SUMMARY OF REPORT NUMBER VI. OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY.

BY CAPTAIN E. D. W. GREIG.

Indian Medical Service.

The work of the Commission in Uganda, up to the present date, by Captain E. D. W. Greig, I.M.S., and Lieutenant A. C. H. Gray, R.A.M.C., has just been published by the Royal Society.

It will be remembered that a Report was published by Colonel D. Bruce, C.B., F.R.S., R.A.M.C., and Captain E. D. W. Greig, I.M.S., towards the close of 1903. In that Report evidence was brought forward which showed: (1) that sleeping sickness is caused by the entrance into the blood and cerebro-spinal fluid of a species of trypanosoma; (2) that this species is probably that discovered by Forde and described by Dutton, from the West Coast of Africa, and called by him Trypanosoma gambiense; (3) that the so-called cases of trypanosoma fever, described from the West Coast, may be, and probably are, cases of sleeping sickness in the earliest stages; (4) that monkeys are susceptible to sleeping sickness, and show the same symptoms and run the same course, whether the trypanosomes injected are derived from cases of so-called trypanosoma fever or from the cerebro-spinal fluid of cases of sleeping sickness; (5) that dogs and rats are partially susceptible, but that guinea-pigs, donkeys, oxen, goats and sheep, up to the present, have shown themselves absolutely refractory; (6) that the trypanosomes are transmitted from the sick to the healthy by a species of tsetse fly, Glossina palpalis, and by it alone; (7) that the distribution of sleeping sickness and G. palpalis correspond; (8) that sleeping sickness is, in short, a human tsetse fly disease.

After the departure of Colonel Bruce for England, in August, 1903, the Commission continued to extend and elaborate the work on similar lines, and this Report contains the results of their work up to the present time. The following is an abstract of the Report:

1 "Report VI." Printed by Harrison and Sons, St. Martin's Lane, 1905. Price 4s. 6d.
"CONTINUATION REPORT ON SLEEPING SICKNESS IN UGANDA. By Captain E. D. W. Greig, L.M.S., and Lieutenant A. C. H. Gray, R.A.M.C. (Sleeping Sickness Commission)."

In the introduction the authors state:—

"Since the departure of Colonel D. Bruce, F.R.S., for England on August 28th, 1903, the work of the Commission was carried on by Greig and Nabarro until November 20th, 1903. On that date Dr. Nabarro left Entebbe for England. The work of the Commission was conducted by Captain Greig until he was joined on March 9th, 1904, by Lieutenant A. C. H. Gray, R.A.M.C.

"Captain Greig left Entebbe for England on November 15th, via the Nile and Egypt.

"This Continuation Report brings the work of the Commission up to the date of Greig's departure for England.

"In this Report evidence is brought forward which affords additional proof of the correctness of the conclusions arrived at in the last Report. Further evidence is brought forward to show:—

"(1) That the disease is at first a specific polyadenitis caused by the T. gambiense.

"(2) That, in addition to enlargement of lymphatic glands, the blood shows a constant lymphocytosis at all stages of the disease.

"(3) That sleeping sickness is the last stage of this disease, and is invariably fatal. It consists, essentially, in a polyadenitis, plus signs and symptoms due to changes in the nervous system; the onset of these signs and symptoms synchronises with the entrance of the T. gambiense into the lymph spaces of the nervous system; this is accompanied by a rise of the mononuclear elements in the cerebro-spinal fluid.

"(4) That the resistance of both men and monkeys to the T. gambiense, as judged by the duration of the early stage, varies greatly, and probably a certain proportion, not yet exactly determined, acquire sufficient immunity to arrest the development of the disease at that stage.

"(5) That the action of arsenic in vita on the T. gambiense is partial. It destroys a number of the trypanosomes, and probably these act as immunising agents. Its administration in the stage of polyadenitis tends to help the natural resistance to combat the disease."
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"(6) That bacterial invasion, chiefly coccal, occurs in some cases, but only in the very last days of the sleeping sickness stage, and therefore cannot determine the onset of this phase of the malady.

"(7) That, in addition to the *T. gambiense*, other varieties of trypanosoma occur in Uganda, which are pathogenic to animals.

"(8) That these trypanosomes differ entirely from *T. gambiense* in morphology and animal reactions.

"(9) That one of these trypanosomes is probably identical with *Trypanosoma brucei*. The other two differ from it, and are, provisionally, unclassed.

"(10) That these varieties of trypanosomes are conveyed from the sick to the healthy by the Uganda tsetse fly (*G. palpalis*), and not by other biting flies (*Stomoxys*).

"The general situation as regards sleeping sickness in Uganda at the present time may be summed up as follows: In the sleeping sickness areas from 50 per cent. to 75 per cent. of the inhabitants are in the stage of polyadenitis, and are carrying on their ordinary work, because the disease at this stage produces few symptoms; but they are acting as reservoirs of the parasite, like the wild animals in the case of Nagana. It is this class of case that is especially liable to infect 'clean' fly belts. The after history of these early cases, as far as we have observed up to the present, is as follows: (1) that they may terminate fatally, either *(a)* by passing into the stage of sleeping sickness, which is the most frequent and usual; *(b)* through some intercurrent affections, particularly pneumonia. In this connection it is interesting to note that Dr. Albert Cook has observed that the admissions for pneumonia to the C.M.S. Hospital, Mengo, have risen markedly within the last two years. (2) That they remain in good health for long periods, indicating that at least a 'tolerance' to the parasite has been acquired. The question then arises: will any of these individuals acquire sufficient immunity to destroy the parasite at this stage? Can they in fact become 'salted'?’; and further, can this immunity by any means be artificially increased?

"From reports just received there is reason to believe that the hitherto 'clean' fly belt on Lake Albert and the Nile has become infected. The suspected district is Bugungu, near Fajao, where the *G. palpalis* was obtained last year. The subject is being further investigated. If the disease is sleeping sickness, the infection must have been either carried across Unyoro from Uganda, or travelled along the Nile from Usoga. As the *G. palpalis* has been found at
Nimule, and probably exists north of that, the disease will involve an extensive tract of fresh country.¹

“A feature in the morbid anatomy of sleeping sickness, to which attention has not previously been directed, is a curious condition found in the stomach. In a number of cases the organ was found to contain a quantity of dark, semi-fluid material. The mucous membrane showed a remarkable alteration; it was studded with areas of varying size, having a dark centre and a light red periphery. They were most numerous towards the pyloric orifice. On microscopical examination they were seen to be petechial hemorrhages into the mucous membrane, which had broken down and formed superficial ulcers. No ova of Bilharzia were seen in the scrapings. A full account is given in the histories of the cases recorded in the Appendix. In all cases in which the stomach was inspected this condition was met with. The condition is comparable with the petechial hemorrhages met with under the endo- and epicardium of the heart in trypanosoma infections . . .”

Under the following headings the work of the Commission is arranged:—

“(1) The lymphatic glands of every case of sleeping sickness are enlarged, and the juice taken by puncture during life contains many active trypanosomes and also disintegrating forms.

“Every case of sleeping sickness here has shown enlargement of the lymphatic glands. The enlargement of the femoral, inguinal, axillary and superficial cervical glands can, during life, be readily determined, and after death the abdominal, thoracic, and deep cervical.

“In the Proceedings of the Royal Society for May, 1904, it was pointed out that the juice of the lymphatic glands, especially the posterior cervical glands, contains many active trypanosomes in all cases and at all stages of sleeping sickness.

“Some of the juice can easily be obtained by puncturing a superficial gland in the posterior triangle of the neck with a hypodermic needle, and sucking it into the needle by means of a syringe. The drop is then blown out on to a slide, covered with a cover-glass, and examined under a low power, 150 to 200 diameters of

¹ “The most recent Reports confirm the original information that sleeping sickness has broken out in this area. Captain Greig is proceeding to England, via the Nile and Egypt, in order to investigate this outbreak, and also to determine the presence or absence of G. palpalis and trypanosomiasis in Egypt. The results of this expedition will be reported on its completion, vide Report 12.”
the microscope—Zeiss 16 mm. objective, and Nos. 12 or 18 eye-piece. The trypanosomes are numerous in the juice, and are readily found after a short search. In stained preparations, in addition to well-formed trypanosomes, there exist a considerable number of disintegrating forms, suggesting that destruction of trypanosomes takes place in the glands. Similar preparations from a drop of peripheral blood were prepared and examined at the same time, but a prolonged search, in the majority of cases, failed to discover the presence of trypanosomes.

"A practical outcome of these observations will be, that the recognition of sleeping sickness in its earliest stages will be a matter of easy accomplishment; the enlargement of the superficial lymphatic glands presents a sign which will arrest the attention of the observer, and the determination, by the above method, of the presence of trypanosomes in them can be very simply carried out.

"The trypanosomes are present in small numbers in the peripheral blood, but, from time to time, an increase in their numbers takes place. This increase suggests that an occasional overflow from the glands, to which they are chiefly confined, takes place. The trypanosomes are sometimes more numerous in the blood taken at night.

"The juice of the gland was found sterile and free from streptococci even at a late stage of the disease. As will be shown later, the streptococcic invasion occurs only when the patient is moribund.

"A point of interest in connection with glandular enlargement due to T. gambiense, is that, in monkeys which have been inoculated with the trypanosoma, glandular enlargement is not so marked as in man, the parasite being found more frequently in the blood of monkeys, the disease being in monkeys more a blood one than is the case in man. This absence of gland enlargement in monkeys might explain why the mononuclear exudation which is present in all cases of sleeping sickness (Mott) is not also seen in monkeys."

"(2) The lymphatic glands of cases of so-called 'trypanosoma fever' are enlarged, and the juice taken by puncture during life contains active and disintegrating trypanosomes.

"The early cases of trypanosomiasis examined here have all presented enlargement of the lymphatic glands, and on puncturing them active trypanosomes have been readily found. At this stage of the disease the condition is essentially a polyadenitis.
"Sleeping sickness is this specific polyadenitis, with signs originating in a derangement of the nervous system, due to changes produced by the presence of the parasites there, super-added.

"The occurrence of enlargement of the lymphatic glands, with the presence of trypanosomes in number, in both early cases of trypanosomiasis and sleeping sickness, affords additional evidence in favour of the unity of the two conditions.

"The natives themselves are alive to the fact that, when the glands in the neck become enlarged, they will, sooner or later, pass into the stage of sleeping sickness, and their custom is, then, to eat up their live stock, goats, chickens, &c.

"From the above observations the next question arises.

"(3) What is the incidence of enlargement of lymphatic glands amongst the general population?

"It seemed important to test the above observations on a large scale. If trypanosomiasis causes adenitis, cases of enlargement of glands should be more numerous in the sleeping sickness areas than in the non-sleeping sickness areas. The incidence of gland enlargement in the sleeping sickness areas would be a gauge of the incidence of trypanosomiasis in the general population in sleeping sickness areas, because the majority of cases, coming from sleeping sickness areas with enlarged glands, have on examination showed the presence of trypanosomes in the glands.

"In the sleeping sickness areas the incidence was obtained by the help of the Rev. H. T. C. Weatherhead, B.A., in the islands of Sese and Kome."

The incidences of gland enlargement of general population.

A.—Sleeping sickness area—Sese Island, Kome Island. The result of the examination of the population of these areas showed, as was to be expected, that the incidence of gland enlargement was remarkably high—50 to 70 per cent.

B.—Non-sleeping sickness areas. Here the incidence was found to be low.

"(4) Lymphocytosis occurs in all cases of sleeping sickness.

"Enlargement of lymphatic glands being a constant feature in sleeping sickness, it was a matter of importance to determine whether the lymphocytes in the blood show an increase in numbers. This point is of interest, further, because the most constant lesion found in the nervous system of sleeping sickness cases is an accumulation of cells of this nature in the perivascular spaces.
In uncomplicated cases of sleeping sickness anaemia does not occur, the number of the red cells, and the percentage of haemoglobin, being normal. Towards the end, in a certain proportion of cases the number of red cells, the percentage of haemoglobin and the specific gravity, rises above the normal. These cases did not present any signs of cyanosis. The examination of the bone marrow in one of these cases showed a very large number of nucleated red cells, chiefly normo-blastic; but some megaloblasts were also present.

Mast cells were present in the blood of all cases to the extent of about 1 per cent.

The eosinophiles, also, form a higher proportion of the leucocytes than is normally met with.

The examination of the blood was made by means of a Thoma-Zeiss blood-counting apparatus and a Gowers’ haemoglobinometer.

It was also found that the trypanosomes were more numerous in the blood at night time.

Following the suggestion of Mr. Plimmer, who found that the trypanosomes were more numerous in the blood of animals at night than in the day time, some observations were made with the object of determining whether this was the case in man. It will be seen to exist in man also. The percentages which are taken as a rough index of the number of trypanosomes present, in a slide, refer to the number of trypanosomes per polynuclear leucocytes counted.

<table>
<thead>
<tr>
<th>Date, 1904</th>
<th>Name</th>
<th>No.</th>
<th>Parasites in blood, daytime</th>
<th>Parasites in blood, night-time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arcadi</td>
<td>303</td>
<td>+ 1 per cent.</td>
<td>+ 8 per cent.</td>
</tr>
<tr>
<td></td>
<td>Asumanie</td>
<td>69/K.P.</td>
<td>+ 1  &quot;  &quot;</td>
<td>+ 10  &quot;</td>
</tr>
<tr>
<td></td>
<td>Tenwa</td>
<td>692</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Juma</td>
<td>69/J.Q.</td>
<td>+ 1 per cent.</td>
<td>+ 2 per cent.</td>
</tr>
<tr>
<td></td>
<td>Hamesi</td>
<td>69/F.V.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Juma</td>
<td>69/J.Q.</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(5) The cells of the cerebro-spinal fluid of sleeping sickness cases taken during life by lumbar puncture are lymphocytes, and are more numerous in the late stages of the disease.

Having seen that the lymphocytes of the blood are increased in number, the next step to take was to determine whether during life these elements were present in number in the cerebro-spinal fluid of sleeping sickness cases. The total number of cells per c.mm.
of cerebro-spinal fluid was determined by means of a Thoma-Zeiss apparatus. Stained preparations were also made of the sediment obtained by centrifuging. The cells were found to be all lymphocytes. There is a progressive rise in the number of lymphocytes in the cerebro-spinal fluid as the disease advances. The following are the averages:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Lymphocytes per c.mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early stage</td>
<td>23</td>
</tr>
<tr>
<td>First stage</td>
<td>257</td>
</tr>
<tr>
<td>Second stage</td>
<td>355</td>
</tr>
<tr>
<td>Third stage</td>
<td>730</td>
</tr>
</tbody>
</table>

"This result is of considerable interest when considered in connection with the post-mortem appearances found in the nervous system of sleeping sickness cases; these were shown by Mott to consist essentially of an accumulation of mononuclear cells in the lymph spaces of the brain . . . ."

"(6) The gland juice in a certain proportion of cases in the last stage of the disease becomes infected by bacteria, especially diplo-streptococci.

"In view of the fact that some importance has been attached to streptococci as playing a part in the causation of sleeping sickness, a series of examinations of the gland juice were made in a number of cases at intervals in the course of the disease, microscopically and culturally. The result of these observations showed that a number remained cases of pure trypanosoma infection to the end; the cultures made from the glands, blood and cerebro-spinal fluid remained sterile. On the other hand, in a proportion of cases an invasion chiefly by diplo-streptococcus, did occur, but by the results of the examination at different stages of the disease, it was possible to locate it to the final stage of the disease, when the patient was practically moribund.

"These cases at this stage of the disease have invariably numerous foci of suppuration on the hands and feet, due to jiggers; also there is frequently before death a purulent discharge from the gums; their vitality and resisting power is a negative quantity . . . ."

"(7) Does the injection of a pure culture of diplo-streptococci obtained from sleeping sickness cases modify the course of the disease produced in monkeys by the T. gambiense?

"Dr. Mott, in a letter forwarded to the Commission, suggested that it would be of interest to test the effect of injection of diplococci obtained from cases of sleeping sickness into monkeys suffering from trypanosoma infection. A pure culture in broth of
a diplococcus obtained from the cerebro-spinal fluid of a case of sleeping sickness was used for the experiments. The injections were made subcutaneously, and a large number of germs were introduced. The effects of the injection were observed in a healthy monkey, a monkey infected with *T. gambiense*, which showed at the time of injection the parasite in the blood and only slight clinical manifestations, and finally a monkey infected with the same trypanosoma, but showing very well-marked clinical signs. This injection did not produce any alteration of temperature or other morbid sign in the healthy animal, nor in the animal infected by the trypanosomes; but at an early stage of the disease, in the monkey which was seriously ill at the time of injection, it produced a local suppuration. It is apparent from these observations that the streptococcus found in the tissues of sleeping sickness cases has very low pathogenic properties, and only gains a footing at all when the resisting power of the tissue is greatly diminished. It does not modify the course of the disease produced in monkeys by the *T. gambiense*.

"(8) Has the so-called trypanosoma fever any connection with sleeping sickness?"

"Since the publication of the last report the observations on the five men in whose blood the trypanosomes were first discovered in March, 1903, have been continued.

"Two of these, Karala Barigi and Bara Risgallah, died of pneumonia, in April and May, 1904, respectively; of the others, Jordien Murjan appears to be, undoubtedly, in an early stage of sleeping sickness. He has gradually developed the characteristic signs of the malady. Trypanosomes are now always found in his cerebro-spinal fluid. Tabula presents some of the features of the disease, but is still able to do his work and has not yet shown trypanosomes in the cerebro-spinal fluid.1 Kumsarsabba is in a similar condition.

"In addition to the above, in order to extend the observations on this most important stage of the disease, five natives were picked out from a batch of prisoners from Usoga, having enlarged glands in the neck. On examination trypanosomes were found in the lymph juice of each. These men are being kept in hospital, and their condition is being carefully observed. We have also observed the action of arsenic on the *T. gambiense* in these men.

1 "Lieutenant Gray writes, February, 1905: 'That Tabula now shows trypanosomes in the cerebro-spinal fluid, and distinct signs of sleeping sickness.'"
None of them show any of the characteristic features of sleeping sickness, and the trypanosomes are not present in the cerebro-spinal fluid. In fact, with the exception of enlargement of the lymphatic glands and slight fever, the general condition of the men is good.

"The importance of this stage of the disease is so great that a full account of the observations on these five natives is given. The diet has been increased; in addition to bananas, a ration of meat is given twice weekly. Up to date they have improved remarkably in general condition and have rapidly put on flesh. The after history of these cases, maintained under the above condition, will be of considerable interest . . . "

"It will be seen from a consideration of the facts brought forward that the essential features of the condition called 'trypanosoma fever' are also met with in sleeping sickness cases. In both polyadenitis is the most constant lesion met with, and the causal agent of this adenitis is in both the T. gambiense. It is apparent from a study of the case of Jordien Murjan, that the onset of this last stage of sleeping sickness synchronises with a marked development of the trypanosomes in the cerebro-spinal system. In following the after history of cases of trypanosoma fever, we have arrived at the following conclusions: (1) that many of them terminate fatally as sleeping sickness cases, which may be regarded as the usual mode of termination; (2) that a certain number die of intercurrent affections, e.g., pneumonia; (3) that a certain proportion remain well for long periods, indicating that a tolerance towards the parasite has been attained. It may be that some of these cases may become in time sufficiently immune to destroy the parasite. The evidence collected so far suggests that this is the case.

The effect of arsenic on the trypanosoma in the blood of patients at this early stage of the disease has been observed. The results of these observations are recorded in the histories of the five cases. The action is somewhat remarkable. The parasites disappear, first from the peripheral blood, and at a later date from the lymphatic glands. After an interval of varying length, the parasites will reappear in the blood, temporarily, and then again disappear; but have not so far returned to the glands. Possibly the glands may store up the arsenic. From a consideration of the following table, it will be seen that after the first destruction of the parasites in the glands and blood by the arsenic, they reappear in small numbers in the blood, and at a
later period finally disappear. This suggests that arsenic acts in two ways: (1) by actually destroying the trypanosomes; and (2) the trypanosomes so destroyed actively immunise the individual, the effect of this not being apparent till later. Through the kindness of Geh. Med. Rath. Prof. Dr. P. Ehrlich in sending to one of us (Captain Greig) 250 grams of trypanroth and tragaroth we will be able to study their action in monkeys and man . . . ."

"Table showing the effects of arsenic on trypanosoma gambiense.

<table>
<thead>
<tr>
<th>Name and Number of Case</th>
<th>Month observations were made, 1904</th>
<th>Trypanosomes in the lymph glands</th>
<th>Trypanosomes in the blood</th>
<th>Amount of arsenic administered as sod. arsenite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of observations</td>
<td>Results</td>
<td>No. of observations</td>
<td>Results</td>
</tr>
<tr>
<td>Tebuwa, 302</td>
<td>June 3+ 4 2+ 2-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>July 11 3+ 9 1+ 8-</td>
<td>As 103 mgs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aug. 11 2+ 9-</td>
<td>As 20 mgs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sept. 8 8- 6 6-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct. 6 6-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kitsame, 303</td>
<td>June 9 9+ 23 16+ 7-</td>
<td>As 74 mgs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>July 13 13- 15 13- 13-</td>
<td>As 100 mgs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aug. 12 3+ 9-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sept. 9 7-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct. 6 6-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manawa, 304</td>
<td>June 1 1+ 1 1-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>July 8 4+ 6 6-</td>
<td>As 105 mgs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aug. 11 8+ 8-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sept. 9 1+ 8-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct. 6 6-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mondu, 310</td>
<td>July 5 3+ 4 1+ 3-</td>
<td>As 85 mgs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aug. 3 3- 11 2+</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sept. 1 9- 9-</td>
<td>As nil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct. 6 6-</td>
<td>As nil.</td>
<td></td>
<td></td>
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</tbody>
</table>

"As well as the cases above mentioned of trypanosoma fever, information has been obtained as to the after history of the men of the general population, mentioned in the last Report, in whose blood trypanosomes were found, but who, then, had no symptoms of sleeping sickness."
It has not been possible to trace out all these men owing to various causes, but the histories of a sufficient number have been obtained. Eighty natives were examined, and trypanosomes were found in the blood of twenty-three. Of these twenty-three, it has been ascertained that, since that date, three have died of undoubted sleeping sickness, one ran away from his shamba and was reported to have died of sleeping sickness. Two died from pneumonia (one was almost certainly in an early stage of sleeping sickness); five are now in an early stage of sleeping sickness. No information has been obtained in six cases. The remainder (six) do not as yet present definite signs of sleeping sickness. These observations strongly support the contention that the so-called trypanosoma fever is an early stage of sleeping sickness. Further, that this phase of the disease may be short or very prolonged, the development of the last stage being dependent on an extension of the invasion of the lymphatic system to the lymph spaces of the nervous system. It will be of considerable interest to follow the further history of the six men showing still no signs.

(9) Are these trypanosomes pathogenic to animals, and can any specific difference be made out between them by animal experiment?

The experiments on the various animals have been continued throughout the year. The additional observations and results obtained strengthen and support the conclusions arrived at in the last Report.

The monkey is the most satisfactory animal for experimental inoculation. The continued observations show that the effect produced in them is in all respects similar, whether the trypanosoma infection is produced by blood from so-called 'trypanosoma fever' cases or the cerebro-spinal fluid of undoubted sleeping sickness cases. As the question of the relationship of these two morbid conditions is an important one, full details of the experiments are given.

The other animals that we have employed for experimental inoculation are, dogs, jackals, cats, rats, guinea-pigs, rabbits, oxen, goats, sheep and donkeys. None of these have shown any marked susceptibility to the disease, and some have remained resistant.

It will be seen that the experiments with the trypanosomes derived from the blood of early cases of sleeping sickness (so-called trypanosoma fever), and those derived from the cerebro-spinal fluid of advanced cases of the disease give exactly the same results, which is strong evidence in favour of the view put forward by Bruce and Greig that these two trypanosomes are one and the same, namely,
T. gambiense. As the result of further experiments we have shown that the guinea-pig is susceptible to trypanosomes from both the above sources; also the cat and jackal react in the same way as the dog to the two strains—that is to say, they are partially susceptible. Oxen, goats, sheep and donkeys remain quite refractory to both strains.

"(10) Further observations on the distribution of G. palpalis.

"Since the last Report further observations have been made on the distribution of the fly and sleeping sickness. The results of these additional observations have been added to the maps of the distribution given in the Further Report, which have been extended in order to embrace them. Its occurrence round Lake Albert is interesting and important. In the light of this discovery, additional significance was given to a case of sleeping sickness coming from this district. The following are the chief points in the case:

"The patient was a Swahili sailor, named Sururu Bin Mze, who was employed on the Government boat running between Butiaba and Wadelai. Two years ago he came from Mombasa and passed through Entebbe, remaining there for a day only, and then proceeded direct to Lake Albert. He remained at his work for two years; being then time expired, he was discharged. On the journey to Entebbe he became ill, and when admitted into hospital here on August 17th, 1904, he had undoubted signs of sleeping sickness, with many trypanosomes in the glands and cerebro-spinal fluid.

"The question arises, did this man acquire the disease locally, or was it an imported case? In any case, an individual harbouring so many trypanosomes could readily have infected flies in the belt in which he was working, and so spread the disease. Further information on this point is being obtained.

"Dr. C. A. Wiggins made a journey from Mumia's to Shirati and ascertained the distribution of the fly and sleeping sickness there. In his Report to the Principal Medical Officer, East Africa and Uganda Protectorates, dated March 30th, 1904, he mentions a point of considerable interest. He states, "I pitched my tent near Omorie's, close to the river, which runs into Hoima Bay, and here I found no tsetse and no sleeping sickness, which surprised me, as I knew sleeping sickness was present on the lake shore. The country here is open plain, more or less cut off from the lake by a chain of small circular hills. Afterwards, when interviewing the chief, he told me that he had had sleeping sickness in his villages nearly three years ago, but there was none now as he had forbidden his people to go to the lake for fish, or to mix with the Wagemi
near the bay. When I told him that sleeping sickness was caused by the bite of the tsetse he and all his men readily believed it.'

"Dr. Wiggins' general conclusions, as a result of his observations on the journey, are: '(1) That where there are trees or bushes near the water the flies are found, and sleeping sickness occurs in these places. Conversely, where there are no trees there are no flies and no sleeping sickness; papyrus does not shelter them; also, that there is sleeping sickness inland, among those tribes who go to the lake for fish, at any point where tsetses are at the lake shore. (2) That sleeping sickness spreads from Uganda and Usoga eastward and southward. (3) That there is no sleeping sickness east of a line drawn from the Maragoli hills down the Maragoli stream to the bay, and then across the bay (Kavirondo) to Hoima; the three or four cases east of this are probably imported. This line is also the eastern limit of the distribution of the tsetse fly, with the exception of Kibuye, i.e., Port Florence District. (4) That the only river which carried the fly inland is the Juja River, which is the only one that has trees at its mouth and thick vegetation along its course.'

"The latest information shows that sleeping sickness is occurring on the shores of the Albert Edward Lake.

"(11) The tsetse flies (G. palpalis), which had previously fed on a case of sleeping sickness or were freshly caught, can produce in the monkey an exactly similar disease to that produced by inoculation of fluid containing T. gambiense.

"Since the publication of the last Report the after history of several of the monkeys in whom the infection was produced, either by freshly caught flies at Entebbe, or flies which had previously fed on sleeping sickness cases, has been studied.

"The result of these investigations shows that the disease, whether induced by the injection of fluid containing the T. gambiense, by the bite of the fresh fly or previously infected ones, is, in the monkey, identical in all respects. These facts strongly support the contention of Bruce and Greig, that the fresh fly trypanosoma is the T. gambiense.

"A point of interest and importance in this connection is that since the hut-tax labourers (one in every two or three of whom had the T. gambiense in his blood), have left the fly belt at Entebbe, it has taken a very much larger number of flies to infect the monkey than it did when they were present. It is therefore fair to assume that the chief source at Entebbe, from which the wild fly obtained its supply of trypanosomes, was the body of men brought in from the various districts for the purpose of hut-tax labour . . . ."
"(12) Are other varieties of trypanosomes found in Uganda?

"In addition to the T. gambiense, trypanosomes from various sources have been studied. In the last Report it was shown that oxen in Entebbe, belonging to the P.W.D., and sent for examination by Mr. Pordage, had trypanosomes in their blood. In the blood of Government cattle at Jinja, Usoga, which were dying at the rate of five or six a day, a trypanosome was constantly found. In the blood of a dog, kindly sent by Mr. R. J. Stordy, P.V.O., Uganda and East Africa Protectorates, trypanosomes were present. This animal had accompanied the Abyssinian Boundary Commission. Lastly, in the blood of a mule of Colonel Sadler's, at Entebbe, a trypanosome was found. The trypanosomes derived from these four sources have been studied side by side here.

"(13) The history and distribution of these trypanosomes in Uganda and East Africa.

"(a) The oxen of Mr. Pordage, as stated in the last Report, came to Entebbe from British East Africa about the end of 1900. They kept well until they were sent to graze in the forest near the Lake, in which G. palpalis is found. Since then they have been sick and Mr. Pordage is of opinion that their illness was contracted whilst grazing.

"(b) The cattle which became sick and died at Jinja, Usoga, and in whose blood a trypanosome was found by us in August, 1903, came from the Bukedi country in May, 1903. They had been in Wamia District to the south-west of Mount Elgon. The route by which they were marched to Jinja, Usoga, was via Igagas, Kibuye, Balemale and Kitindis. They halted at each of these places, and at all of them a species of tsetse fly is found (G. pallidipes). To determine whether a trypanosoma occurs in the animals stationed at any of these places, the blood of animals was examined at Kibuye. Mr. Grant kindly made slides from a number of animals in December, 1903. Of ten slides from different domestic animals, trypanosomes were found in two, one in a slide from a donkey, and one from a cow. We were thus able to demonstrate that the necessary factors for the infection of the cattle were present at the halting places.

"(c) A number of animals which accompanied the Abyssinian Boundary Commission became sick and died, and an examination of one of the sick animals showed that trypanosomes were present in the blood. The animals affected were eleven Boran and Abyssinian ponies, as well as several camels and five English dogs. These all died. None of the Abyssinian donkeys or mules were
affected. The English dog, examined on August 26th, 1903, was half Airedale and half bull terrier. The animals marched from the boundary to Lake Rudolph and thence via Baringo to Nakuru. Two ponies died at Nakuru. The journey from Baringo to Nakuru only occupies four days, so that, probably, the infection occurred further north. Austen in his 'Monograph,' p. 326, records that G. fusca has been found on the north-east shores of Lake Rudolph.

"(d) A mule used by Colonel Sadler was found in September, 1903, to have trypanosomes in his blood. This animal had been about five years in Africa, firstly, in the East Africa Protectorate, and for the last eighteen months in Uganda.

"(14) Can any differences be made out microscopically between these varieties of trypanosomes occurring amongst the domestic animals in Uganda?

"As the same species of trypanosoma varies in size, shape, &c., in the blood of different experimental animals, too much importance cannot be attached to the morphological characters as affording a means of establishing the identity of different trypanosomes. Speaking generally, it may be safely stated that the trypanosomes found in the blood and cerebro-spinal fluid of cases of sleeping sickness, and in the blood of men showing no signs of sleeping sickness, are always smaller than those of the Jinja cattle, Abyssinian or Entebbe mule disease. The variety met with in the mule showed an unusually large number of short 'tadpole' forms. This was especially well seen when the blood containing this variety was injected into a guinea-pig. The variety met with in the Jinja cattle was, as a rule, larger than the others. It is, however, on morphological grounds only, impossible to arrive at a final conclusion, as to the identity or otherwise of the various 'strains' brought under our notice. Accordingly, in addition to this means of distinction, their differentiation was approached by a study of their reactions in a series of animals. These reactions were contrasted and compared. Further, the injection of animals proved to be immune to one species with the blood containing another strain of trypanosomes, was used as a means of arriving at a conclusion on the question of the identity or not of the various species. In the drawing of the specimens, the morphological characters of the trypanosomes are shown.

"(15) Are these trypanosomes pathogenic to animals, and can any difference be made out between them by animal experiment?

"In the case of the trypanosoma found in Mr. Pordage's ox, it produced a very chronic malady in the animals under observation;
the animals became extremely emaciated, with abnormal temperature. They became gradually weaker and finally died.

"The trypanosoma obtained from the Jinja cattle produced few symptoms. There was a general enlargement of the lymphatic glands. As a rule, the animals died in fairly good condition. On post-mortem examination, the cervical and supraclavicular lymphatic glands were enlarged and congested. The heart showed yellow, jelly-like material at the base, and often petechiae on its external and internal surfaces. The spleen was slightly enlarged. The native name of the disease is Sutoko, and has been considered an internal form of Mukebi. The trypanosoma was first found in the herd of cattle in August, 1903. The cattle at the station at Jinja were infected to the extent of 24 per cent. of their number. At Kitindi’s, near Jinja, 20 per cent. were infected. Mr. A. G. Boyle, Sub-Commissioner of Usoga, reports ‘that since March, 1904, the cattle have ceased to die amongst the herd.’ The herd has been kept at Kitindi’s, at which place the G. palpalis is found. These cattle were again examined in September, 1904. The result of the examination showed that 50 per cent. of these cattle had the trypanosoma in their blood. This examination was made to determine whether the cattle were fit to sell or not. The results show the necessity for such examinations before arriving at a definite opinion on the subject. It is further of interest, as showing that the symptoms of the disease amongst these cattle had undergone considerable modification during the year. In August, 1903, the disease ran a very acute course, the animals dying before any marked signs had developed, whilst in September, 1904, although a larger number of cattle were affected, yet none of them were dying. This fact could be explained in two ways: (1) that the parasite had become attenuated; or (2) the animals had become more immune; or it might be a combination of both factors.

"The trypanosoma obtained from the animals which became affected on the Abyssinian boundary caused the death of some eleven Boran and Abyssinian ponies, as well as camels and five English dogs. The Abyssinian donkeys and mules did not suffer. One native (Abyssinian) dog, which was the companion of the English dogs, and had accompanied them on the expedition, remained quite healthy. This animal was, however, susceptible to infection, as was proved by injecting it with blood containing this variety of trypanosoma.

"The mule at Entebbe in whose blood a trypanosoma was found, when brought to the laboratory in September, 1903, had slight fever
and swelling of the lymphatic glands. A few days later it was brought in in a moribund condition. No trypanosomes could be found in the peripheral blood microscopically, but injection of susceptible animals proved the presence of the parasites in the blood.

"(16) Are we dealing with one or more than one species of trypanosoma?

"As has been shown, the T. gambiense differs in morphological characters from the animal varieties studied here. The difference is more marked in their behaviour when inoculated into the various experimental animals. From a consideration of the results obtained, the first conclusion that will be arrived at is, that the trypanosomes found in the animals in Uganda are different from those found in sleeping sickness cases, and in men showing no signs of sleeping sickness; the two latter trypanosomes being identical, being, in fact, the T. gambiense of the West Coast. As to the nature of the animal trypanosomes, the facts may be summed up as follows: the trypanosoma of Mr. Pordage's ox, when inoculated into a monkey and dog, failed to appear in the blood of either. It further appeared and developed in the blood of an ox. In these results a difference is established between this variety of trypanosoma and the T. gambiense. Owing to the fact that it did not 'take' in the experimental animals, it was not possible to study this 'strain' so fully as the others.

"With regard to the other varieties it will be at once obvious that the Jinja trypanosoma marks itself off from the other two in its behaviour when inoculated into animals. It runs a more acute course and is capable of developing in all the experimental animals except the baboon, whilst the Abyssinian and mule do not develop in the blood of sheep, oxen and goats. Thus, a distinct difference is constituted between the Jinja trypanosoma, on the one hand, and the Abyssinian and the mule on the other. This was also established by inoculating animals resistant to the two latter varieties of trypanosoma with the Jinja 'strain'.

"The reactions in animals of the trypanosoma found in the mule at Entebbe and that obtained from the Abyssinian boundary, are in all respects similar. The Jinja trypanosoma most closely approaches the classical African type (Nagana), and is, probably, identical with it. The other two differ from this type, and may be provisionally included under the unclassed varieties of African trypanosomes. It may be briefly stated that the species of trypanosomes which have been met with here are: (1) T. gambiense, which
is identical with those found in sleeping sickness cases and in cases of so-called 'trypanosoma fever'; (2) *T. brucei*, or a very closely related species, with which the Jinja cattle trypanosoma is identical; (3) a trypanosoma which occasioned the death of mules in Abyssinia and a mule in Uganda, and which is provisionally unclassed; (4) the trypanosoma of Mr. Pordage's ox . . . ."

"(17) Can the G. palpalis convey the trypanosoma found in the Jinja cattle, Entebbe and Abyssinian mules, to healthy animals?"

"The animal employed for these experiments was the monkey. The dog is quite unsuitable owing to the difficulty, already mentioned, of obtaining an animal free from ankylostomes. The method employed was to feed tsetse flies on an animal suffering from the above diseases, and then, after varying intervals of time, to place the same cage of flies on a healthy monkey. Only the flies which had filled themselves were counted as having fed . . . ."

"(18) Can other biting flies (*Stomoxys*) convey the trypanosoma found in the Jinja cattle, Entebbe and Abyssinian mules, to healthy animals?"

"Exactly similar experiments to the above were carried out, the only difference being that instead of *G. palpalis* another common biting fly met with in Uganda (*Stomoxys*) was used; these experiments are given in full . . . ."

"As a result of the above experiments it may be considered proved that the *G. palpalis* can convey the above trypanosomes from the sick to healthy animals, and so propagate the disease. Apart from the great practical importance attached to this, it is also of considerable interest to note that the *G. palpalis* can convey not only the *T. gambiense*, but other varieties. So it is reasonable to suppose, also, that other varieties of *Glossina* will convey the *T. gambiense*. Thus it will be evident from Mr. Austen's map that a very extensive tract of country will be involved. At Igaga's and Kibui, halting places of the Jinja cattle, a variety of tsetse fly (*G. pallidipes*) was found.

"It may be further considered proved that *Stomoxys* cannot convey these trypanosomes from the sick to the healthy animals. This is a matter of great practical importance also, because these flies abound in Uganda.

"Some observations were made on the length of time which the various trypanosomes remain active in the stomach of the fly. The contents of the stomach, food reservoirs and salivary glands have been studied, both fresh and by staining, but no definite life cycle has been observed in the parasites. In the ventral food reservoir,
active trypanosomes have been seen up to twelve hours after feeding. This is interesting in view of the fact stated by Schaudinn, that mosquitoes discharge the contents of the sac into the wound; in fact, the irritation is produced by these contents.¹

"Experiments were made to see whether the G. palpalis can convey any of these varieties of trypanosomes after longer intervals (five days and over). These remained entirely negative. So it would appear that if the trypanosoma undergoes any transformation in the body of the fly, as Schaudinn's work suggests, it must be a short one.

"A point of considerable interest in connection with the flies is the tendency which they have to 'abort' in captivity. Mr. Austen drew attention to the great variation in size of the pupæ in some specimens sent to him, and put forward the above explanation. To test this a number of pupæ have been placed in suitable places, and their development noted. It was found that the small, undersized specimens underwent no further alteration, whilst the larger and normal looking pupæ hatched out as usual. This would suggest that the small pupæ had been prematurely laid and were not viable."

(To be continued.)

¹ "This portion of the investigation which is very technical, will be elaborated by Professor Minchin, who has gone to Uganda for this purpose."