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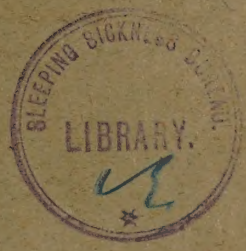
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EXPERIMENTAL KALA-AZAR IN
THE GREY MONKEY OF THE
SOUDAN (*CERCOPITHECUS*
SABÆUS). By LIEUTENANT W. E.
MARSHALL, R.A.MC.

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EXPERIMENTAL KALA-AZAR IN THE GREY MONKEY OF THE SUDAN (*CERCOPITHECUS SABÆUS*).

BY LIEUTENANT W. E. MARSHALL.

Royal Army Medical Corps.

(*From the Floating Laboratory of the Wellcome Tropical Research
Laboratories, Khartoum.*)

SUCCESSFUL experimental inoculation of animals with kala-azar has become a well-recognized fact in the case of the parasite of infantile kala-azar (*Leishmania infantum*). Nicolle and his colleagues, pioneers in this work, have frequently infected monkeys and dogs. So far they have only succeeded in infecting these animals by intraperitoneal inoculation, and subcutaneous injection has only given a local reaction without general infection. They also found that passage of the disease from monkey to monkey attenuated the virus, but the same phenomenon did not occur in dogs. They recognize differences in the infection in these two animals. In the monkey there is marked enlargement of the spleen, and the disease resembles the human type of the disease. In the dog the spleen is only slightly enlarged and the infection is insidious. One of the dogs inoculated with the disease, and which died seventeen months after inoculation, showed no symptoms for fifteen months. They also found that in a series of dogs there was great variation in the susceptibility of the individual animal. With regard to immunity they have found that in an animal incompletely cured, or only just recently recovered, a second inoculation produces a severe and fatal result, whereas in an animal cured for some time immunity is conferred. Immunity is also produced by the virus of Oriental sore. Excision of the spleen in the dog has produced no effect on the course of the disease.

Nicolle and his colleagues have also found that Leishmaniosis occurs as a natural infection in the dog, and this has recently been confirmed by Gabbi and Basile in Italy, Critien in Malta, Alvarez in Lisbon, and the Sergents in Algiers. Basile recognizes two different forms of natural infection in the dog, the acute and chronic, and he thinks that the former, which affects principally young dogs and lasts from three to five months, plays a more important part in the spread of the disease. He was able to infect three young dogs by keeping them beside cases of infantile kala-azar, and he thinks the flea (*Pulex serraticeps* and perhaps

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P. irritans) is the likely transmitting agent. Jemma, Gabbi, and Alvarez and Pereira da Silva have all succeeded in infecting dogs intraperitoneally with *L. infantum*, and Jemma has also produced infection by intravenous inoculation.

Laveran and Pettit, besides infecting dogs, have tried infecting rats and mice, but could not produce a general infection.

Novy, by the use of large and repeated doses of cultures of *L. infantum* on Novy and MacNeal's medium, has been able to produce infection in the dog.

Patton, working with Indian kala-azar, failed to infect dogs even with repeated inoculation, but probably he killed his animals too soon to say definitely that infection does not occur.

PRESENT INVESTIGATION.

The following experiments were carried out with parasites obtained from cases of kala-azar in Sennar Province in the Anglo-Egyptian Sudan. In this province the disease affects chiefly children about 12 years of age, though adults are also occasionally infected. So far we have found the best method of infecting monkeys is from a splenic puncture during life. An ordinary sterilized hypodermic syringe is taken, washed out with sterile citrate solution, and spleen puncture carried out in the ordinary way, the contents of the syringe being immediately injected into the peritoneal cavity of a monkey.

Twelve monkeys were used in these experiments, and the results are shown in the following table. Eight monkeys were infected with kala-azar, in three the experiment failed, and in one the result still remains doubtful.

INFECTED CASES.

Of the eight infected cases, five were infected intraperitoneally, one subcutaneously, one subcutaneously and intravenously, and one was naturally infected.

Of those infected intraperitoneally, monkey B was chloroformed on the 62nd day, when the spleen was found to be enlarged, and Leishman bodies were present in moderate numbers; no parasite could be found in the liver, so probably the spleen becomes infected before the liver.

Monkey D died on the 65th day from a mixed infection of tuberculosis and Leishmaniosis, and it is interesting to record the degenerate nature of the parasites in this case. The parasites

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were most degenerated in the spleen, which was heavily infected with tubercle, and were best preserved in the bone-marrow, where the tubercles were scanty. The post-mortem examination was done immediately after death, so the change in the parasites could not be a post-mortem one.

Monkey E was infected by the 54th day, became very ill with diarrhoea on the 143rd day, and was chloroformed on the 145th day. It was heavily infected with parasites.

Monkey N, inoculated intraperitoneally from monkey E, remained quite well, but, owing to Leishman bodies being present in monkey S (*vide infra*), liver puncture was carried out, and doubtful Leishman bodies were seen 35 days later, the 155th day; the monkey died, and the post-mortem examination showed the spleen to be enlarged, and parasites were found in the spleen, liver, and bone-marrow. There was a small ulcer in the ileum.

Monkey P, which died on the 134th day, was heavily infected with parasites.

Of those infected subcutaneously, in the case of monkey R the injection was made into the subcutaneous tissues of the thigh with a post-mortem splenic emulsion from a case of kala-azar. On the 150th day the spleen was found to be enlarged, and liver puncture showed the presence of Leishman bodies. On the 156th day the peripheral blood was examined, and in the fifth slide three parasites were found inside a large mononuclear cell. This monkey is still alive.

Monkey L received an intravenous and subcutaneous injection; it was intended to inject intravenously, but only the first portion of the virus entered the vein, the remainder entering the subcutaneous tissues of the forearm. Examined on the 161st day the spleen was found to be enlarged, and spleen puncture showed the presence of Leishman parasites. This monkey is also still alive.

The remaining monkey, which was a very young animal, was put in the same cage as monkey N on the day that the latter was inoculated intraperitoneally from monkey E. It remained in that cage, and on the 112th day it became ill. On the 117th day liver puncture showed the presence of Leishman bodies, and on the 121st day it died, thirty-four days before monkey N. It was very heavily infected, parasites being present in large numbers in the spleen, liver, and bone-marrow.

With regard to the negative cases, monkey H was inoculated with a splenic emulsion from a case of kala-azar, the emulsion being made eight hours after the death of the patient, when no

Animal	Where inoculated and date of inoculation	Material inoculated	Course of disease							Result	Organs infected	Remarks
Monkey B..	Liver and peritoneum 9.1.10	Post mortem spleen puncture. Case 4	30th day liver puncture negative 53rd day liver puncture negative 62nd day chloroformed							Infected	Spleen + Liver -	Spleen slightly enlarged; parasites present in moderate numbers.
Monkey C..	Peritoneum 12.3.10	Post mortem spleen emulsion. Monkey B	8th day peripheral blood negative	22nd day peripheral blood negative	33rd day peripheral blood negative	45th day peripheral blood negative. Liver puncture negative	66th day liver puncture negative	94th and 95th days liver puncture negative	108th day chloroformed	Not infected	Spleen - Liver - Bone-marrow -	
Monkey D..	Peritoneum 21.4.10	Spleen puncture. Case 3	14th day peripheral blood negative	55th day liver puncture negative	63rd day Ill.: Dyspnoea, splenomegaly. Liver puncture negative. Spleen puncture negative			65th day died		Infected	Spleen + Liver + Bone-marrow +	Miliary tuberculosis also present; spleen markedly infected with tubercle.
Monkey E..	Peritoneum 23.4.10	Spleen puncture. Case 1	14th day peripheral blood negative	54th day liver puncture positive	58th day peripheral blood negative	78th day Spleen enlarged. Spleen puncture positive. Liver puncture negative. Peripheral blood negative	143rd day diarrhoea. Liver puncture positive	145th day chloroformed		Infected	Spleen + Liver + Bone-marrow +	Monkey emaciated; spleen enlarged; liver enlarged and cirrhotic; bone-marrow red; intestinal intussusception present.
Monkey H..	Peritoneum 21.6.10	Post mortem spleen emulsion. Case 1	65th day chloroformed							Not infected	Spleen - Liver - Bone-marrow -	The spleen emulsion in Case 1 was made 8 hours after death.

Monkey K..	Sub-cutaneous tissues 11.7.10	Spleen puncture. Monkey E	160th day spleen enlarged. Liver puncture negative	161st day spleen puncture negative	163rd day spleen puncture negative	—	—	Still alive.
Monkey L..	Intra-venous and sub-cutaneous 11.7.10	Spleen puncture. Monkey E	161st day spleen enlarged. Spleen puncture positive			Infected	—	Still alive.
Monkey N..	Peritoneum 11.7.10	Spleen puncture. Monkey E	120th day liver puncture doubtful	155th day died		Infected	Spleen + Liver + Bone-marrow +	Small ulcer in small intestine, ileum ; smears and sections of this negative.
Monkey O..	Peritoneum 26.7.10	Post mortem spleen puncture. Case 10	42nd day found dead			Not infected	Spleen — Liver — Bone-marrow —	Spleen not enlarged.
Monkey P..	Peritoneum 31.7.10	Post mortem spleen emulsion. Case 11	123rd day liver puncture positive	134th day died		Infected	Spleen + Liver + Bone-marrow +	—
Monkey R..	Sub-cutaneous tissues 31.7.10	Post mortem spleen emulsion. Case 11	150th day spleen enlarged Liver puncture positive	156th day peripheral blood positive		Infected	—	Still alive.
Monkey S..	Not inoculated	Kept in cage beside Monkey N	112th day ill	117th day liver puncture positive	121st day died	Infected	Spleen + Liver + Bone-marrow +	Lived continuously with Monkey N ; became naturally infected, and died before Monkey N.

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parasites were visible in the spleen smears (monkey E was successfully infected from the same case during life).

Monkey C was inoculated with a spleen emulsion from monkey B, but was not infected by the 108th day, when it was chloroformed. Probably, as Nicolle has pointed out, the parasite tends to lose its virulence when inoculated from monkey to monkey, and this might account for the non-infection in this case, though the positive results obtained in monkeys L and N show that infection can be conveyed from monkey to monkey. In the case of monkeys L and N, however, the inoculation was made from a splenic puncture during life, and in the case of monkey C a post-mortem splenic emulsion was used.

Monkey O died 42 days after inoculation. There was no apparent cause of death, and no Leishman bodies were found.

Monkey K, which was inoculated subcutaneously, and which is still alive, shows definite enlargement of the spleen, but one liver puncture and two spleen punctures have so far given negative results.

The Presence of Parasites in the Peripheral Blood of Infected Monkeys.—Parasites are present in the peripheral blood of infected monkeys, but are difficult to find. In two infected monkeys, still alive, we have searched carefully for parasites in the peripheral blood. In one monkey we found in the fifth slide three parasites inside a large mononuclear cell; in another monkey, out of seven slides examined, we found one parasite inside a polynuclear cell.

Captain Archibald, R.A.M.C., has also successfully infected the red monkey of the Sudan (*Cercopithecus ruber*) with the disease.

We have tried inoculating dogs, but so far have failed to infect that animal as, with one exception, all the animals died soon after inoculation. We have also not yet met with spontaneous Leishmaniosis in the dog.

In the Sudan form of kala-azar the parasite is present in the peripheral blood in over 80 per cent of the infected people. The parasite morphologically resembles that of Indian kala-azar and infantile kala-azar; growth into flagellate forms has been obtained in 10 per cent. citrate, on Novy and MacNeal's medium, and on Nicolle's modification of that medium. Full details of these investigations will appear in the reports of the Wellcome Tropical Research Laboratories at Khartoum.

CONCLUSIONS.

(1) The ordinary grey monkey of the Sudan, *Cercopithecus sabæus*, can be infected with kala-azar.

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(2) It can be infected by intraperitoneal or by subcutaneous inoculation of the parasite.

(3) It can also be infected naturally, an uninoculated monkey, living in close contact with an infected monkey, having contracted the disease.

(4) The parasites are present in the peripheral blood of infected monkeys.

(5) So far we have found that the best method of infecting the monkey is by injecting into the peritoneal cavity the contents of a spleen puncture taken during life.

I beg to acknowledge my indebtedness to Captain D. S. B. Thomson, R.A.M.C., who was associated with me in investigating kala-azar in Sennar Province, for help and advice, and to Captain R. G. Archibald, R.A.M.C., for carrying on the experiments while we were both absent on leave.

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