior extremity forward, it usually progresses with the flagellum to the front,—feeling its way, as it were, among the corpuscles.

Inoculation experiment. A white rat was inoculated intraperitoneally with blood containing living trypanosomes, but no infection followed.

Attempts at cultivation have so far proved unsuccessful.

2. *Bacillary-like bodies in the red blood corpuscles.* These are doubtless identical with the bodies described by G. S. Graham-Smith (x. 1905). His photographs (Plate XIV) leave no doubt as to the identity of the organism described by him and those here referred to. I have not yet seen anything comparable to the forms depicted in his coloured plate. I find that they stain with all the ordinary bacterial stains:—Carbol-thionin, dilute carbol-fuchsin, methylene blue, as well as by various modifications of the Romanowsky method. They are not acid fast and they do not stain by Gram.

Inoculations of the blood from the heart of an infected mole into a rabbit (intravenously), a rat and a guinea-pig (intraperitoneally), and a mouse (subcutaneously), all gave negative results.

The relative proportion of infected moles and the degree of infection seems to be much greater in the neighbourhood of Elstree than in that of Cottenham near Cambridge, where Graham-Smith found them. Out of a total of 102 moles examined by him 10 were found to be infected, and in only two of these ten was the infection large. Of 14 moles examined here during May and June of this year 12 were found to be infected, some largely so, and all to such a degree that there was no difficulty in finding infected corpuscles. No mention is made of the time of the year when Graham-Smith found his infected moles, so that it is just possible that the difference may be seasonal.

3. *New form of intracorpuseular parasite.* The form now to be described was found in two out of the 14 moles examined. As seen in stained preparations it is larger than a normal red blood corpuscle. The corpuscle in which it lies is stretched and reduced to a mere shell, and what remains of haemoglobin is altered, as indicated by its staining of a somewhat paler and more yellowish tint than adjacent normal corpuscles. The parasite itself varies in shape. When completely enclosed in the remains of a red blood-corpuscle it is more or less oval in outline. Where the corpuscle has become ruptured the parasite may assume various irregular amoeboid outlines, as seen in Plate X, Figs. 12 and 15. The body protoplasm stains by Giemsa's method a pale blue, while the nucleus gives the usual chromatin reaction. The nucleus varies in appearance. In some it is irregularly ring-shaped, the thickness of the ring too varying much in different parts. In others it is more diffuse, irregularly vacuolated or almost reticular in appearance. When ring-shaped or coarsely reticular, the parts within the ring or in the meshes of the reticulum usually stain pale blue like the body protoplasm. In the body protoplasm of most, but not of all, are to be seen small round sharply circumscribed particles almost black in colour (? Melanin).

Vassal (1905) describes an intracorpuseular parasite found by him in the blood of a squirrel (*Sciurus grisiemanus*), in Annam. Ring-forms, schizonts and gametocytes are described as being present in the blood at the same time; and the ring-forms are said to be indistinguishable from the ring-forms of the parasite of malignant malaria. The gametocytes of the parasite discovered by Vassal grow to about the same size as the parasite of the mole here described, though there are

morphological and other differences that need not here be detailed. Vassal attaches great importance to the resemblance between the parasite of the squirrel and that of human malaria, as suggesting the possibility of the squirrel being an alternative host to man for the parasite of malignant tertian fever.

In new countries where malaria attacks pioneers of civilization, and in those intensely malarious districts already occupied, where disturbance of the soil is followed by severe outbreaks of fever, it would be well in searching for the intermediary host in the absence of man, to examine the blood of animals such as the mole, that live and burrow in the soil.

In the blood of the two moles that harboured this parasite trypanosomes were also found, while they were the only two out of fourteen examined that did not show the bacillary-like form described by Graham-Smith. Trypanosomes were found associated with the bacillary-like forms in one case out of the twelve.

Smears from various organs.—Brain, bowel, bone-marrow, spleen, liver, lymphatic gland, etc., were examined but though prolonged search usually revealed a parasite or parasites in proportion to the number of blood cells present, no variation in the appearance was observed.

Though the material at disposal is insufficient to establish the life-history of this parasite, and though the protozoal nature of the bacillary-like forms first described by Graham-Smith may not be thoroughly established, certain points are of sufficient interest to raise the question of a possible relationship and to merit brief discussion. Granted that the number is small it is still a very significant fact that of a total of fourteen moles examined no less than twelve were found to be infected by the bacillary-like form, while the only two that were not so infected harboured the large amoeboid form. A great drawback to the further study of these forms will be the difficulty of keeping moles alive. They seem to die very readily in captivity. It is, therefore, interesting to note that the mole in which the large intracorpuseular parasite was first found escaped, but was recaptured (unfortunately killed) on the 15th day after its escape, and that in the blood films taken before and after, the same appearances were found. The parasite, therefore, must either have a life-cycle of 14 to 15 days or it remains in the form in which we have seen it for some considerable time.

Analogy with the crescent of the malignant malaria parasite might suggest that these large forms appear late in the infection, persist in

the blood after all former bodies have disappeared, subserve the interest of the species and require for their further development the intervention of an intermediary host. The fully developed crescent, however, is a resting form while the form we are now discussing,—if we may deduce this from appearances seen in stained preparations,—seems still capable of amoeboid movements. (Plate X, Figs. 12 and 15.)

It is hardly probable, though still possible, that the organism, whatever it is, had just completed its life-cycle in the period during which the mole was at large. On the other hand, it may be a parasite with a very long life-cycle, so that at the stage in which we have seen it no appreciable change is to be detected in 14 days. Allowing that the forms seen may be developing gametocytes they may still be stages in the life-history of some parasite of which no other stage has yet been seen. This large form must have entered the red blood corpuscle at a very much earlier stage and must have grown in its interior to the size we now see it.

The fact that 14 infected moles have been examined without our being able to demonstrate transition forms argues against the likelihood of a relationship between the bodies described by Graham-Smith and this new form. On the other hand, the evidence in favour of the view that this new form is a parasite *sui generis* rests so far on insufficient data. A much larger number of moles harbouring this parasite would require to be examined before reliable conclusions could be deduced. The association of this haemocytozoon with trypanosomes in the blood of the two moles in which it has been observed may be mere coincidence; but in view of Schaudinn's observations on *Trypanosoma noctuae* and of the relationship claimed by him to exist between endocorpuscular (*Halteridium*) forms and free (*Trypanosoma*) forms of that parasite in the blood of the owl (*Athene noctua*), further classification may well be delayed until something more is known regarding its life history.

REFERENCES.

- LAVERAN, A. and MESNIL, F. (1904). *Trypanosomes et Trypanosomiases*. (Paris, Masson et Cie.)
- PETRIE, G. F. (IV. 1905). Observations relating to the structure and geographical distribution of certain trypanosomes. *Journ. of Hygiene*, v. pp. 191–200, Plate VIII.
- GRAHAM-SMITH, G. S. (x. 1905). A new kind of parasite found in the red blood corpuscles of moles. *Journ. of Hygiene*, v. pp. 453–459, Plates XIII–XIV.
- VASSAL, J. J. (1905). Sur un hématozoaire endoglobulaire nouveau d'un mammifère. *Annales de l'Institut Pasteur*, Vol. 19, p. 224.

DESCRIPTION OF PLATE X.

TRYPANOSOMES OF THE MOLE.

- Figs. 1, 3, 4, 6 and 7. Trypanosomes with centrosome and nucleus compact and sharply defined. Fig. 6 also shows the posterior extremity partially retracted; and Fig. 7 the appearance of a vacuole in front of the nucleus.
- Figs. 2 and 5. Trypanosomes with diffuse chromatin-staining granulations in which the centrosome and nucleus are more or less obscured.

NEW FORM OF INTRACORPUSCULAR PARASITE FOUND IN THE MOLE.

- Figs. 8—12. Parasites with irregularly-shaped nucleus showing various degrees of vacuolation; and with dark granules in the body protoplasm varying in number and arrangement. Fig. 12. Free parasite. In most cases the remains of the corpuscle is seen to surround the parasite.
- Figs. 13—15. Parasites with nucleus irregularly ring-shaped. Fig. 15. Free parasite with remains of red blood corpuscle still clinging to one end.

NOTE.

- Figs. 1, 3, 4, 6 and 7. From blood films fixed in methyl alcohol and stained by Giemsa's method.
- Figs. 2 and 5 also 8—15. From blood films fixed in absolute alcohol and stained in a freshly prepared mixture of weak watery solutions of Giemsa's azur II and of eosin. All specimens magnified 1200 diameters.