REPORT ON A CASE OF EXPERIMENTAL SLEEPING SICKNESS IN A MONKEY (MACACUS RHESUS).

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Since 1903, when Colonel Bruce first demonstrated that the causal agent of sleeping sickness was a trypanosoma, more and more evidence has been forthcoming to confirm the truth of this observation, but up to the present one link in the chain of evidence has been missing, namely, a convincing animal experiment.

Mott, in 1900, in describing the pathological changes in the brain and cord of three cases of sleeping sickness drew attention to the presence in all three cases of a perivascular infiltration, this infiltration consisting principally of large and small lymphocytes, and some large mononucleated granular cells; since 1903, he has examined the brains of twenty-six cases of sleeping sickness, and in all has found the same lesion. So it may be taken that unless this condition is present we are not dealing with a true case of sleeping sickness; indeed, the cardinal symptom which gives its name to the disease is in all probability due to an anaemia of the brain matter caused by the pressure of this accumulation of lymphocytes occluding the smaller vessels.

So far no animals, experimentally inoculated, either with blood or cerebrospinal fluid from cases of sleeping sickness, have shown this particular and essential lesion. Prior to Colonel Bruce's arrival in Uganda, the Portuguese Commission and Dr. Castellani had found a coccus, usually a diplococcus, in the cerebrospinal fluid of a large proportion of cases, 80 per cent., and were inclined to attribute the disease to this organism. Dr. Castellani, by centrifuging the cerebrospinal fluid, demonstrated the presence therein of a trypanosoma, but, as he was aware that trypanosomata are to be found in the blood of a large number of the natives of the district, he did not connect this parasite with the disease, and placed it in the same category to which the Filaria perstans has now been relegated; and still looked on the coccus as the causal agent. This contention has, apparently, been borne out by the fact that many animals inoculated with cerebrospinal fluid from cases of sleeping sickness have died at varying intervals from a cocal infection.

Dr. Mott also has found in many of the recent cases of sleeping
sickness a coecal meningitis, but he is of opinion that this is an acute infection superimposed on a chronic lesion, the latter being due to an irritant acting through a considerable period of time.

It is well known that in acute inflammations we get a migration of polynuclear cells; in chronic cases, on the other hand, mononuclear cells are attracted. In the discussion at the British Medical Association Meeting on "The Rôle of the Lymphocyte," the majority of the speakers held the opinion that the lymphocyte was capable of active ameboid movements, and when attracted to a particular spot could pass through the wall of the blood-vessels and to a considerable distance through the surrounding tissues; and that in this migration, in contradistinction to the polynuclear cells, large numbers were not necessary, for the reason that the lymphocytes are capable of multiplication by division in connective tissues.

The condition in the brain of a sleeping sickness patient is a case in point, the chronic irritant present is the trypanosoma and the products of its metabolism; attracted by this, the lymphocytes migrate through the walls of the vessels into the perivascular canalicular spaces which are normally filled with cerebrospinal fluid. This fluid in health is destitute of cellular elements, but in sleeping sickness contains numbers of large and small mononuclear cells. In cases, however, in which death is due to a secondary coecal infection, large numbers of polynuclear cells are found, and, indeed, the fluid may become almost purulent. It has been proved over and over again that in cases of trypanosomal fever in man, although trypanosomes are numerous in the finger blood, they are not to be found in the cerebrospinal fluid; these cases are common in sleeping sickness districts, rare in districts where this disease is unknown. Again, in all cases of sleeping sickness the trypanosomes are found in the cerebrospinal fluid.

How is it that the trypanosomes can live in the blood of man for months or years, and give rise to little more than slight anaemia and transient edema, whereas shortly after gaining entrance to the cerebrospinal fluid they cause grave symptoms and finally kill?

The train of events may conceivably be of the following nature. While it is in the blood the parasite lives upon the plasma; the products of its metabolism can be neutralised by the white cells of the blood and also by the cells lining the blood-vessels, these, however, being damaged somewhat in the process. When the parasite enters the cerebrospinal fluid, it has to live upon a fluid differing in its origin and chemical constitution from the blood plasma, and which contains no cellular elements; the products of
the metabolism of the parasite alter the chemical composition of
the fluid, the result being that the nutrition of the brain is interfered with. In an attempt to correct this there is a migration of lymphocytes from the blood-vessels into the perivascular spaces, where they remain and cause compression of the capillaries, and thus the condition becomes aggravated.

The earlier stages of the disease, then, would be due to an alteration of the chemical composition of the cerebrospinal fluid caused by the trypanosomata, the lethargic stage due to the presence of the perivascular infiltration resulting in anaemia of the brain and its sequelæ. In this connection it must also be remembered that in sleeping sickness there is invariably an affection of the lymph glands throughout the body; these are enlarged and haemorrhagic, and are the seat of an immense increase of mononuclear cells; the bone marrow also is affected and shows the same changes.

Further, a large number of the lymphocytes seen in smears from the brain show vacuolation of the protoplasm now it has been noted that large lymphocytes secrete bacteriolytic and cytolytic substances, and the evidence of this is vacuolation of the protoplasm. If these substances are discharged into the central nervous substance, it is possible that they have a share in producing the degenerative changes therein.

In speaking of animal experiments, Dr. Mott says "the brains of monkeys inoculated with blood from sleeping sickness cases do not afford conclusive evidence of the trypanosoma being the cause of sleeping sickness. First, because only a few of the monkeys died; secondly, there is no proof that the animals that died really suffered from sleeping sickness, but rather that they behaved like animals profoundly ill; thirdly, no blocking of capillaries with trypanosomata nor any lymphocytic reaction around the vessels could be found; fourthly, in two or three cases there was an obvious diplococcal infection."

I hope to show in the following history of the case of a monkey lately under observation here that some of these conditions were fully fulfilled. This monkey was inoculated in Uganda by Colonel Bruce, in September, 1903, with 10 cc. of cerebrospinal fluid from a case of sleeping sickness. He came under observation here first on October 9th, 1903, he was then fit and well, no trypanosomata were found in his blood, but some "malarial" parasites were seen.

On October 23rd trypanosomata were first discovered in his blood, almost exactly six weeks from the date of his inoculation. The parasites continued in his peripheral blood until November 19th;
Experimental Sleeping Sickness in a Monkey

Fig. 1.—Photomicrograph of a section of sleeping sickness monkey's brain, from the region of the internal capsule. Stained by Leishman's method. Showing three small vessels with the perivascular infiltration. Magnification, 100 diameters.

Fig. 2.—Photomicrograph of a section of monkey's brain. Stained by Leishman's method. To show filling of perivascular canalicolar spaces with mononuclear cells. (During the process of hardening and cutting the sections, the mass of cells has been partially torn away from the wall of the space.) Magnification, 500 diameters.

(Photographs by Corporal Gibbons, R.A.M.C.)
after that date, in spite of careful scrutiny, none were found until December 16th, when they again appeared and were found up to January 4th, 1904, when they again disappeared. The blood was examined daily for some weeks from that date, but trypanosomata were not again found.

Thereafter, blood films were examined fortnightly throughout 1904, but no trypanosomata were seen. I am of opinion, however, that had it been possible to continue the close scrutiny of the blood, parasites would have been found in small numbers from time to time. During this period, although he got somewhat thinner, his health was otherwise good and he was very lively.

In February, 1905, it was first noticed that he was becoming weaker, the weakness affecting primarily the hind limbs; he also developed a slight puffy swelling about the lips and nose. The blood was again submitted to daily examinations, but it was not until March 5th, three days before his death, that one trypanosoma was found in a film made from finger blood.

The weakness was progressive, and his disposition also changed; he became irritable and nervous, snapping at any one who attempted to touch him, a thing he had never been known to do previously, and chattering loudly as long as any one remained in the room. His attendant declared that the tone of his voice had altered; this last symptom has also been noted in man, and is supposed to be due to an oedema of the vocal chords.

The hind limbs finally became completely paralysed, and this was followed by complete loss of power in the upper limbs. For the last three days of his life he lay prone on the ground, unable to move or eat; thirty-six hours before death he became comatose, and this condition of coma deepened into death.

The points of interest about the autopsy were, first of all, the presence of several much enlarged glands in the groin and axilla, which were of a deep purple colour, an evidence of a chronic trypanosomal infection.

The spleen was much enlarged and dark in colour, resembling a piece of black india-rubber in appearance and consistence.

Culture tubes inoculated with peritoneal fluid and spleen pulp remained sterile.

The lungs were healthy, there was no sign of tubercle or pneumonia, some clear fluid was present in the pericardium; cultivations from this fluid remained sterile and there was no deposit on centrifuging. A small quantity of fluid was drawn off from the base of the brain; this was slightly turbid, and on centrifuging the deposit
was found to consist of large and small lymphocytes and a few red corpuscles; no trypanosomata were found.

The skull-cap was then removed and it was found that there was no inflammation of the dura mater; on exposing the brain the convolutions were seen to be flattened and the vertex mapped out by turgid superficial vessels. About 5 cm. of fluid were withdrawn by means of a pipette from the lateral ventricles, and a film preparation made; on this slide four fully-developed trypanosomata were found. At the same time thick films from the heart blood were stained by the "wet" method, but although several were gone over carefully, no parasites could be found. Dry smears were also made from the cut surface of the brain, but the search here was again unsuccessful.

Sections were cut from the cerebrum, both cortex and base, also from cerebellum medulla and cord; in all of these the typical brain lesions of sleeping sickness were found, namely, a chronic meningo-encephalo-myelitis, the leading characteristics being a filling of the perivascular canalicular spaces with large and small lymphocytes, also an increase of connective tissue and degeneration of the neurones.

Sections of the brain were sent to Dr. Mott by Lieutenant-Colonel Leishman, and he agreed that the changes present were similar to those found in a well-marked case of sleeping sickness.

Cultures were made from the brain pulp and also from the cerebrospinal fluid, but remained sterile, and in none of the sections were any cocci seen.

To sum up, we have here a monkey inoculated with 10 cc. of cerebrospinal fluid from a case of sleeping sickness. He harbours the parasites in his blood for a period of eighteen months; during the first twelve months he is little inconvenienced, during the last four he becomes wasted, and suffers from a progressive weakness culminating in complete paralysis, coma and death.

At the autopsy the only film in which trypanosomata could be readily demonstrated was that from the fluid taken from the lateral ventricle of the brain. Sections of brain medulla and cord showed in a marked degree the typical and specific lesions of chronic sleeping sickness. Also to be noted is the entire absence of any bacterial infection.

With regard to the blocking of the capillaries by masses of trypanosomata, this does not occur in true sleeping sickness; it is found in the brain of animals dead of nagana and surra, and is not accompanied by perivascular infiltration. That trypanosomata
were present in the heart blood in this case was proved by the fact that a monkey inoculated with 5 cc. of heart blood showed trypanosomata in his blood three weeks later.

Cultivation experiments were made on McNeal and Novy's medium, but so far without result.

Note.—The animal experiments were performed by Lieutenant-Colonel Leishman.

REFERENCES.

3. Royal Society Reports of the Sleeping Sickness Commission, 1902.