FURTHER EXPERIMENTAL INVESTIGATION INTO SUDAN KALA-AZAR.

By Captain W. E. MARSHALL.
Royal Army Medical Corps.

In previous communications on Sudan kala-azar it has been shown that this form of Leishmaniasis has the following characteristics:—

(1) It affects people of all ages, but is much commoner in late childhood and early adult life.

(2) The *Cercopithecus sabæus*, the ordinary grey monkey of the Sudan, can be infected with the disease by intraperitoneal or subcutaneous injection of the parasite.

(3) The parasite grows readily on 10 per cent citrate, on Novy and MacNeal's medium and on Nicolle's medium.

(4) The parasite can be demonstrated in the peripheral blood of the infected in a large percentage of the cases.

In the study of the method of transmission of infantile Leishmaniasis considerable progress has been made. The similar geographical distribution of canine and human Leishmaniasis and the occurrence of human cases in close contact with canine cases made it extremely probable that infection was conveyed from dog to child. There is now considerable experimental evidence in support of this theory, the probable transmitter being the *Ctenocephalus canis*, the dog-flea. Basile was successful in transmitting the disease from infected to uninfected pups, and was also able to infect pups with fleas brought from an infected district. The parasite has also been demonstrated in the interior of fleas by Basile, Sangiorgi, and Alvarez and Da Silva. Basile, La Cava and Visentini have found them also in *Pulex irritans*, the human flea. Franchini and Gabbi, on the other hand, have failed to find any intracorporeal development in the body of the flea. In support of the dog-flea theory it may be mentioned that the Sergents, Lombard, and Quilichini have recently found an infected kitten living in the house containing an infected child and an infected dog.

Present Investigation.

The following experiments are a continuation of the work already published with regard to the clinical features of the disease. There is little to add, but it is necessary to modify the statement

that the parasite is always present in the peripheral blood in small numbers, as two cases were seen where the parasites were present in considerable numbers. In one case, shown in the plate, there were two mononuclear cells in one slide, one containing thirty-two parasites and the other twenty-nine. This was early in the course of the disease. In the other case the parasites were extremely numerous, but were more uniformly distributed among the white blood-cells, the majority being inside polynuclear cells. This was from a case in the advanced stages of the disease.

The diminution or absence of eosinophile cells from the peripheral blood is usually well marked, and is of some help in differentiating kala-azar from malarial infections where usually a slight eosinophilia exists.

No drug has been found which in any way affects the course of the disease, and all cases seen since our last report have terminated fatally; "606" was tried in one case, but without any benefit.

Experimental Kala-azar in the Dog.

Our previous inoculation experiments with dogs were inconclusive, as with one exception all the dogs died soon after inoculation. We have therefore again inoculated four dogs with the parasites and in each case a positive infection was obtained.

Dog 1, ♂, injected intraperitoneally with post-mortem spleen emulsion from infected monkey.

82nd day: Liver puncture positive.

145th day: Chloroformed, infected with Leishmania.

Dog 2, ♂, pup, injected intraperitoneally; material obtained by post-mortem spleen puncture from a human case.

Died on 128th day; heavily infected with Leishmania.

Dog 3, ♂, pup, injected intraperitoneally with post-mortem spleen emulsion from Dog 1.

Died on 95th day; infected with Leishmania.

Dog 4, ♂, pup, injected intraperitoneally with material obtained by spleen puncture from a human case.

Died on 74th day; heavily infected with Leishmania.

There is therefore no doubt that dogs are susceptible to Sudan kala-azar. They can be infected from the monkey, from another infected dog, or from human cases. Young dogs are more susceptible, and in them the disease runs a more acute course. Dog 1, which was an adult, showed very few symptoms, and was in fairly good condition on the 145th day when it was chloroformed. As in the monkey, probably the best way of producing an infection is from

a spleen puncture of a human case during life. Dog 4 was inoculated in this way, and died with a severe infection on the 74th day.

Owing to Basile's discoveries it was decided to try to convey the disease from dog to dog by means of fleas, the *Ctenocephalus canis* being used.

Dog 5, ♂, was put in the room beside Dog 1 after the latter was known to be infected. The dogs were in contact for thirty-seven days, and fleas were frequently placed in the room and were constantly present on both dogs. No infection occurred. This dog was chloroformed five months later, when no parasites were found on post-mortem examination.

Dog 6, ♀, pup, was put in the box beside Dog 2, four days after the latter had been inoculated. They were together for 124 days and fleas were frequently placed in the box. No infection occurred. This dog was chloroformed two months later; the post-mortem examination showed filaria, but no Leishman-Donovan parasites.

So far, therefore, we have been unable to convey infection from dog to dog by means of the flea. It must be borne in mind, however, that these experiments were carried out in the summer, when human cases are less frequent, and when the temperature is probably less favourable for infection.

A similar experiment was carried out to determine if ticks could convey the disease from dog to dog.

Dog 7, ♂, pup, was put in box beside Dog 3, when the latter was inoculated. The dogs were 95 days in contact. Ticks were introduced at frequent intervals. No infection occurred in Dog 7.

Experimental Kala-azar in the Grey Monkey (*Cercopithecus sabæus*).

Further experiments were carried out to endeavour to determine by what means the disease may be conveyed from monkey to monkey.

In one experiment an infected and a healthy monkey were freed from all insects and were kept together in a wire-gauze cage. They were fifty-four days in contact. No infection occurred in the healthy monkey.

In a second experiment similar precautions were taken, but monkey fleas were placed in the cage. Unfortunately the infected monkey died twelve days later, so they were only a short time in contact. No infection occurred in the healthy monkey.

In a third experiment an infected and a healthy monkey were

freed from all insects and kept in an open cage. They were seventy-one days in contact. No infection occurred in the healthy monkey.

In a fourth experiment no precautions were taken to render the monkeys free from insects, and one infected and two healthy monkeys were put together in an open cage. There was a doubtful infection of one healthy monkey, but the slides were unsatisfactory, the post-mortem examination being made some time after death.

In a fifth experiment, similar to the fourth, no infection occurred in the healthy monkeys.

In a sixth experiment numerous lice (*Pediculus capitis*) were fed at intervals on a human case and then transferred to a healthy monkey. No infection occurred.

These results are therefore all negative, with the possible exception of one monkey in Experiment 4. In that experiment lice were the only insects actually found. [The monkey louse (*Pedicinus*) is quite different from the human species, having a very elongated head and only three instead of five joints to the antennæ.] In this experiment also the infected monkey was removed from the cage for a time in order to see if the *Ctenocephalus canis*, the dog-flea, would infect the monkey, though with apparently negative results. As the cage was an open one mosquitoes also were not excluded, so that even if this monkey were infected, the louse, the flea, and the mosquito are all possible carriers of infection.

In the autumn many of the monkeys suffered from the plasmodium of monkey malaria, and we lost many animals.

Though one can, in the majority of cases, be sure of infecting monkeys, we had one animal which showed a natural immunity from infection. It was injected intraperitoneally from spleen puncture of a human case, but no infection occurred. Six months later it was again inoculated intraperitoneally with a spleen emulsion from Dog 1, and again no infection took place. Another monkey inoculated with the same splenic emulsion contracted the disease. Perhaps, as Delanoë has recently shown occurs with cultures of Leishmania, the immunity is purely phagocytic.

Conclusions.

(1) Dogs can be experimentally infected with Sudan kala-azar.

(2) They can be infected from human cases, from infected monkeys, or from other infected dogs.

(3) Young dogs are more susceptible, and in them the disease runs a more acute course.

(4) Experiments to transmit the disease from dog to dog by means of the *Ctenocephalus canis*, the dog-flea, gave negative results.

(5) The Leishman-Donovan parasite is occasionally present in large numbers in the peripheral blood of human cases.

LITERATURE.

ALVAREZ and PEREIRA DA SILVA. *Medicine Contemporanea*, 1911, June 18, No. 25, p. 197.
Ibid., July 2, No. 26, p. 216.
BASILE. *Rendiconti della R. Accademia dei Lincei*, 1910, November 20, p. 523.
Ibid., 1911, January 8, p. 50.
Ibid., February 19, p. 278.
Ibid., March 19, p. 479.
Ibid., June 18, p. 955.
Ibid., July 16, p. 72.
BASILE and VISENTINI. *Rendiconti della R. Accademia dei Lincei*, 1911, April 23, p. 590.
BASILE, LA CAVA, and VISENTINI. *Rendiconti della R. Accademia dei Lincei*, 1911, August 6, p. 150.
DELANOË. *Annales de l'Institut Pasteur*, 1912, March 25, p. 179.
FRANCHINI. *Studi intorno alle Malattie Tropicali dell' Italia meridionale e insulare e delle colonie*, 1911, fasc. ii, p. 1.
GABBI. *Malaria e Malattie dei Paesi Caldi*, Rome, 1911, October, No. 10, p. 285.
MARSHALL. *Journal of the Royal Army Medical Corps*, 1911, xvii, No. 3, p. 255.
Fourth Report, Wellcome Tropical Research Laboratories, Khartoum, 1911, vol. A, p. 157.
SANGIORGI, *Pathologica*, 1911, January 15, No. 53, p. 23.
Ibid., March 1, No. 56, p. 89.
Ibid., May 15, No. 61, p. 231.
SERGENT (EDMOND) et SERGENT (ETIENNE), LOMBARD, et QUILICHINI, *Bull. Soc. Path. Exot.*, 1912, Février, p. 93.
THOMSON. Fourth Report, Wellcome Tropical Research Laboratories, Khartoum, 1911, vol. A, p. 143.

Drawings.—Fig. 1. Large mononuclear cell from peripheral blood, case of kala-azar; it contains thirty-two parasites. × 1,500. Giemsa's stain.

Fig. 2. Cell from same slide, containing 29 parasites. × 1,500. Giemsa's stain.

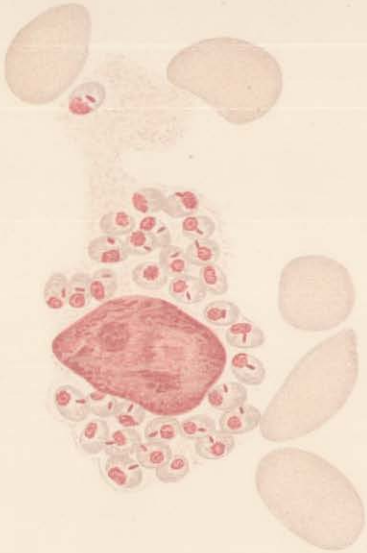

Fig. 1. Peripheral Blood. × 1,500 × × ×

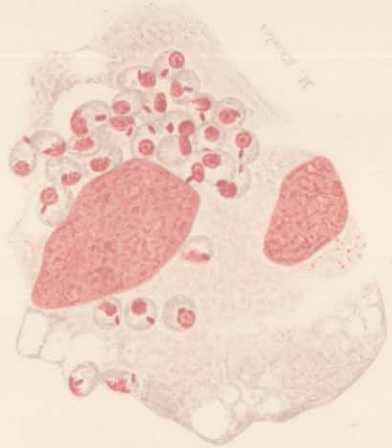

Fig. 2. Peripheral Blood. × 1,500 × × ×

To Illustrate "Further Experimental Investigation into Sudan Kala-Azar."
By Captain W. E. Marshall, R.A.M.C.