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

By Captain E. D. W. Greig.

Indian Medical Service.

(Continued from page 491.)

"12."

"REPORT ON SLEEPING SICKNESS IN THE NILE VALLEY. By Captain E. D. W. Greig, I.M.S.

"In the introduction to the Reports it was mentioned that Captain Greig left Entebbe for England via the Nile; this Report contains the record of his observations on the distribution of sleeping sickness and tsetse fly in the Nile Valley.

"About a year ago the exact distribution of Glossina palpalis was marked out on Lake Albert for the Commission by Mr. W. Y. Wyndham, then Collector, Wadelai. At that time no report of the presence of sleeping sickness in that district had been received. That the 'belt' had become infected was suggested, in the first instance, by a case of sleeping sickness (Case 69, Zururu bin Mza) which was admitted into Entebbe Hospital from Lake Albert on August 17th, 1904. This case is recorded in Appendix I. Later, a report was received by H.M. Acting Commissioner, Mr. George Wilson, C.B., from Mr. T. Grant, Collector, Hoima, stating that a disease, resembling sleeping sickness, had broken out amongst the inhabitants of Bugungu on the north-eastern shores of Lake Albert. It was, therefore, of great importance to determine (1) if the disease in Bugungu was sleeping sickness; (2) how far north the disease extended; and (3) the distribution of G. palpalis along the Nile banks.

"The co-operation of the Government of Egypt having been obtained, it was possible to make observations from Lake Albert down the Nile to Khartoum under specially favourable conditions.

"I left Entebbe on November 15th, 1904, and proceeded direct to Hoima, arriving there on November 25th, 1904. Halting there till December 6th, the cases of suspected sleeping sickness from Bugungu, collected there for me by Dr. Pooley, Medical Officer, Hoima, were all carefully examined. From Hoima the march was continued to Butiaba on Lake Albert. From there I proceeded to Bugungu by sailing boat 'James Martin.' At this point I was met by the Government steam launch and proceeded towards the Victoria Nile, examining on the upward journey the south bank
as far as the Murchison Falls, and the north bank on the return journey. This occupied four days. Proceeding down the Nile, Wadelai was reached on December 11th. Halting here two days, some of the general population were examined for trypanosoma infection, and the villages for actual sleeping sickness. From Wadelai the journey was continued down the Nile to Nimule. Halting here for four days, an investigation of a number of the general population for trypanosoma infection was made. From Nimule the march was continued along the right bank of the Nile to Gondokoro. This was reached on December 27th, 1904. At Gondokoro I was joined, on December 30th, by Dr. Sheffield Neave, sent by the Egyptian Government to co-operate with me. The gunboat 'Abu Klea' was placed at our disposal to investigate the banks of the Nile as far as Bor. From here the journey was continued through the sudd in the post-boat 'Amka.' Khartoum was reached on January 21st, 1905. This completed the investigation.

"As it is of extreme importance to ascertain whether *G. palpalis* is present or absent, not only on the banks of the Nile but throughout the whole Sudan, an arrangement was made by which each official of the Sudan Government stationed in the various districts will receive a specimen of the *G. palpalis* with a memorandum requesting information as to the presence or absence of this fly or flies resembling it (collections to be sent for identification to headquarters), and as to the character of the country, &c., should the fly or one resembling it be found. The results of the investigation on the banks of the Nile are recorded on the two maps which accompany this report. The red dots represent the distribution of the *G. palpalis* on the one map and of sleeping sickness on the other. It may be briefly stated that the following facts were ascertained:

"(1) The disease on Lake Albert from which the people were dying was undoubtedly sleeping sickness.

"(2) The disease could be traced, in diminishing severity, along the south and north banks of the Victoria Nile, below the Murchison Falls, and as far north as Wadelai.

"(3) Examination of the lymph glands of the general population of Nimule showed that the proportion of enlarged cervical glands was low, and the examination of the juice of these glands was negative as regards trypanosomes. No case of sleeping sickness has been recorded here.

"(4) The distribution of *G. palpalis* coincides with the area of
sleeping sickness. It terminates on the Nile banks a little north of the point where the fourth degree cuts the Nile. Here the character of the country begins to alter, open spaces and sparse vegetation giving place to undergrowth and trees.

"(5) G. palpalis was not found on the banks of the Nile in the Sudan.

"(6) G. morsitans has been found in the Bahr-el-Ghazal Province. This interesting observation was made by Colonel Griffith, D.S.O., P.V.O., who states 'that he found G. morsitans in the Bahr-el-Ghazal Province on the banks of the Pongo River, where the road to Deim Zubeir crosses it.'

"(1) Sleeping sickness is present in the 'Fly belt' at Bugungu, Lake Albert.

"Eighteen cases were collected at Hoima by Dr. Pooley from Mwanga's shamba, Bugungu.

"These were, clinically, typical cases of sleeping sickness at different stages of the disease. Trypanosomes were found in the gland fluid of every case. The cerebro-spinal fluid was examined in several cases and the trypanosomes found in every case. Dr. Pooley reported on December 13th, 'that six of the above cases had since died.'

"It is interesting to note that a blood-sucking maggot is found in Unyoro. Specimens were brought in to Dr. Pooley by the natives. Specimens of the maggot have been sent to Mr. Austen for identification. A curious feature was, that the dogs in the sleeping sickness area died in considerable numbers of a wasting disease. Two sick dogs were sent to Entebbe, to be kept under observation. Lieutenant Gray, R.A.M.C., writes on January 19th, 1905, 'that one of the dogs shows a trypanosome. Of the two monkeys and the guinea-pig which we infected from this dog: (a) the guinea-pig has not yet shown trypanosomes; (b) monkey showed trypanosomes eleven days after infection; (c) second monkey has not yet shown trypanosomes.' It will remain to be seen from further observations to what variety of trypanosoma this belongs.

"(2) Sleeping sickness is present in the 'Fly belt' as far north as Wadelai.

"At various villages on the south and north banks of the Victoria Nile and the right bank of the Nile to Wadelai, the general population was examined, and the chiefs questioned regarding the occurrence of sleeping sickness. The method of investigation was by examination of the lymphatic glands as recorded in page 7 of the Report.
"The following table shows the village or station examined and the presence or absence of sleeping sickness in the general population.

<table>
<thead>
<tr>
<th>Name of village or station</th>
<th>Situation</th>
<th>Sleeping sickness</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borigi</td>
<td>South bank, Victoria Nile, 15 miles from mouth</td>
<td>Present</td>
<td>Two early cases.</td>
</tr>
<tr>
<td>Fajao</td>
<td>Near Murchison Falls</td>
<td></td>
<td>One case reported.</td>
</tr>
<tr>
<td>Kimori</td>
<td>North bank of Victoria Nile, 7 miles from mouth</td>
<td></td>
<td>Sixteen men examined. Thirteen had enlarged cervical glands with rapid pulse. Chief reports eight persons died in his village last month of sleeping sickness. Fifteen of the general population examined. Four had enlarged cervical glands. Trypanosomes found in one. One case of undoubted sleeping sickness.</td>
</tr>
<tr>
<td>Wadelai</td>
<td>Right bank of Nile</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"(3) Sleeping sickness at the present time does not occur as far north as Nimule.

"At this station the cervical glands of eighty-seven males of the general population were examined, namely, sixty Nubian Askaris and twenty-seven Askaris from Afudda. A few of these showed slight enlargement of the cervical glands, but microscopic examination of the juice was negative as regards trypanosomes.

"Through the courtesy of Commandant H. V. Calseyde, I was enabled to examine a number of the general population of Dufile in the Enclave. One case of trypanosoma infection was found. This was imported from the interior. This observation is of considerable importance as indicating a route along which the infection might enter the ‘fly belt’ of the Nile.

"No cases of sleeping sickness have occurred at Gondokoro or in the Sudan.

"(4) Glossina palpalis extends along the banks of the Nile 30 to 50 miles north of the point where the 4th degree cuts the Nile.

"The red dots on the map indicate the position where the G. palpalis was actually found; at some points it was extremely numerous; this was especially so at Fajao, on both sides of the Nile at the Falls. G. palpalis is found all along the banks of the Nile in Uganda territory. It only ceases to occur a short distance south of Gondokoro. It is interesting to note that, at the point where the fly ceases, the character of the country alters completely. It
becomes more open, the undergrowth is not found, and the trees are further apart, and therefore affording much less shelter from the sun.

"(5) Glossina palpalis was not found on the banks of the Nile between Gondokoro and Khartoum in the Sudan."

"I examined both banks of the Nile at each possible landing place as far as Bor, but with negative results. From Bor the journey was continued through the sudd. No specimen of G. palpalis was found, nor at any point on the journey to Khartoum. Dr. Sheffield Neave will continue and extend the observations on these lines in the Sudan under the direction of Dr. A. Balfour.

"(6) Glossina morsitans occurs in the Bahr-el-Ghazal province of the Sudan.

"The observation of Colonel Griffith shows that the G. morsitans exists on the banks of the Pongo River. Mr. Brown, of the Imperial Institute, who has recently been in the Bahr-el-Ghazal, considers 'that the fly is more numerous on the west bank. There is a forest of trees on the west bank. The trees are more scattered on the east.' He also states 'that the fly occurs on the Jur River near Wau.' It is of great importance that this belt should be accurately defined. Dr. A. Balfour, Director of the Gordon College Laboratory, Khartoum, has found trypanosomes in the blood of animals from the Bahr-el-Ghazal.

"(7) Has the Nile 'Fly belt' become infected from Uganda or the Congo?

"It is obvious that the infection must have been carried in from one or other of these areas of sleeping sickness. Its greater severity in Unyoro and gradual diminution north, appears to suggest that it gained an entrance from the Uganda side, but cases are found in close proximity on the left bank of the Nile. It is impossible, therefore, to definitely answer the question.

"The general situation as regards sleeping sickness in the Nile Valley is, that sleeping sickness is slowly spreading in the 'fly belt,' and will extend to its northern and southern limit. As the northern limit does not extend into the Sudan there will not be a direct extension of the disease along the Nile into this country. As, however, a closely related species (G. morsitans) exists in the Bahr-el-Ghazal province, it will be of the utmost importance to prevent the introduction of people from sleeping sickness areas into this 'belt,' and to accurately define the limits of the 'belt.' . . ."

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1 "Vide Map of Africa showing distribution of tsetse flies, by Mr. Austen."
"Although our knowledge of the distribution of the eight species of tsetse flies is still very far from complete, it is nevertheless possible, owing in large measure to the special attention that has been paid to the genus *Glossina* within the last two years, to make an attempt to illustrate the distribution of the various species by means of a map. In view of the possibility that the trypanosome of sleeping sickness may be conveyed by other species of *Glossina* besides *G. palpalis*, it is the more important that this should be done, especially since no such attempt has hitherto been made. The map published in the writer's Monograph of the Tsetse Flies (1903), merely showed what was then known of the distribution of the genus as a whole, without attempting to discriminate between the species, and although a map showing the distribution of the different species was exhibited by the author at Oxford in July, 1904, in connection with a paper on Tsetse Flies¹ read by him in the section of Tropical Diseases, at the Annual Meeting of the British Medical Association, it was unfortunately not found possible to reproduce the map when the paper was printed. The accompanying map, in the preparation of which the writer has been most kindly assisted by Mr. A. J. Engel Terzi, will, it is hoped, at least serve as a basis for future work.

"Since it may now be assumed to be well understood that tsetse flies are not met with continuously throughout broad tracts of country, but are confined to relatively narrow 'belts,' which are frequently discontinuous, and are usually to be found along the margins of water-courses, rivers and lakes, it is perhaps hardly necessary to explain that a particular species must not be supposed to occur everywhere within the areas marked on the map. The latter only shows broadly what is at present known of the *relative distribution* of the different species, which, in view of the scale used, was all that was possible. Similarly, where a species of tsetse is shown as occurring along a river or on the margin of a lake, the map must not be taken as giving any indication whatever of the

distance from the water to which the fly is to be found, which in some cases may be merely a few yards. Moreover, the fact that any particular locality lies within the limits of the occurrence of a species of tsetse, as shown on the map, is not to be taken as implying that the fly necessarily exists there to-day. The areas marked are in accordance with records of the localities of actual specimens, but in some instances, as has certainly happened in the case of G. morsitans in parts of the Zambesi Valley, owing to the retreat of big game or other causes, tsetse flies are no longer to be found in places formerly infested by them. When isolated areas are marked as the home of one or more species, it is to be understood that specimens have been received from these localities, or else that there are apparently reliable records of the occurrence there of the species concerned; in many cases, more complete collections or fuller information would doubtless prove their existence in intervening localities also.

"With these introductory remarks the map may be left to explain itself, but the following notes on certain of the species of Glossina will perhaps be of interest:—

"Glossina palpalis, Rob.-Desv.—This species has recently been reported by Laveran as occurring at Sengaleam, in Senegal, about six miles from Rufisque, and thirty from Cape Verde; this is the most northerly locality yet recorded for any tsetse fly. In West Africa the limit of the range of G. palpalis towards the interior is entirely unknown, so that no attention should be paid to the inner boundary of the area shown on the map. In this region most of the specimens and records are derived from localities near the coast, and it is consequently impossible to say how far the species extends into the interior, although it may reasonably be supposed to occur throughout the valleys and basins of the majority of the rivers that fall into the Atlantic within the limits of the Tropics. Since we now have records of the occurrence of G. palpalis at various points between Sengaleam and the Congo inclusive, the species is shown on the map as occurring throughout this area, for, although the evidence is not yet complete, there is no reason whatever to imagine that it will not ultimately be found to exist in all suitable localities

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1 "For information as to 'fly-belts' and their extent, and the distribution and limits of tsetse within these areas, cf. 'Monograph of the Tsetse Flies,' p. 9, et seq."

within these limits. According to our present knowledge therefore, the distribution of G. palpalis extends from Cape Verde in the north-west, through West Africa to an unknown distance into the interior, and southwards to the Congo. In the equatorial region the eastern limits of the species, as at present known, are the River Omo, which falls into the northern end of Lake Rudolf, and the eastern shore of Lake Victoria. It was encountered by Dr. Brumpt from the sources of the Welle to the mouth of the Congo, and since Laveran\(^1\) states that he has identified it among specimens from Katanga, in the south-east corner of the Congo Free State (the most southerly record at the present time), it is probably to be found throughout the Lualaba-Congo system as well. South of the Congo G. palpalis doubtless occurs throughout the greater part, if not the whole, of Portuguese West Africa, since, although actual records of the occurrence of the fly are at present lacking and no collections have as yet been made in this region, according to Dr. H. Rey\(^2\) sleeping sickness has been observed from Benguela northwards.\(^3\)

"Glossina morsitans, Westw.—In the paper already referred to, Dr. Laveran records the identification by him of this species among material from French Guinea, the Rivers Assinie and Comoë (Ivory Coast), and Katanga in the Congo Free State, to the south-west of Lake Mweru. Collections received at the British Museum last autumn from Mr. Robert Codrington, Administrator of North-Eastern Rhodesia, show that G. morsitans may be said to be distributed throughout North-Eastern Rhodesia.

"As regards G. morsitans in the Bahr-el-Ghazal province of the Sudan, the locality shown on the map is that of the specimen obtained by Colonel Griffiths in 1903, on the Pongo River, between Wau and Dem Zibehr, where the species appears to be very abundant; Dr. Andrew Balfour, of the Gordon College Laboratories,"

\(^{1}\) "Loc. cit., p. 662."

\(^{2}\) "Quoted by Christy, 'Reports of the Sleeping Sickness Commission,' No. iii., November, 1903, p. 7."

\(^{3}\) "Since these notes and the accompanying map were prepared, the British Museum has received from Dr. F. Creighton Wellman a form of G. palpalis taken by him in November last on the Katumbela River, Benguela; the specimens are somewhat different from the typical form, and represent a new sub-species, which the author has described as G. palpalis wellmani. In the Congo Free State, according to information furnished by the Rev. W. Holman Bentley, of the Baptist Missionary Society, G. palpalis is abundant some eighty miles to the south-east of Lutete."

Khartoum, in a letter to the writer, dated January 9th, 1904, said that, during a recent journey to Uganda, a native officer informed him that the fly is found six miles inland from Shambe, on the Bahr-el-Jebel. Dr. Balfour is inclined to think that in the Egyptian Sudan, *G. morsitans* is 'limited to the Bahr-el-Ghazal province, and does not extend further north than the river of that name.' Major Penton, R.A.M.C., whom the writer has lately seen, is disposed, as the result of experience gained during recent service with the Egyptian Army, to agree with Dr. Balfour, and thinks that, at any rate, *G. morsitans* is not to be found to the north of Fashoda.

"*Glossina tachinoides*, Westw.—This species is recorded by Laveran¹ from the River Bani, a tributary of the Niger, in the French Sudan. The same author (ibid., p. 659) also speaks of its occurrence on the Lower Rio Nunez, French Guinea; but, since this is an isolated record, it is not shown on the map.

"*Glossina pallidipes*, Austen.—In October, 1904, specimens of this species were forwarded from Gosha, Jubaland, East Africa Protectorate, by Major L. H. R. Pope-Hennessy, 3rd King's African Rifles. Writing from Kismayu on October 11th last, Major Pope-Hennessy states that the natives say that this fly is deadly to cattle and camels, and adds that 'should recruits with the germ of sleeping sickness in them be obtained from Uganda, and be bitten by this fly, the disease may be propagated in Gosha, and perhaps annihilate our only hard-working section of the inhabitants. Apart from questions of humanity, this would put an end to any opening-up of the country.'

"*Glossina longipalpis*, Wied.—A specimen of this species, obtained long ago by Sir John Kirk and labelled 'Zambesi,' is in the British Museum collection, but since the precise locality is unknown, the species is not shown on the map as occurring in the region in question. It is recorded by Laveran² from French Guinea and Katanga, Congo Free State.

"*Glossina fusca*, Walk., is now known from a number of widely distant localities, and its area of distribution, in addition to being in all probability co-extensive with that of *G. palpalis* in West Africa, also extends to Central and East Africa. Apart from previous records, the writer has recently seen a specimen from Usagara, German East Africa, obtained by the Rev. A. North Wood, in 1904.

¹ Loc. cit., p. 661.
A specimen in the British Museum, collected by Sir John Kirk, is simply labelled 'Zambesi,' but the occurrence is not recorded on the map for the reason stated above in the case of G. longipalpis. As regards West Africa, the latest record is one by Laveran (loc. cit.) from French Guinea.

"G. fusca was met with in July, 1904, fifteen miles north-east of Chiromo, British Central Africa, by Major F. B. Pearce, Deputy Commissioner, British Central Africa Protectorate. Writing from 'The Residency, Zomba, British Central Africa,' on November 8th, 1904, Major Pearce says: 'I have arranged to have a few head of cattle kept within the fly (G. fusca) zone, so as to arrive at some conclusion with regard to the question whether G. fusca is dangerous to live stock. In this connection, you may perhaps be interested to know that a herd of Government cattle has been kept for years at Chiromo, and it is not an unusual occurrence for them to graze in the Elephant Marsh, actually in sight of buffalo. The Chiromo cattle have always done very well, and none have ever been lost from 'fly' sickness. The same may also be said concerning the cattle of the chief Makwira, who has a large number of cattle, which always graze in the 'Marsh,' where buffalo are common. If, therefore, the only species of 'fly' in the Elephant Marsh game reserve is G. fusca, it would seem that that species is not dangerous to live stock.' It may be noted that Major Pearce's statements as to the apparent harmlessness of G. fusca to domestic animals are supported by Stuhlmann's observations on the same species near Dar-es-Salâm.¹

¹ "Cf. Austen, 'Monograph of the Tsetse Flies,' p. 300."

(1) Can the trypanosome of sleeping sickness multiply in the stomach of Glossina palpalis?

The following is an outline of the experiments made to prove this. The flies used were brought in daily from the lake shore at Entebbe. It appeared that a dry atmosphere affected the vitality of the caged flies, and also had a marked effect on the length of time during which the trypanosomes survived inside them. To counteract this the flies, from the time they were brought in, were kept in cages, placed on a bed of absorbent paper, constantly saturated with water from a reservoir with a syphon attachment.

The flies were kept either twenty-four or forty-eight hours after they were brought in. They were then fed on monkeys infected from the cerebro-spinal fluid of sleeping sickness cases. These monkeys showed trypanosomes in varying numbers in a blood film, though never more than one trypanosome to six fields of a 2 mm. objective. Forty-eight hours later they were fed on a fresh normal monkey ‘A.’ Forty-eight hours later they were fed on another fresh monkey ‘B.’ Forty-eight hours later on monkey ‘C’, and so on. This interval was selected because, from previous trials, it seemed a natural one for the fly, and nearly all the flies would re-feed after forty-eight hours.

An enormous increase occurs sometimes in the number of trypanosomes taken in by the fly, so much so, that the blood in the intestine of the fly literally swarms with them. In this case the appearance of a fresh preparation can only be compared to a similar one made from the blood of a rat dying of Nagana, when the number of parasites equals that of red corpuscles.

This increase was first seen in flies ninety-six and one hundred and twenty hours after infection, and was thought to occur first at these periods.

Later on it was found that the same increase occurred, and that the same enormous numbers of trypanosomes were found, twenty-four hours after the fly had fed on the infected animal.

When these flies were re-fed in the way described, each successive feed of blood seemed to act as a fresh supply of culture medium, and we have found these greatly increased numbers maintained up to two hundred and eighty-eight hours (twelve days) after...
the infective feed. It is very probable, therefore, that the increase first found at ninety-six and one hundred and twenty hours after infection was only the continuation of one which had occurred in the first twenty-four hours. After it had been found that this increase could occur in the first twenty-four hours, observations were made on two monkeys. When examined twenty-four hours after feeding this multiplication was observed in a total 10 per cent. of all the flies.

"On some days a considerable number of flies would be examined and the increase would not be found in any of them, though they were kept under the same conditions and fed on the same monkey, and though there was no perceptible difference, either in numbers or in morphology, of the trypanosomes as seen in a blood film. For instance, of the flies which fed on monkey 350 on March 23rd, five out of fifteen showed this great increase when examined twenty-four hours later. On the next day the increase was not seen in any of twenty-nine flies examined. On the next day eighteen negative flies were examined, and on the day following ten. Three days later, when another box of flies fed on the same monkey was examined, the increase was found in two out of nine flies examined. This increase, which is found in 10 per cent. of flies twenty-four hours after feeding, is continued at later periods up to two hundred and eighty-eight hours, in a total of 5.6 per cent. of them. Probably, if a much larger number of flies could be fed and examined, it would be found that the increase was continued in the same proportion of flies as showed it originally.

"The proportion of male flies brought in is very much greater than that of females. This increase has, however, been observed in one female fly.

(2) What proportion of freshly-caught flies in the neighbourhood of Entebbe contain trypanosomes?

"The following method was used in order to try and find this out:

"The flies were kept for twenty-four hours after they came in. They were then fed on an uninfected normal monkey. Twenty-four hours later they were dissected and examined for trypanosomes. Out of 200 flies examined up to the present, two contained in their intestines the same enormous numbers of trypanosomes as were found in 10 per cent. of flies which had been fed on an infected monkey twenty-four hours previously.

(3) Morphology of the trypanosomes seen in the fly.

"The forms of trypanosome seen in the fly vary from very small
The most striking variation from the ordinary form seen in the blood, however, is the different position of the micronucleus. This is very rarely seen at the extreme blunted end of the parasite. It varies from a position midway between the posterior extremity of the trypanosome and the macronucleus, to a position on the anterior or flagellar side. In trypanosomes from the fly, the most common positions for the micronucleus are, either anterior to the macronucleus or at the side of it. A very common dividing form was seen which would give rise to two trypanosomes, one with an anterior micronucleus and the other with a micronucleus at the side of the macronucleus. The very small forms have been observed to be formed by unequal division of a large trypanosome. No vacuole is seen in any of these trypanosomes. The blue-staining granules in the protoplasm are present, as in the ordinary forms from the blood. What seems their natural method of progression is with the flagellum foremost. They then move very rapidly along a straight course, with only the flagellum and undulating membrane vibrating, the rest of the trypanosome having no lateral movement at all. They can also move with the blunt posterior extremity first, but, in this case, they move very slowly; their path is zig-zag instead of straight, and they advance by a series of contractions which bend one half of their body at right angles to the other. With greatly increased numbers of trypanosomes in a fly at any period after infection, there is, in most cases, a large proportion of forms with anterior micronucleus. In some cases, however, all the trypanosomes found in a fly are practically normal in appearance, the micronucleus being near the posterior extremity. Of the two 'fresh' flies which contained trypanosomes, one contained forms almost all of which had an anterior micronucleus, the other showed almost 'normal' trypanosomes. Rosettes of trypanosomes have been seen in both fresh and stained preparations. In these rosettes the trypanosomes are joined directly by their posterior extremities; there is no central mass of protoplasm. They vary from very distinct rosettes of four to seven trypanosomes, to large loosely-woven masses of fifteen to twenty, most of which are joined at their extremities, but some of which, either naturally, or in making the preparation, are a little separated and lie entangled among the others. When observed in a fresh preparation these rosettes become smaller from breaking away of some of the individuals; there is nothing in the nature of agglutination. In some rosettes every trypanosome
belonged to the type in which the micronucleus is anterior. Other rosettes were composed of forms with the micronucleus either at the side of the nucleus or touching it posteriorly. One stained preparation showed a mass of trypanosomes visible with a hand lens. It consisted of a long strip of trypanosomes lying side by side, closely opposed to each other, and four or five deep. It had the appearance of a mass or colony formed by progressive multiplication. Several oval forms of trypanosome have been observed, with a darkly-staining blue protoplasm, macro- and a micro- nucleus. These oval forms frequently have a capsulated appearance, possibly due to the remains of the flagellum. In the examination of these flies the whole gut was dissected out in each case, and its various parts mixed with normal saline examined afresh. If examined soon after re-feeding the fly, the trypanosomes are confined to the dark altered blood in the lower gut, but later on, they swarm throughout the blood in the whole alimentary tract. As in the case with cerebro-spinal fluid or gland juice, the medium surrounding the trypanosomes in the fly was found to hinder staining of the chromatin. Accordingly, films were made and fixed while still wet in osmic vapour. They were then treated with an application of fresh blood serum, as recommended by Lieutenant-Colonel Leishman for sections containing trypanosomes. This was then washed off and they were stained by Leishman's stain. This method gives a very clear staining of the chromatin elements, and they are not obscured by the granules in the protoplasm, which stain a deep blue.

"(4) Can infection be conveyed to an animal by inoculating these trypanosomes from the intestine of the fly?"

"The following experiments have been done in connection with this point. Monkey 380 was injected with the intestinal contents of a fly which had been fed on an infected monkey one hundred and twenty hours previously, and re-fed in the usual way. This fly contained enormous numbers of trypanosomes. The monkey was frequently examined, but never found infected. Forty-nine days later the contents of 10 flies, which had fed twenty-four hours previously, were injected. A drop of the fluid injected showed numerous active trypanosomes, but the monkey remained uninfected. Monkey 381 was inoculated with the contents of 20 flies which had been infected ninety-six hours previously (and re-fed): Forty-nine days later the animal died. Its blood was frequently examined up to the time of death, but never showed trypanosomes. Death, in this case, was probably due to a long
Report of the Commission on Sleeping Sickness

captivity. Monkey 382, a duplicate experiment to 381, has never shown trypanosomes. Monkey 395 was injected with the contents of 10 flies which had fed twenty-four hours previously. It died twenty-one days later, never having shown trypanosomes. Monkey 396 was inoculated with the contents of 10 "twenty-four hour" flies. This animal was also uninfected. The natural conclusion is, that infection cannot be produced by inoculation of trypanosomes from the intestine of the fly, and this same conclusion was arrived at by Colonel Bruce when experimenting with the trypanosome of Nagana.

"(5) Can trypanosomes travel from the intestine to the salivary gland of the fly?

"(a) The salivary gland of a fly, which had been infected one hundred and forty-four hours previously (and re-fed as usual), was dissected out. This fly contained great numbers of trypanosomes in its intestine, many of them showed forms with an anteriorly placed micronucleus. The salivary gland, on examination, showed numbers of actively motile trypanosomes. On staining, most of these trypanosomes appeared to be the ordinary forms as seen in the blood, but there were a few forms similar to those seen in the gut.

"(b) In the 'fresh' fly noted above, which contained numerous trypanosomes of almost the ordinary form in its gut, the salivary gland was also found to contain numbers of trypanosomes. The salivary gland was broken up in normal salt solution and injected into a monkey, but it had been kept for some time before this was done, and the trypanosomes had lost most of their activity. In a stained preparation, these trypanosomes were like the forms ordinarily seen in the blood of man or injected animals. Up to the present, fifteen days, this monkey has not shown trypanosomes. None of the series of monkeys on which the flies were re-fed have as yet shown trypanosomes."

The photographs on page 597 show the enlargement of the lymphatic glands in case of No. 69 ZN Arcadi on June 5th, 1904.

[This summary is taken from Report No. VI. of the Sleeping Sickness Commission of the Royal Society, and is published, with the illustrations, by permission of the Controller of His Majesty's Stationery Office.]
FIG. 1.—BLOOD OF DOG SUFFERING FROM JINGA CATTLE DISEASE.

FIG. 2.—BLOOD OF DOG. ABYSSINIAN TRYPANOSOME.
BLOOD OF GUINEA PIG. MULE VARIETY OF TRYPANOSOME.

Bole & Daniëlssoon, L.M. (ed.}
PORTION OF STOMACH OF GEERUDE, CASE OF SLEEPING
SICKNESS, SHOWING ULCERATION OF MUCOUS MEMBRANE.

Baird & Davidson, Ltd.
MAP SHOWING THE DISTRIBUTION OF GLOSSINA PALPALIS.

The Red Dots show localities where Tsetse Fly was obtained.
AFRICA
SHOWING THE DISTRIBUTION OF THE
TSETSE-FLIES
AS AT PRESENT KNOWN.