LEAGUE OF NATIONS

Health Organisation

INVESTIGATIONS

ON

HUMAN TRYPANOSOMIASIS

IN MOZAMBIQUE

Report submitted to the Second International Conference on Sleeping-Sickness (Paris, November 5th to 7th, 1928).

by

Professor Dr. Ayres KOPKE

Professor at the Lisbon School of Tropical Medicine

Geneva, 1930

LEAGUE OF NATIONS

PUBLICATIONS OF THE HEALTH SECTION

COMMISSION FOR THE STUDY OF SLEEPING-SICKNESS AND TUBERCULOSIS IN EQUATORIAL AFRICA

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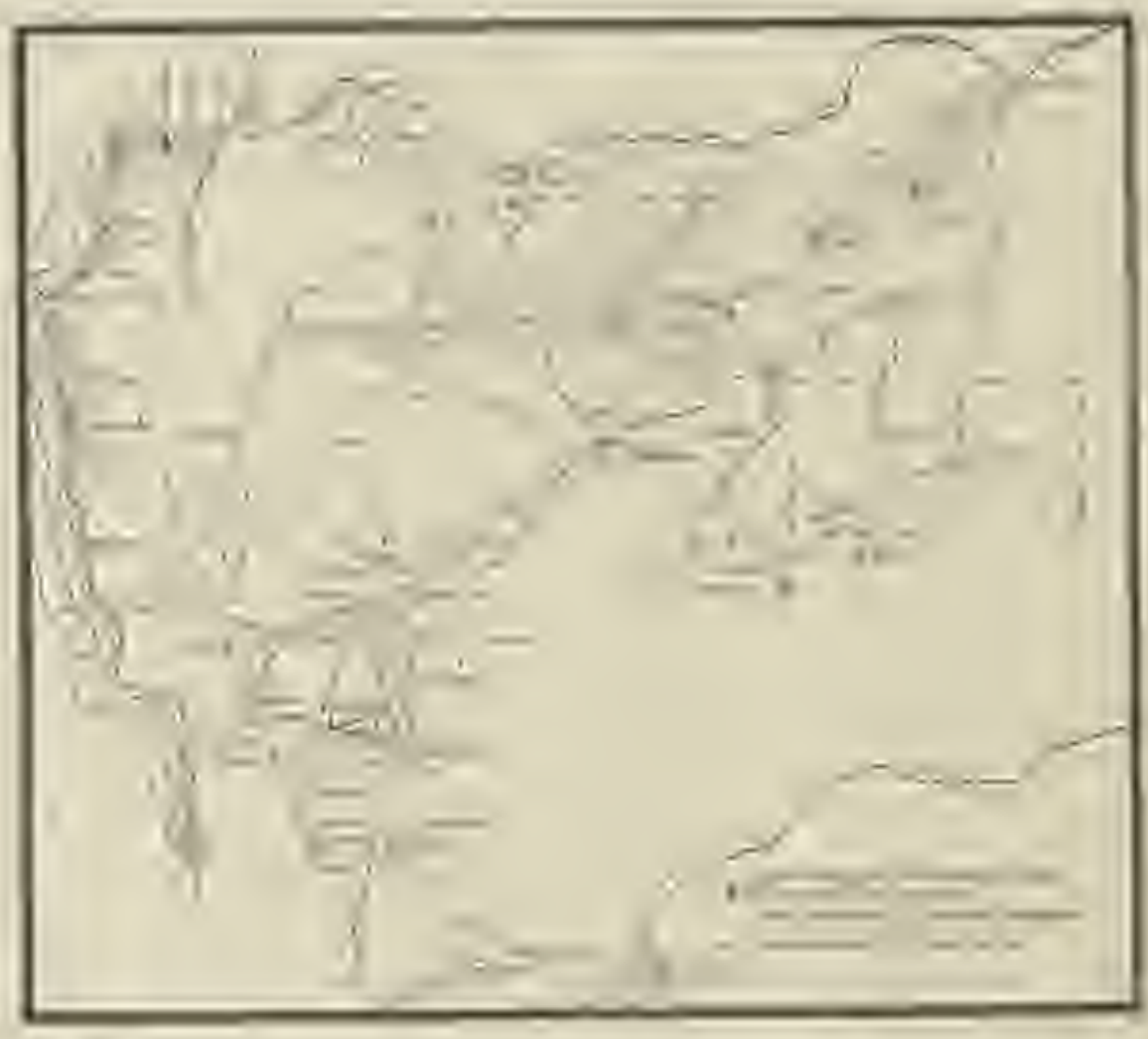

Investigations made in the Western Zone of the Nyassa Company's Territories by the Kopke Mission in 1927.

Investigations on Human Trypanosomiasis in Mozambique.

Research Work carried out by the Medical Mission in Mozambique.

Some thirty years ago, sleeping-sickness had assumed an epidemic form in regions where it had till that time been only insignificant, sporadic and endemic.

On the initiative of Professor Miguel Bombarda, President of our Medical Association, and Dr. Ramada Curto, Director of the Health Department of the Ministry of the Colonies, a new stage in the study of this tropical disease was begun ; and, in 1901, the Minister, Dr. Teixeira de Sousa, sent our first Medical Mission to Africa, under the leadership of Professor Annibal de Bettencourt, the Director of the Câmará Pestana Bacteriological Institute.

My object in recalling this fact is to draw attention to the desire our country has always displayed to place itself on an equal footing with other colonising nations in this field of scientific medical research, as well as in others.

It is true that this first mission, in spite of strenuous and conscientious work of which its report bears evidence, did not succeed in determining the true cause of the disease ; but other investigators, working before and during the same period and with the same object in view, failed in like manner.

Our early laboratory training was mainly bacteriological and not protozoological ; but it may be noted that parasitologists, including some of the most distinguished in the world, as Sir Patrick Manson was in the field of tropical medicine, formulated hypotheses regarding the etiology of sleeping-sickness, which the future was to disprove. Manson assumed that *Filaria perstans* found in blood preparations from sleeping-sickness patients, and distributed over a geographical area which at that stage of the enquiry appeared to coincide with that of sleeping-sickness, was the cause of the disease ; subsequent investigations proved that this was not the case.

Eventually, the discovery of the cause of sleeping-sickness was not made by any of the Missions which specially and deliberately set out to study its etiology. Forde, and Dutton, who were at that time (1901-02) studying in Gambia a disease which was thought to be peculiar to that region, found in the blood of their patients a flagellate protozoal parasite, a trypanosome, to which they gave the name of *Trypanosoma gambiense*.

This was the first time that the existence of a human disease caused by this flagellate was definitely proved. Naturally, the demonstration attracted the attention of the whole medical world, and subsequent researches were orientated in that direction.

It was after Dutton's discovery in 1903 that Professor Aldo Castellani, who was studying sleeping-sickness in Uganda and, like the first Portugese mission, believed it to be caused by a streptococcus infection, found trypanosomes in the cerebro-spinal fluid of a sleeping-sickness patient, and subsequently in that of many others. He gave it the name of *Trypanosoma ugandense*, which Kruse subsequently changed to *Trypanosoma castellani*.

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Sir David Bruce then put forward the hypothesis that sleeping-sickness was caused by this trypanosome — which subsequent research showed to be completely correct — and that the Gambia fever was simply the first stage of the disease. It was thus established that the disease was due to the trypanosome discovered by Forde and Dutton and very properly named *gambiense*.

Since that time, research on tropical diseases, both of man and of animals, has actively continued.

Various trypanosomes pathogenic for the larger mammals had been previously described : the trypanosome of Indian surra discovered by Evans in 1880, that of the nagana of Zululand by Bruce in 1895, that of Caderas' disease in Brazil by Elmassian in 1901, and *T. equiperdum* (the cause of dourine) by Doflein, also in 1901.

After the discovery of *gambiense*, many other trypanosomes were described — *equi*, *Venezuelense*, *Sudanense*, *elephantis*, *vivax*, *cazalboui*, *uniforme*, *pecaudi*, *congolense*, *dimorphum*, *confusum*, *pecorum*, etc. — but subsequently many of these species came to be considered as mere varieties, or even in some cases as identical forms. Thus these flagellates, the causal agents of disease in mammals, were reduced to a few main types of which the others are held to be simply synonyms. These types are : *equiperdum*, *evansi*, *vivax*, *congolense* or *dimorphum*, and *brucei*.

Lavier even carries this tendency to simplification so far as to suggest that all trypanosomes may be regarded as having a single common ancestral origin, and he derives the *vivax* and *congolense* types from the *brucei* type. He admits, however, that this view is at present mainly hypothetical.

Two new human trypanosomiases were subsequently discovered, one by Chagas in Brazil in 1907, the causal agent of which, *Trypanosoma cruzi*, was described in 1909, and the other in Rhodesia in 1910 by Stephens and Fantham, the causal parasite of which is known as *Trypanosoma rhodesiense*.

This last parasite was thought to be different from *gambiense*. The morphology is different ; blood preparations from small laboratory animals which have been inoculated yield forms with displacement of the nucleus, generally towards the posterior extremity. The virulence is greater ; the disease kills sufferers and experimentally infected animals more rapidly, is more resistant to treatment, and prevails in small endemic foci without attaining the alarming epidemic expansion observed in the case of the *gambiense* virus, which called the attention of the medical world to sleeping-sickness. It is transmitted by glossinæ of the *morsitans* group, and not by those of the *palpalis* group.

All these differences seem gradually to have lost their importance. Forms with posterior displacement of the nucleus have been met with, though more rarely, in *T. gambiense* (Prates, Lavier, and Kleine, working for the Entebbe Commission of the League of Nations Health Organisation). Virulence is variable. Parasites of identical origin may present different degrees of virulence, and Professor Kleine assumes that the number of forms with an abnormal position of the nucleus is determined by the power of multiplication of the more virulent strains, both of *gambiense* and of *rhodesiense*. The method of propagation — endemic or epidemic — is also not a reliable basis for a distinction ; it is obvious that the great epidemics of trypanosomiasis must have been due to an increase in virulence and in the facilities available for infection by strains which previously caused only a small number of

cases. In Mwanza, in Tanganyika, south of Lake Victoria, an epidemic due to *Trypanosoma rhodesiense* (Balfour, Maclean, Swynnerton and Duke) commenced in 1918.

Shortly after the discovery of *Trypanosoma rhodesiense*, analogies were observed between this flagellate and the *T. brucei* of nagana (a disease of wild and domestic animals in Africa). The morphology is absolutely identical — indeed, the two species are indistinguishable in blood preparations made from laboratory animals inoculated with one or the other of these strains. Bruce and his collaborators came to the conclusion that the two viruses were identical and that, consequently, the human trypanosomiasis of Rhodesia was the same as nagana. This view is almost an absolute conviction to certain observers ; indeed, Wenyon, in his recent book (1926), described the causal agent of East African sleeping-sickness as the human strain of *Trypanosoma brucei*.

It was subsequently found, however, that the geographical distribution of nagana and of the Rhodesian type of sleeping-sickness did not coincide. The animal disease is much more widely distributed ; the explanation may be that only rare strains of the *brucei* virus become adapted to man. In some parts of Africa, such as Zululand, animals suffer very seriously from *T. brucei*, and the transmitting agent, the tsetse fly, is abundant, but there are no cases of human trypanosomiasis ; in other parts, the two diseases exist side by side, but the number of cases of Rhodesian sleeping-sickness in man is much smaller. On the assumption that the two trypanosomes are identical, all this goes to prove that man has a greater power of resistance to the infection, and consequently can seldom be successfully inoculated by infected glossinæ.

Other observers have not accepted the identity of *T. rhodensiense* and *T. brucei*. Experimental inoculations have been carried out in man, chiefly by Taute and Huber. Taute inoculated himself with the blood of an animal suffering from nagana, and submitted himself to bites by infected flies ; he and his collaborator, Huber, inoculated themselves and 129 natives with blood from nagana-infected animals. None of the persons thus experimentally inoculated contracted the disease, but the animals died.

We must, however, remember that trypanosomes which are not generally pathogenic to a given animal species may — either because the resistance of an individual of that species is lowered or because the parasite finds means of overcoming the normal resistance — succeed in infecting an individual of the species, and, if subsequently the virus is passed through a series of animals of the same species, its pathogenicity for that species may become accentuated.

This possibility of adaptation applies also to chemical substances injurious to the virus. Thus strains of trypanosomes have developed which are resistant to arsenic, antimony, etc., and it is equally possible that strains may evolve which, though not originally pathogenic for man, may become and remain so if subsequent inoculations be made direct from man to man. Ordinarily, however, human trypanosomiases are not propagated by direct contagion, but by the agency of the fly, in which the parasite passes through a cycle of evolution during which the recently acquired powers of adaptation may be lost, as was shown by Gronder in 1911 for certain strains of arsenic-fast trypanosomes.

Bearing this in mind, an explanation is forthcoming for the observed facts. *T. brucei*, the virus of nagana — a disease of animals to which man is naturally resistant — may, in certain special circumstances not yet determined, overcome

this resistance and develop in certain individuals who have been bitten by infected flies. These individuals will then contract the disease and will constitute a small number of scattered cases in foci where the disease will remain in an attenuated endemic form, if the great majority of the trypanosomes do not retain, throughout their cycle of evolution in the fly, their newly acquired power of infecting man. If, on the other hand, the majority of the trypanosomes could retain their pathogenicity for man throughout the cycle, this would be an important factor in the subsequent propagation of the disease in epidemic form.

These various interpretations are of interest in view of the work done by the Entebbe International Mission,[1] whose preliminary reports were published in March 1927. Dr. Duke succeeded in producing arsenic-fast strains of trypanosomes which retained that character even after their cycle of evolution in the tsetse fly, and down to the third generation.

We see, therefore, that there is a school which believes that *Trypanosoma gambiense* of West African sleeping-sickness, *Trypanosoma rhodesiense* of East African sleeping-sickness, and *Trypanosoma brucei* of nagana are one and the same, and gives them the name of *Trypanosoma polymorphum*. If this be so, a comprehensive campaign against sleeping-sickness must take count of nagana, and in this campaign, on which the future of many parts of Africa depends, the medical and veterinary services must co-operate.

Proposed Mission to Study Sleeping-Sickness in the Province of Mozambique.

When the International Congress on Tropical Medicine was held at Loanda in August 1923, some of these problems were already attracting the attention of students of tropical pathology. I then proposed to His Excellency Dr. Francisco Ferreira dos Santos, Chief of the Health Department that a medical mission should be sent to the province of Mozambique. On returning to Lisbon, I also put the idea before Professor Silva Teles, Director of the School of Tropical Medicine, who took it up at once. He submitted it with his own recommendation to the High Commissioner, His Excellency Victor Hugo de Azevedo Coutinho. All these authorities displayed great willingness to further the idea of the mission, and an appropriation of £1,000 was made for the purpose in the estimates for the colony. It proved impossible, however, to send out the mission in that year (1924), when it would really have been very valuable.

Our mission left Lisbon at the first opportunity after the publication of the order establishing it by the then Minister of the Colonies, Commander Joao Belo, but it was not until July 5th, 1927, that we reached Mandimba, where there was no laboratory — and, of course, no possibility of establishing one — suitable for detailed scientific research such as the Uganda laboratory at Entebbe. From the date of our arrival at Mandimba to the date by which the delegates from Lisbon had to be back (April 1st, 1928), we had only seven and a-half months in which to work together.

As soon as I reached Lourenço Marques, I explained my scheme of work

[1] Sent out by the Health Organisation of the League of Nations.

for the mission to the Governor-General, Lieut.-Col. José Cabral, saying that, so far as sleeping-sickness was concerned, it was proposed to concentrate our investigations in the Nyasa Company's territory around Metarica, where cases of sleeping-sickness among Europeans had occurred, as stated by the Chairman of the Board of Directors of the Company on December 22nd, 1926. It was my intention to visit, if possible, the districts south of the River Rovuma. This part of the programme was the outcome of indications given by Dr. Andrew Balfour, based on the League of Nations enquiry into the geographical distribution of sleeping-sickness in Central and East Africa.

Lieut.-Col. Newham, of the Royal Army Medical Corps, Adviser on Tropical Medicine, who served in the British Expeditionary Force in East Africa during the war, published his report in 1919 (No. 4 of the *Journal of the Royal Army Medical Corps*). The work done by the Germans before the war showed that Tanganyika was infected with sleeping-sickness. The basins of the rivers Malagarazi and Rusisi were infested with tsetse. In Urundi, the percentage of flies containing trypanosomes was from 60 to 90, and of 200 natives examined at Kirando (80 miles north of Bismarckburg) 15 had trypanosomiasis. In 1910 and 1911, Wolf demonstrated the existence of sleeping-sickness, noting an abnormal mortality among the natives living along the river Rovuma near its junction with the Sassawara ; the disease was of the Rhodesian type and was propagated by *Glossina morsitans*.

Professor Beck asserted that 8 per cent of the glossinæ in the Rovuma area were infected with trypanosomes.

Wolf further stated that the German authorities working in the Rovuma area were convinced that the main source of sleeping-sickness infection was to be found in the villages in the valley of the River Lugenda, which runs through the Portuguese Nyasa territory and falls into the Rovuma near Negomano.

Newham notes that the German doctors were confident that the Portuguese bank of the Rovuma was also infected. He was, however, unable to find any reports by Portuguese doctors on investigations into the distribution of human trypanosomiasis in these areas.

In the maps published by the League of Nations Committee of Experts in its preliminary report published in 1924 and in its later report of April 1925, our Nyasa territory, from the neighbourhood of Porto Amélia through Lucinge and the Lugenda Valley as far as south of the River Rovuma, to the east and west of Negomano, is shown as infected with sleeping-sickness.

Newham, who accompanied the British military column which entered our territory at Porto Amélia and continued in the direction of Lake Nyasa through Anquabe, Mêdo, Balama and Lucinge as far as Negomano through the Lugenda Valley, describes cases of sleeping-sickness in members of the white race who had undoubtedly contracted it between Bandari and Anquabe — quite close, therefore, to Porto Amélia — and another case in the Lugenda Valley. According to McGregor, glossinæ of the *morsitans* and *pallidipes* species were to be met with from Mahiba onwards in the direction of Anquabe.

I endeavoured to ascertain whether, during our military operations in Portuguese Nyasa, our doctors had made any mention in their reports of cases of sleeping-sickness contracted in the area. I was unable to obtain any information from the Chief of

the Health Department or the Chief of the General Staff of the Mozambique Colony. These reports, if any such were made, are not to be found in the archives of the Miguel Bombarda Hospital or in the military offices at Lourenço Marques.

My colleague Archer e Silva stated that in 1913, when he was at Mtengula, on our shore of Lake Nyasa, he observed two cases of sleeping-sickness in natives. Both were isolated in the small hospital that he established at Mtengula. One escaped, and the other died in hospital a few months later ; blood preparations were made from the latter and confirmed the diagnosis.

With reference to these cases reported by Archer e Silva, Professor Anibal de Magalhaes obtained the following information : the lake in the Serra do Manda, known as Dengere, is roughly opposite Cobué (a post of the Lake Nyasa district on the Portuguese side of that lake). There had formerly been a settlement there, but the majority of its inhabitants died (according to the native report) through witchcraft, and others emigrated. The two natives observed by Archer came from this settlement.

I submitted my report to the Minister for the Colonies on June 30th, 1925, urging that an energetic campaign against sleeping-sickness should be carried on in our African territories, especially along our frontiers with other colonial Powers. With regard to technical work, I pressed once more for our medical mission to be sent out and to co-operate, as Balfour had suggested, in the work of the Entebbe Commission, with a view, so far as human trypanosomiasis was concerned, to helping in the determination of the relationship between *gambiense*, *rhodesiense* and *brucei* viruses. Our mission would also make a study of bilious haemoglobinuric fever.

After the London Conference, the Mozambique Health Department expressed itself in favour of our being represented on the Entebbe Commission, and stated that it still desired that the Portuguese medical mission, proposed long since, should be sent out, and should include professors from our School of Tropical Medicine.

With regard to the institution of a more active campaign against sleeping-sickness, especially in the neighbourhood of our frontiers with other colonial Powers, the Health Department stated that investigations were being continued on the frontier of the Tete district to ascertain the existence of glossinæ.

An order was voted by the Council of Medicine and Hygiene and confirmed by provincial Decree No. 207 of October 31st, 1925, for the establishment of a medical committee to collect all information that might be of use to the future Portuguese mission. This Committee consisted of the Director of the Central Laboratory and a veterinary surgeon selected by the directors of the various services. The Committee drew up another questionnaire to be circulated to all medical inspectors and sub-inspectors and veterinary inspectors ; but owing to unavoidable circumstances, including the departure of the Director of the Laboratory for Entebbe, the Committee was unable to furnish the mission with the valuable assistance that had been expected.

Most of the laboratory material used by the mission was supplied by the School of Tropical Medicine and a large part of it was left in the colony for the use of future missions.

I planned to set up in the Nyasa territory a small base laboratory for the preliminary examination of samples of blood, glandular secretions and cerebro-spinal fluid from patients and suspects met with in the districts through which

members of the mission were to travel. Blood samples from domestic and wild animals and specimens of tsetse and other blood-sucking insects would also be examined. These examinations would probably have to be completed by us later on in the Lourenço Marques laboratory, and possibly in the Lisbon laboratories. This turned out to be the case, for we have a large number of preparations, and if we are to establish beyond question that they are negative, the examination will have to be extremely thorough and repeated by several different persons.

In the decree establishing the mission, the Chief of the Health Department proposed that Luis Fontoura de Sequeira and José Saraiva de Aguilar should be members of the mission, and provision was made for the necessary auxiliary staff.

The approval of the Colonial Financial Council was obtained on June 17th, 1927. My assistants, Professor Anibal de Magalhaes and M. Sequeira and M. Aguilar, promptly became busy in obtaining, preparing and arranging almost everything the Mission required. Thanks to their efforts, we were able to leave Lourenço Marques on June 24th for Beira, with the intention of proceeding to Portuguese Nyasa via Blantyre and Fort Johnston.

On the basis of the information given me as soon as I reached Fort Johnston by the medical officer for the Nyasa Company's western zone, Dr. Alves Sardoeira, and by the agent for the Mandimba district, Jaime Hermogene Chaves — to both of whom the mission is indebted for much valuable and whole-hearted assistance — it was decided to establish the base at Mandimba, where a small laboratory was to be set up in which Anibal de Magalhaes and I should remain to make a preliminary examination of the material collected in various places by Fontoura de Sequeira and Saraiva de Aguilar. A more leisurely and thorough examination, the execution of drawings and the preparation of photographs, and other longer operations which might be necessary, were to be done later at Lourenço Marques and Lisbon.

At that time, I was not in good health and, as I subsequently grew worse, my colleagues thought it necessary that I should go back from Mandimba, and I left on July 15th for Mozambique. I stayed ten days in the hospital there, and reached Lourenço Marques on August 6th.

As my health improved, the Chief of the Health Department decided that I should remain at Lourenço Marques to direct the work. Specimens of all the samples of blood, glandular secretions, etc., stained or unstained, were to be sent to me, together with flies and other blood-sucking insects collected in the bush, so that a comparison might be made of the results of all the examinations carried out by me, by Professor Anibal de Magalhaes and by our other colleagues, in order to make the conclusions drawn as reliable as possible.

On August 15th, 1927, a month after my departure from Mandimba, in collaboration with the veterinary services under the direction of Dr. Joao Botelho and with the assistance of the district veterinary officer, Dr. António Ayres, I commenced a study of the problem of animal trypanosomiases in the south of the colony, and travelled through part of the Lourenço Marques district as far as its southern frontier with Zululand and through a large part of the Inhambane district, finally arriving at Mecoque, which is in the Mozambique Company's territory. This work will be described later.

While I was incapacitated, Professor Anibal de Magalhaes temporarily assumed

the direction of the work on the general lines I had laid down. I have nothing but praise for the admirable manner in which he acquitted himself of these duties. The Chief of the Health Department also returned to Lourenço Marques on November 14th, 1927, and we continued together to study the material which was sent us from the bush. Fontoura de Sequeira arrived on December 26th and Saraiva de Aguilar, having accompanied the mission's equipment through Mozambique, arrived at the end of January 1928.

The last batch of samples brought by these two were studied by me with the assistance of Fontoura de Sequeira. We both examined all the glossina specimens captured, and then made our diagnoses according to Newstead's method from dissections carried out by Sequeira under my instructions.

Itinerary of the Mission.

Fontoura de Sequeira and Saraiva de Aguilar had travelled through various parts of the western zone according to an itinerary based on the information given by the Nyasa Company regarding sleeping-sickness cases occurring in 1926 among Europeans, on the statements made at the second London Conference, and on the publications of English and German doctors. They left Mandimba on July 15th in the direction of Amaramba, having crossed the river Lugenda near Lake Chiuro ; a good number of mosquitoes, including many anophelines, and of *Glossina morsitans* were observed. On the 18th they returned to Mandimba, and on the 23rd they started on their long tour, which was successfully carried through to the end.

They went in a north-north-westerly direction, passing through Medinde, Mitambe and Maparilo, and reaching Mtonia on the 26th. This is an elevated region (1,000 metres), relatively cool, healthy, fertile and fairly well populated. Flies were seen from Mandimba as far as Medinde, but not afterwards. None were found at Mtonia, and we were informed that there were none there even during the rainy season. My assistants are of opinion that this would be a good neighbourhood for European settlement.

From Mtonia they turned eastwards as far as the right bank of the river Lunguene, a tributary of the Luambala, which they crossed, and arrived at the post of Catur on the 30th. Flies began to appear near the rivers, and were abundant at Catur, where they attacked persons even indoors.

On August 2nd they started through Namessinge for the post of Luambala, which is in the district of Metarica, where they knew that there had been cases of human trypanosomiasis. They crossed the river Luculese, and afterwards the Luambala itself. They inspected numerous tribes in the neighbourhood, which were under the control of that post, and came to the conclusion that it was not a satisfactory practice to tell the chiefs to assemble all their subjects at some particular place for examination. They accordingly had the healthy ones sent for, and let the sick stay in their huts ; if the natives had been told to bring the sick, they would not have done so, but would have said that they had none. Consequently, my colleagues decided to pay surprise visits to the villages and to ask the chiefs to tell off a native (headman) to show them the different huts ; the inmates could then be examined and their replies translated by an interpreter who was with the party.

This is in accordance with Professor Kleine's experience : in his report he states that it is no use confining one's attention to the cases who come of their own accord ; a thorough search must be made in the villages. The first step in the campaign against the epidemic must be this strict search for cases.

Throughout this zone, flies were found but no cases of sleeping-sickness were discovered.

On returning to the post of Luambala, they met M. Medeiros de Moura, agent for the Metarica district, and learned from him that this district, which covers a wide area, is very sparsely populated and lacks supplies of food, that it is difficult to obtain supplies for porters and other natives, and that in some of the posts the chief authorities are native headmen, most of whom do not speak Portuguese well. For these reasons they decided that, in order to carry out the tour and make such observations and collect such material as were necessary, they would have to separate, each travelling to selected posts, and meet from time to time and make any necessary urgent examinations of the samples obtained.

The route followed by Fontoura de Sequeira was as follows :

On August 24th, he left Luambala for Metarica, near the river Lugenda, crossing the Luangua and subsequently two small streams. He found a fair number of flies, which at Metarica came even inside the huts. He obtained information which showed that a European had contracted sleeping-sickness at the capital of the district, and that near the post of Lucinge natives had died with symptoms suggesting trypanosomiasis. He visited a number of villages, including Chirumba, which is on an island in the river Lugenda.

He then started eastward in the direction of Lucinge on August 30th, crossing the river Luleco and visiting a number of villages. At Metarica-Munde, he met with the first case, Saize, observation case No. 80, whose symptoms were rather suspicious. On examining the blood, he found trypanosomes ; he inoculated two guinea-pigs and then treated the native with Bayer 205.

He then continued his journey, stopping at various villages. Flies continued plentiful throughout, and were abundant at the post of Lucinge near the river of that name, which is one of the main tributaries of the Lugenda.

On September 11th, he left Lucinge for Cumela, where he found his second case of sleeping-sickness, Nacope, observation case No. 123. The blood examination was positive. He was informed that Nacope's sister had died of the same disease (they call it ligono) some two years earlier. This patient was also treated with Bayer 205. The people of the region in which he found these cases are accustomed to travel to Nyasaland, and occasionally beyond the Rovuma and to Porto Amélia.

Sequeira wrote to Mandimba that he had found these two cases of human trypanosomiasis and asked for instructions. Anibal de Magalhaes wrote to me on September 26th, saying that he thought it dangerous to transfer these cases from their own tribal areas to Mandimba, because flies were common throughout the region and isolation would be difficult. The Chief of the Health Department thereupon telegraphed to our mission at Mandimba that the cases found should be collected in a concentration camp set up at a place to be chosen by the Nyasa Company's medical officer, so that the latter could keep them under treatment and observation. The Governor of the Company was also telegraphically notified.

Sequeira continued north-eastward, collecting information in a number of tribal areas, and then came to the river Gondosse, which he followed as far as Chiloega ; thence he turned westward in the direction of the river Lugenda, which he crossed near Nantusia. He then followed this river, always bearing westward, investigating a number of villages, and on October 2nd he reached Namungune, on the left bank of the Luleco tributary.

The chief of Namungune said that nearby there was a case of ligono (sleeping-sickness), a young man who lived in a hut near Lugenda.

This was correct, and on October 4th Sequeira saw his third case, Cêpo, observation case No. 169 ; the diagnoses was confirmed by microscopic examination of the blood. He inoculated a guinea-pig and treated the patient with Bayer 205.

Throughout the journey there were plenty of flies.

He returned to the banks of the Lugenda and went on to the post of Mecula, situated on the slope of a ridge, where he saw no tsetse, though he was told that on very still days they came as far as the post.

This seemed to him a suitable place for a concentration hospital for the treatment of sleeping-sickness cases.

He left Mecula for Buena-Usse, near the river Metapir, to fetch supplies, arriving on October 11th and meeting Aguilar. He examined blood preparations obtained by the latter and sent a good many to Mandimba.

On leaving Buena-Usse, he went to the river Lugenda by way of Nocote, continuing as far as Chilanga and then northward through Cumumba to Mecula again. Thence he started north-eastward on October 23rd for Macanga, Nantuego and Luonda meeting with flies throughout the journey — not only *morsitans* but also *pallidipes*. He passed through Luambala to the river Lugenda, which he followed as far as Negomano, at the confluence of that river with the river Rovuma, where he arrived on October 30th. He saw a fair number of flies on the way.

He returned to Mecula, where he again met Aguilar on November 7th.

Following an agreement made between them, he continued westward in the direction of the river Chiulaze, which he reached at Candaia on the 10th ; on the way he collected a number of flies to study. He then travelled through villages on the banks of the Chiulaze, and at Macaloje his attention was drawn to one of the chief's sons, who, in addition to the usual appearance of sleeping-sickness patients, had clearly visible cervical adenitis and fever. Microscopic examination of the blood revealed trypanosomes (Matete, observation case No. 256).

He went on towards Lussanhando on November 15th, first along the Chiulaze as far as Xixoca, and then directly westward.

From Lussanhando he turned south-west, passing through Mataca, where the tsetse were already becoming much rarer. He then began to climb into a high mountainous area, free from glossinæ and with a good climate. This in his opinion, would be an excellent place for a concentration camp for sleeping-sickness cases, especially as it is not very far from the infected area.

He continued his journey in this rough country, which covers a considerable area, and which, he said, would be an excellent centre for Portuguese colonisation. He thought it would be a good thing to build a railway to it from the coast (Porto Amélia).

He continued as far as the river Luchulingo, which he crossed near Urango, there being nearby a delegation from the English mission at Likoma. He learned that this mission was then without its doctor, who would not arrive until the first fortnight of December. He accordingly decided not to go to Metengula, but to return to Mandimba through Metarica, concluding his tour on November 29th, 1927. From Medinde onwards he again met with flies.

He spent four months on this long tour, and in that short time he carried out strenuous and fatiguing work of great value and importance.

The rainy season was beginning, when work of this nature becomes impossible in those parts, and he accordingly returned via Blantyre to Lourenço Marques, where he arrived on November 26th, 1927.

The separate journey made by Saraiva de Aguilar was as follows :

He returned to Mandimba on August 24th, 1927.

Early in September he left for Luambala, passing through a number of villages, the chief of which were Nhumua, Mucuaila, Conge, and Calambo, and crossed the Luchinua, a tributary of the Lugenda. There were many flies near this river and beyond. He then set out for Metarica. Near Tambala, he met ten natives coming from beyond the Rovuma, where they had been for a year.

He then left for the post of Lussanhando, passing first through the post of Lucumba, crossing the river Luatize, and following fairly closely the river Massanisse, leaving it later and reaching the end of this stage in three days. Flies were frequent as far as the Massanisse, afterwards became rarer, and then again increasing in numbers in the neighbourhood of Lussanhando.

He inspected various villages south-west of Lussanhando, between the river of that name and the Xissangesse, on the borders of the chief Mataca's territory. On September 17th, he started north-eastward, closely following the river Lussanhando through Caputue and Marrabo as far as Achala, at the confluence of the Lussanhando and the Rovuma. At Marrabo, he examined a suspected case and took blood samples, which were later examined by Sequeira. Trypanosomes were found (Bitamarrabo, observation case No. 198).

He returned to Abêdo and then started for Intelele, on the Portuguese bank of the Rovuma, an important crossing-point for traffic into the former German territory. He met twenty-two natives coming from Catur with Pass No. 288, dated August 22nd, 1927, and intending to cross into the territory beyond the Rovuma at this spot.

He then set out for the confluence of the river Lucholingo with the Rovuma, reached Matola and returned to Caputue, on the Lussanhando river again, finally arriving at Checomochira. On September 25th, he left this village for Buena-Usse (the present headquarters of the post of Mecula), passing through a series of villages under that post.

On October 1st, he started another tour through Matola, Capunga and Macaloje, where he found a case of human trypanosomiasis which had already been discovered on Sequeira's tour.

He visited a number of villages near Macaloje, and then went on through Metapir to Metarica. Near this post, he inspected several localities to the south-west, along the river Lugenda, where he found many flies. He returned from Metarica

to Buena-Usse, passing through Assir, crossing the river Metapir and thence to Muchalisse, Saide, Lipede, Luntupa and Chesuma.

Here he met Sequeira on September 11th. They exchanged views and examined some of each other's preparations.

On the 14th he left Buena-Usse, proceeding north-west in the direction of Ganguevene and then turning east for Nematologo and Cangana ; a case of trypanosomiasis was discovered in each of these two places — Cicomoja, observation case No. 252, and Climacavene, observation case No. 238.

He went on through Chiroma as far as Messane, where he struck the river Chiulaze, which he followed in a northerly direction, inspecting various villages on the way. At Tonene, he was told that someone had recently died from sleeping-sickness, and, after much questioning, the same informant finally told him that there was another sufferer living in one of the huts along the left bank of the river ; he went there and found the patient Cornebase, whom he thought to be clinically suspect, but this diagnosis has not yet been confirmed.

While following the river Chiulaze, he found another native at Mavele who appeared to be clinically suspect, although his symptoms were less pronounced than those of the patient mentioned above. Neither has this diagnosis yet been confirmed by microscopic examination. He came across a fair number of glossinæ on this tour. He continued northwards and struck the Rovuma at Chirange, where it joins the Chiulaze.

He followed the course of the Rovuma westward as far as Socolilo, which is on the river Lumelilo. He noticed that the point at which the natives cross into the former German territory is at Sibokue. He continued to follow the river Lumelilo as far as Mucuinda, leaving it at this point and turning eastwards ; he returned to Candaia and then to Mecula. On this tour, he met natives coming from Porto Amélia and others coming from beyond the river Rovuma, where they had been living for nearly two years.

On October 29th he left Mecula for Maombe, proceeding in a north-easterly direction as far as the village of Mahango filho and returning through Macanga and Mepape to Alari-Lumbe, where he diagnosed a further case of sleeping-sickness, which was confirmed by microscopic examination — Alimo, observation case No. 244. He then made a tour through Omar, Mepate and Mecoco, afterwards returning to Mecula, where he again met Sequeira.

On November 8th, he passed through Buena-Usse on the way to the post of Lussanhando, which he left on the 17th, turning north-west ; he crossed the tributary stream Xissangesse at Chituto, followed the course of that river as far as Suber, struck the river Lucholingo, which he crossed at Omar, making for Macaloge ; he then crossed the river Livolece and turned west at Jamanda for Timo-Timo. A road runs direct to this village from Metengula, which is near our shore of Lake Nyasa ; this road then runs straight on to Chicolo, which is on the left bank of the Rovuma in the former German territory.

Aguilar might have proceeded direct to Mitomone by taking the cutting through the bush from Timo-Timo, but he purposely went the other way in order to investigate the Chicolo neighbourhood and reached the Portuguese bank of the Rovuma at the place opposite the village of Chicolo mentioned above.

He then continued to follow the Rovuma as far as Mitomone, where it joins the river Messinge, and then proceeded westward, following our boundary line and passing through Chibuto, Capungo (which is in the mountains), Cacecatula and Chevinde (which is on our shore of Lake Nyasa). There are no glossinae in the mountainous district of Capungo. At Cacecatula, near the lake, he found his last trypanosomiasis patient, a man named Mecombe ; the diagnosis was confirmed by microscopic examination.

He continued along the lakeside as far as Metengula, and then passed through Metonia to Mandimba, which he reached on December 6th, 1927. No tsetse were found on the shores of the lake or until he reached Metonia.

His tour was as extensive and as difficult as that of Sequeira, and his investigations were carried out with equal thoroughness. The facts which he ascertained and his clinical diagnoses, which were confirmed by us in blood and other preparations which we kept and took with us to the Lisbon School of Tropical Medicine, have been of great value in framing the proposals for further investigations based on the work of our mission.

In short, we see that a large part of the Nyasa's Company's western area was covered, from the Gondosse, a tributary of the Lugenda, along this river from its source in Lake Chiura to its junction with the river Rovuma at Negomano, through various districts on our bank of the Rovuma, which for a large part of its course separates our territory of Nyasa from the former German colony, and also following the boundary line from the Rovuma to the lake. Samples were collected from 267 natives, who were examined in the bush by Sequeira, and a large number of samples were also examined in Mandimba and Lourenço Marques by Anibal de Magalhaes ; all these were subsequently checked by me. As a result of this examination, the diagnosis of sleeping-sickness was confirmed in nine cases, and there were two other suspected cases, but no trypanosomes were found in their blood. The nine cases were those of Saize, Nacope, Cêpo, Matete, Bitamarrabo, Sicomoja, Climacavene, Alimo and Mecoma, of the Metarica-Munde, Cumela, Namungune, Lucumbo, Palango, Panguissa, Palango, Alari-Lumbe and Capungo tribes from the posts of Lucinge-Metende, Lucinge, Mecula, Metarica, Lussanhando, Metapir, Mecula and Mitomone.

The previous findings of German and English medical men in this western district were thus confirmed.

On examining the strains preserved by means of successive passages through animals (guinea-pigs, rabbits, and dogs) which I was able to take to Lisbon alive, and which were also passed through rabbits and guinea-pigs on my return journey, we found that human infection was due to the *rhodesiense* virus.

Sequeira and Aguilar shot a number of large and small wild mammals and birds in the bush — 92 in all. Some birds and rare mammals were found to be infected with trypanosomes ; none had flagellates of the *brucei* type, but only of the *congolense* type. In the preparations we also found piroplasma, halteridia, leucocytozoa, plasmodia and ambryo filariæ. In human blood we diagnosed, in addition to trypanosomes, spirochaetæ, malaria parasites, mainly of the *Laverania malariæ* type, but also a few *Plasmodia vivax* and, more rarely, *Plasmodia malariæ* ; we also found embryo filariæ.

A large number (nearly 2,700) of glossinæ were collected. Professor Anibal de Magalhaes had tsetse collected in and around Mandimba, on the road leading to Maracotera in the neighbourhood of this district, and in and around Catur, the total number obtained being 997, which he identified as *morsitans* (Austen's criteria) ; this classification was subsequently confirmed by Sequeira and myself by the same method and in part by Newstead's method. He also examined 535 specimens sent by his colleagues from the bush, and which he afterwards forwarded to me, and when Sequeira and Aguilar returned to Lourenço Marques they brought with them a further 1,160 flies from various localities, which I examined in conjunction with Sequeira.

The great majority of these glossinæ from the bush were *morsitans* ; some found in the districts north of Mecula (Nantuego, Auambala, Negomano) were *pallidipes*, and a few belonged to the *submorsitans* and *paradoxa* varieties. Tsetse were found in large numbers near the rivers, becoming scarcer at the foot of the mountains and disappearing altogether in places at a high altitude.

Professor Anibal de Magalhaes made smears from the salivary glands and other parts of the digestive organs of 300 glossinæ without finding trypanosomes, and similar dissections were carried out by Sequeira in the bush, likewise with a negative result. These investigations will have to be carried out with recently collected flies if we are to obtain the desired success ; naturally, the conditions under which we were working made it impossible for us to experiment by causing susceptible animals, particularly macaques (ceropithecus), to be bitten by the tsetse captured in the various parts of the bush covered. This would have been the best form of ascertaining whether they were infected with *T. rhodesiense* or *T. brucei*.

Magalhaes examined the blood of thirty-three animals from Mandimba and the neighbourhood ; the majority were domestic animals, the few wild ones being macaques, bush-hens and a gazelle captured by Sequeira in Amaramba and Mandimba. Practically all the mammals in this group, with the exception of one dog and one sheep, were free from trypanosomes.

The dog was examined on August 16th, 1927, and trypanosomes were found to be present. The morphology of the parasites in this animal's blood showed them to be of the *brucei* type (polymorphism and nuclear ectopy) and this diagnosis was fully confirmed after inoculation into guinea-pigs, whose blood was examined by us at Lourenço Marques. Their virulence was very great, especially in the case of the dogs experimentally inoculated at the Central Laboratory, these animals dying within an average period of eight days.

The dog was naturally infected by being bitten by glossinæ in the bush near Mandimba during the tour made nine days previously along bypaths in the direction shown in the map on page 2, from Cheabudo, Champwina, Congerenge, O'Mesito, Chesota, Macangaga, the Lusangasi, Melinde, Lubimbino, Mesinge, Rwaka and N'goma streams being crossed. Magalhaes was unable to collect blood from the natives living in the villages in this district for microscopic examination, as the supply of microscope slides for which he had telegraphed failed to reach him.

It was unfortunate that the co-existence of sleeping-sickness *(rhodesiense)* and nagana in this small area could not be accurately determined.

Trypanosomes found in the sheep were inoculated into guinea-pigs, but did not

produce infection. The blood preparations of this animal, which I also had occasion to examine, revealed long forms with free flagella and short forms with flagella not free, with displacement of the nucleus, principally towards the anterior extremity. As there are only a few smears of the sheep's blood, no reliable diagnosis could be made ; it is probable that the trypanosomes are also of the *brucei* type, but it should be noted that inoculations gave a negative result, which is strange. The sheep was treated with Bayer 205, but suffered a relapse and, as it was not easy to isolate it from the tsetse in the locality, it was decided to have it killed as a precautionary measure.

Before leaving for Nyasa, I had had an opportunity of exchanging views with Dr. Joao Botelho, the Director of the Veterinary Department of the Colony of Mozambique, and examined some of the stained blood preparations on slides in his collection which had been made by the veterinary staff of this province ; as regards the veterinary service of Inhambane, they were made by Albino Fernandes and Cunha Fajardo ; in the case of Vila de Joao Belo (Chai-Chai), by Silva Pinto and, of Quelimane, by Jacinto Martinho ; preparations were also collected in the northern district of Mozambique by Fernao Napoles, a high percentage of wild animals (45 out of 84, *i.e.*, 53 per cent) being infected with trypanosomes.

Dr. Botelho and my colleague Ferreira dos Santos, Chief of the Health Department told me that cases of animal trypanosomiases have for some time been noticed in the southern parts of the colony, in the district of Lourenço Marques and Inhambane, where no tsetse have been discovered, except for a few rare specimens of *brevipalpis* in the extreme south, on the Zululand border.

On my return from Mandimba, I was shown every consideration by Dr. Joao Botelho. He expressed a desire that I should collaborate with the Veterinary Department in the further study of this important problem, to which our compatriots and foreign investigators, particularly Walter Jowett and Dr. Theiler, have already devoted their attention.

As already stated, I consider this collaboration of the Medical and Veterinary Departments to be of the highest value. Suggestions to this effect were included in the proposal made by the Lisbon School of Tropical Medicine with reference to the establishment of our mission and were accepted by the Minister, Commander Joao Belo, in the decree drawn up by him. This co-operation might well take the form of an investigation into an important problem — namely, the possibility of trypanosomes, which are usually transmitted by tsetses being regularly carried to regions in which these flies have not been discovered. This leads to the asumption that the flagellates in question have become adapted to live in other flies or blood-sucking agents, in which they pass through the cyclical stage, and that the arthropoda in question do not merely act as vehicles of mechanical and accidental dissemination. This hypothesis is of great importance in view of its bearing on human pathology.

During the tour of inspection, which I began an August 15th, 1927, and until I left Lourenço Marques on May 15th, 1928, I was accompanied by Dr. Antonio Ayres, the veterinary officer, who was of great assistance to me.

I first studied trypanosomiasis in domestic animals in the districts near Lourenço Marques — Umbeluzi, the Goldsbury, the Empresa Agricola (run by Dr. Saldanha) farms and the Government's experimental farm. I then proceeded to the south of

this district, leaving it on October 22nd and passing through the capital of the Maputo area at Bela Vista, where every assistance and facility was given me by M. Serra Cardoso, the Administrator. A few hours later, we left for the Catuane post, which is situated in the extreme south of the district, on the Zululand border, near the river Maputo. M. Alves Martins, the chief of this post, did his utmost to make our stay profitable. This district is extremely deficient in natural resources ; the only water obtainable for drinking is rain water ; good communications are lacking ; there are a number of large lakes and numerous mosquitoes, while the land is very marshy. The result was that shortly after our return some of our companions, including M. Costa Fialho, the Customs Collector, suffered from intermittent fever.

Both at Goldsbury, at Dr. Saldanha's farm and in the Catuane tribal areas, where we inspected the cattle, we obtained blood from adult and adolescent bovines, asses and dogs, making thick and thin film preparations. In some cases, a vein was punctured, the large quantity of blood thus obtained being repeatedly centrifugalised by Martin's process and the sediment examined for flagellates ; the small laboratory animals at our disposal — rabbits, guinea pigs and dogs — were then inoculated.

As regards the staining of thick preparations, hæmolysis was obtained by Giemsa solution diluted to half strength (one drop to every cubic centimetre of distilled water), the preparations then being stained by the classic Lieshman process ; all the other preparations were stained direct by this method. Excellent results were obtained as soon as we used distilled water carefully rectified with reference to its acidity.

In Catuane, in the forests of the Matope and on the open ground along the right bank of the Maputo, known as the Chucha, a number of wild animals — *Changos (Servicapra arundimum)*, *pallahs (Aepyceros melampus)*, bush kids and one cercopithecus — were shot by our companions.

I was unable to find trypanosomes in the blood of these animals ; I found plasmodia in the preparations obtained from the macaque and gonderia mutans in those of one of the changos *(Servicapra arundimum)*.

The number of cattle inspected in these districts was 270 making, if we take the thick- and thin-film and thin-film preparations alone, a total of nearly 1,000 slides to examine, apart from specimens obtained from centrifugalisation and from the blood of inoculated laboratory animals.

We attempted to leave Catuane on October 30th, but owing to stormy weather we were obliged to turn back. We started out again on the 31st. The journey was very difficult and it took us 18 hours to cover a distance which, under normal conditions, can be covered in one-third of the time by motor.

On April 12th, I left Lourenço Marques to study cattle trypanosomiases in the Inhambane district, to collect flies and other blood-sucking insects, to obtain blood from wild animals and to inoculate laboratory animals (rabbits and guinea-pigs). I was given every facility by the Government agent in charge, a Buick motor-car and a small cart being placed at my disposal.

We continued our journey through Marracuene, Manhiça, Chinavane, crossed the river Incomati, passed through Bilane, the Gaza territory, went on past the river Limpopo and reached Vila de Joao Belo (Chai-Chai), where we stopped and were

received by M. Manoel Sepulveda, the Administrator, who showed us every consideration.

We continued our journey on the following day, passing through the Changuene, Machacaze and Zavala territories. We then passed the Lake of Inharrime and arrived at the village of the same name, where we found our colleagues Eliseo Gandara e Calixto and the veterinary officer Pedro Fajardo, acting head of the zootechnic post of Inhamussua ; we reached the town of Inhambane shortly afterwards.

While in the Inhambane district, we visited Maxixe, Homoine, Inhammussua, Maimela, Mocoduene, Massinga and, lastly, Vilanculos in the extreme north. We collected samples from the cattle belonging to various farms in these places, including those of M. Cunha Vaz, M. Alberto Rocha (Jogô and Savanguana tribal areas), of M. Mendes Silva (Inguane tribal area), of the Swiss proprietor Albert Hedgar (Panga area), of M. Alberto Abrantes in the Vilanculos area, and that belonging to the native Flan, at Mangarilane.

On April 26th, I proceeded to the northern boundary of this district, together with my colleagues Gandara, Cunha Cardoso, Luciano Pinto and the veterinary officers Fajardo and Antonio Ayres. We passed parallel 22 and entered the Mozambique Company's territory, stopping in the first village, Mucóque. We were received by M. Anibal de Rezende, the Administrator, who told me (and this information was confirmed by my colleague Cunha Cardoso) that there was a belt of glossinae 150 kilometres to the north, in the Govuro area, this belt being crossed by the road under construction from Vilanculos to the Beira. This tsetse belt runs through a forest in which game — antelopes, buffaloes etc. — are to be found, and the forest continues southwards, where it joins other forests in which the same wild animals are to be found. Cattle are not kept in the tsetse fly belt in question ; M. Anibal de Rezende told me that of the yoke oxen which he had been obliged to send to that district one had died there and the others died shortly after their return ; the disease did not spread, however, in Mucóque, where there are no glossinæ.

I must express my gratitude to my colleague Cunha Cardoso who, after my return and at my request, visited this belt where he caught flies which he believed to be glossinæ and sent them to me in Lisbon. They are, in fact, glossinæ of the *morsitans* group.

Cunha Cardoso is inclined to think that, owing to the opening of the road, this tsetse belt has moved southward towards the district of Inhambane. He did not discover anything bearing on the existence of human trypanosomiasis which is suspected in the Govuro zone.

I am grateful to all the administrators and other persons in the Inhambane district for the kindness and consideration shown me, and in particular to my colleague Luciano Pinto at Morrumbene, who gave us every assistance and accompanied us on many of our visits of inspection.

I returned to Vila, and thence to Lourenço Marques, which I reached on May 1st, 1928.

As the date of my return to Portugal, which took place 15 days later, was drawing near, and I wished to visit the scientific institutes in the Transvaal, I was obliged to leave the examination of almost the whole of the material obtained in Inhambane, part of that relating to animal trypanosomiasis in Lourenço Marques, that relating to bilious hæmoglobinuric fever, the blood preparations from the wild animals and

glossinæ collected by the veterinary officer Fernao Napoles in the northern part of the Mozambique district, one-half of which are to be sent to me at the School of Tropical Medicine, after my return to Lisbon. As regards animal pathology, these researches will be continued simultaneously by the Directorate of the Veterinary Department at Lourenço Marques and by myself at Lisbon, and we shall communicate to each other the results obtained.

In the blood of the cattle which I examined in the places mentioned above, I found trypanosomes whose morphological structure was that of the *T. congolense* and others of the *vivax* type, both pathogenic flagellates. I also diagnosed in preparations of varying origin (Goldsbury, Katuane) *Trypanosoma theileri*, which is innocuous.

Generally speaking, the slides on which the *T. congolense* was found did not contain *T. vivax*.

On examining the samples collected during my work, I frequently came across *T. congolense* in Goldsbury samples and a greater number of *T. vivax* in samples from Dr. Saldanha's farm ; the former were very rare in cattle from Katuane and the neighbouring tribal areas, where animal trypanosomiasis was formerly very prevalent.

Nevertheless, in one of the blood preparations from Goldsbury cattle the infection was very intense ; side by side with a large number of typical forms of *T. congolense*, which were fairly short with the flagella not free, there were other decidedly longer forms with free flagella and which were of another strain. When the laboratory animals — rabbits, guinea-pigs and dogs — were inoculated, positive results were only obtained in the case of a few rabbits in which flagellates of *T. congolense* type alone appeared.

In all the districts through which we travelled and in which it was stated that there were no tsetse, we found this to be correct ; we obtained a good number of specimens of other blood-sucking flies — stomoxys, hippobosca, hæmatopota and other tabanidæ — as well as other arthropoda, principally ticks. In many villages in the Inhambane district, it is thought that a blood-sucking fly, which I found to be a hæmatopota, is responsible for propagating animal trypanosomiasis. We found it in various places and in large numbers near the farm of the Swiss, Hedgar, at Panga. Finally, we endeavoured to put into practice at Goldsbury the birdlime process introduced by Bulhoes Maldonado ; when the cattle went to graze in districts suspected, owing to their appearance and vegetation, of harbouring tsetse, or when they went to drink at the river Umbeluzi, we covered them with black cloths having a layer of birdlime on their outer surface ; the bird-lime obtained was, however, of bad quality and did not give the desired result. The Directorate of the Veterinary Department will have this experiment repeated with better quality bird-lime.

It is curious that in the exact vicinity in the north of Inhambane where we were informed that tsetse existed, we should find them without difficulty, and I was able, as already stated, to identify as *G. morsilans* the specimens sent to me at Lisbon by Cunha Cardoso, the medical officer at Vilanculos.

With regard to the phenomena observed by us, two hypotheses might be formulated on which to base our future work. One, which might have important effects on human pathology, is that *T. congolense* and *T. vivax* (in view of the persistence of the disease in animals in districts in which no tsetse have been found)

have become adapted to living in other blood-sucking flies, in which they pass through their cycle of evolution, and that those flies are thus constant carriers of the infection.

The other hypothesis is that the trypanosome in the districts of Lourenço Marques and Inhambane is a different flagellate, and that in blood preparations it presents various forms, some resembling the *T. congolense* and others the *T. vivax*.

All this points the way to a programme of research to be carried out as a result of the work done by this medical mission and other previous missions.

I was instructed to draw up this programme by telegram (February 24th, 1928, from the Minister to the Governor-General), which stated that I was to arrange for the continuance of the work.

In my view, the programme should be as follows :

1. The eastern zone of the Nyasa Company's territory should be inspected as carefully as was done in the case of the western zone — from the post of Lucinge to the coast and from the Rovuma to the Lurio. Efforts should be made to ascertain the endemic foci of human trypanosomiasis and to find out whether those already reported by foreign authorities, including the Health Organisation of the League of Nations, still exist.

For this purpose, blood films should be obtained from patients and suspects, both thin-film preparations made by the Jancso-Rosenberger process and also thick-film preparations being collected. Thin preparations should be stained by some modification of the Romanowsky process (I found the Leishman method most successful).

Thick preparations should be hæmolysed by the use of Giemsa solution diluted in the proportion of one drop to two cubic centimetres of distilled water, accurately rectified with reference to its acidity ; they should be carefully washed in a container, but should not be placed under a jet of water ; they should then be stained by the Leishman process.

The slides should never be blotted but should be allowed to dry themselves. This was the method I found to give the best results.

A similar process should be followed with the juice obtained from gland puncture.

If possible, trypanosomes would be sought in the blood obtained by veni-puncture and subjected to repeated centrifugalisation by Martin's process.

In advanced cases, it is advisable to analyse the cerebro-spinal fluid obtained by lumbar puncture.

The slides should be very carefully examined, the whole area of the preparations being covered, because it often happens that trypanosomes are very rare in the blood of infected persons and sometimes in the glandular secretions also.

The whole difficulty lies in stating definitely that any given suspected case is negative.

An examination of this kind will also reveal pathogenic blood parasites, such as malaria parasites, spirochaetæ, embryo filariæ, etc.

2. A study of the geographical distribution of the various strains of glossinæ in this territory should be undertaken at the same time and they should be classified by Austen's and Newstead's methods.

3. Recently collected specimens of these insects should be dissected to ascertain

whether they are infected with trypanosomes and in what parts of the digestive organs these flagellates occur.

4. If possible, these investigations should be supplemented by the inoculation, of animals, preferably macaques (cercopithecus, and not cynocephalus) and dogs and their blood should be sucked by recently collected glossinæ.

In my opinion, these animals are most suitable for experiments with trypanosomes of the *brucei-rhodesiense* strain, which, from the point of view of the disease in man, it is most important to investigate.

5. Blood samples should be obtained from domestic and wild animals and smear preparations made on slides, (especially from the lungs, liver and spleen), for the purpose of studying animal trypanosomiases and as a minor point noting whether they are infected with other parasites, whether protozoa or metazoa, piroplasma, halteridia, proteosoma, leucocytozoa, filariæ, etc.

For staining these preparations, I advise the above-mentioned processes.

6. Similar investigations should be undertaken in the Tete district, especially in the unexplored regions near the frontiers of Northern and Southern Rhodesia and Nyasaland, the crossing-points of the native emigration movement being noted.

7. Similar investigations should be undertaken in the Mozambique district, principally in the northern area near Nyasa.

8. These investigations should also be carried out in the district of Quelimane, especially where it joins Portuguese Nyasa and Nyasaland.

9. Information should be obtained from the Mozambique Company regarding the investigations already undertaken by its medical officers or other specialists into the existence of human and animal trypanosomiases in its territories and the geographical distribution of glossinæ and other blood-sucking insects.

The medical and veterinary personnel in the Company's service should co-operate with the missions and with the Veterinary Department of this province in studying and combating endemic and enzootic diseases.

10. An adequate medical, veterinary and auxiliary staff should be employed for this purpose, the number provided for in the respective estimates being increased or special contracts concluded so that the campaign against these diseases may be as effective as possible, and the Nyasa and Mozambique Companies should be required to take similar steps to co-operate, as is their duty, in all this work.

11. The personnel undertaking these arduous and dangerous investigations, many of which require special and highly technical knowledge and qualifications, should receive adequate remuneration. As I have already suggested, in the case of doctors and veterinary officers, this should never be lower than that granted to officials serving on boundary commissions.

12. To enable the prophylactic measures suggested by the enquiries undertaken by the missions to be put into practice and pursued, means of communication must be provided, so as to make it easier for the permanent medical and veterinary staff in those regions to inspect their various districts.

With reference to the Nyasa Company, I suggested in my notes, dated October

19th, 1927, January 3rd and February 3rd, 1928, that the construction of a railway linking Porto Amélia with our shore of Lake Nyasa (plans for which have already been drawn up, the line being shown on the map) was essential and that motor roads should be built from the principal stations on this railway northwards as far as the Rovuma, along this river and thence southwards as far as the boundary of the Mozambique district.

I also pointed out the enormous advantages which would be derived from the execution of this plan, which would enable our territory to be exploited more efficiently, the health services to be improved — better protection being thus afforded both to colonists and natives — and a satisfactory international policy to be developed.

13. In the areas in which endemic foci of human trypanosomiasis have been discovered, concentration camps should be established in such places as the medical officers in charge of these investigations and the health inspectors may select. As regards the Nyasa Company's territory, I consider that there should be two, one for the eastern zone and the other for the western zone.

Small laboratories should be set up in these concentration camps and provided at any rate with the essential equipment for examining blood preparations, glandular secretions, etc., which should be sent them by the doctors and veterinary officers investigating the various parts of the territories in which cases of trypanosomiases, are being looked for, proper examination and collection of specimens of glossinæ and other blood-sucking arthropoda and for the constant and adequate examination, especially from the point of view of parasitology, of patients in these camps.

Doctors, veterinary officers and other personnel going into the bush to look for cases of trypanosomiasis should also be supplied with sufficient material for collecting blood, glandular secretions and cerebro-spinal fluid, for making preparations from these products and for their preliminary examination.

In addition, they should have the necessary equipment for collecting tsetse flies and other bloodsucking insects.

It is obvious that they should have at least as much camping equipment, as many ambulances, fire-arms for shooting wild animals, native bearers, etc., as I had for the medical mission in my charge which carried out investigations in the western zone of the Nyasa Company's territory.

I would point out once again that Zeiss microscopes with sliding stage should be used, that the staining materials (Leishman powder, Giemsa solution) should be of the best quality (from the firm Gruber) and that the distilled water should be carefully rectified with reference to its acidity.

14. Both the doctors and veterinary officers undertaking investigations in the bush and the doctors in charge of the concentration camps should report on their observations and the conclusions reached and should send these reports and specimens of the material collected to the Central Laboratory of the Miguel Bombarda Hospital and to the Veterinary Departments Laboratory, where they should be examined.

15. The Director of the Central Laboratory of the Miguel Bombarda Hospital should act as head of any medical missions of members of the colonial staff that may be formed. He should draw up the programme of any supplementary investigations undertaken in continuation of those carried out by the medical mission in my charge

and should propose measures for combating endemic diseases, sleeping-sickness in particular.

16. It should continue to be the duty of the Directorate of the Veterinary Department to propose any measures which it may consider necessary and which the reports sent to that Department by the veterinary officers co-operating in the work of the medical missions may suggest, and also to examine the preparations and specimens sent by them.

17. With reference to the investigations to be continued in the southern part of the province, in districts where animal trypanosomiases exist and where no tsetse flies have been found or where the number of these flies is insufficient to explain the intensity and persistence of these diseases, every possible means should be employed to make certain that in some of these localities no glossinæ, not even a few rare specimens, exist — *i.e.*, to prove beyond a doubt that they are entirely absent.

Flies and other blood-sucking insects in these districts should be captured and classified. Recently collected specimens should be dissected for the purpose of discovering which of them contain trypanosomes or forms evolved from trypanosomes in their digestive organs and which in those regions propagate the disease in cattle. These arthropoda should be collected alive, and after two days at least, to avoid any risk of their being direct transmitters ; they should be made to suck the blood of cattle duly isolated and previously examined and definitely found to be free from infection with trypanosomes ; after they have been bitten by these arthropoda, the cattle should be kept under observation, to see whether any of them develop pathogenic trypanosomiases, identical in character to those observed in the regions in which the investigations are being carried out. If so, this would prove that the trypanosomes have become adapted to living in these arthropoda and pass through a cycle of evolution in them.

It is possible that, if care and patience were exercised in carrying out this part of the programme, the problem in question, which is of great importance from a scientific point of view, might be solved, and if the cycle of evolution to which I have referred could be proved, this would be of great practical value.

In the telegram mentioned above, the Minister instructed me to initiate these further investigations before my departure.

I consider that it is absolutely essential that they should be begun as soon as possible. I therefore propose that, in accordance with my programme, and with the indications of the Directors of the Department of Medicine and Hygiene and of the Veterinary Department, the staff of the new missions should shortly be appointed. I suggest that the head of these missions should be granted the necessary credits to defray the expenses, under the same conditions as in the case of the credit granted me for the purpose of carrying out the work undertaken by the mission of which I had charge.

My colleagues Fontoura de Sequeira and Saraiva de Aguilar have agreed to continue in the Tete district the investigations already carried out by Pereira Lapa, Firmino Sant'Ana and Morais de Sousa, in view of the extension of human and animal trypanosomiases and the advance of the tsetse belt, both in our territory and in the neighbouring territory of Nyasaland. At my suggestion, Francisco

Ferreira dos Santos, the Chief of the Department of Medicine and Hygiene, has invited them to carry out this work, and I have recently received letters from these former members of my mission, informing me that they are leaving immediately for that district and have been authorised to purchase the necessary material.

I was thus able to carry out, in this connection, the decision of the Minister of the Colonies and to leave one of the new missions duly established.

I consider that work in the eastern zone of the Nyasa Company's territory should be carried out under the immediate direction of Maximo Prates, who has already returned to his post as Director of the Central Laboratory of Lourenço Marques. In view of his excellent work for the Entebbe International Commission, this gentleman is eminently qualified to take charge of this new mission — a post of great responsibility.

Proposal for the Enlargement of the Central Laboratory at Lourenço Marques and its Conversion into a Medical Research Institute.

The various problems connected with tropical diseases should be studied with a view to their solution, since, notwithstanding the active research undertaken by all colonial Powers, there are many diseases which are still obscure, either, as in the case of bilious hæmoglobinuric fever, from the point of view of etiology, or as regards therapy, or the manner in which they are conveyed. The institute should, of course, give every assistance to clinics, whether hospital or private, and should co-operate in health matters, criminal investigations, etc., by carrying out any necessary laboratory analyses. Its organisers should never forget, however, that the activities of the personnel should not be confined to carrying out these analyses. It should be possible for them to devote a great deal of their time to the scientific work mentioned above, so that our country may undertake similar investigations to those which are constantly being carried out by other nations. Each of the sections mentioned should have its own director and assistant directors, together with a sufficient number of laboratory assistants and workers to enable all necessary analyses to be undertaken and vaccines and therapeutic sera to be prepared, without prejudice to the principal object — *i.e.*, the work of scientific research — for which the director of each section should be specially responsible. The institute should be controlled by the medical director of one of the sections, who should inform the Chief of the Health Department and the Directorate of the Hospital which branches of research or investigation are being dealt with by each of the sections. In conjunction with the directors of the other sections, the head of the Institute should draw up an annual report on the work. This report should be published in Portuguese and in French or English, for distribution to all the scientific institutes in our country, all the health delegations and sub-delegations in our colonies and by foreign institutes dealing specially with problems connected with colonisation ; in exchange, the institute would receive the publications of these various centres of scientific research, which would enrich its library and help it to decide on the course of its enquiries. Each section should, of course, have its own private library for consultation purposes and should be supplied with the most important national or foreign periodicals dealing with its special

subject. The whole staff, including both the directors and the assistant directors of the various sections, should receive adequate remuneration, since they will not have time to attend to a clinical practice or to carry out other paid work for the authorities or the public. For this purpose recourse should be had to any legal provisions already in existence, or new measures should be introduced and the respective sources of revenue improved — for instance, by increasing the percentage reserved for the personnel on the receipts from analyses, sera, vaccines and other work carried out for the public, and for which payment may legally be received. The laboratory assistants should undertake technical work only ; all accounts, etc., should be dealt with by clerks and typists. The services should be installed in such a way as to enable the institute's full programme to be carried out. As, under existing legislation sera and vaccines other than anti-smallpox vaccine have to be prepared, cattle should be procured and housed under satisfactory conditions. The doctor in charge of the institute should draw up the general programme of work and inform each of the sections of the special branch to which it is to devote its activity. The heads of sections may undertake the study of any scientific problem on their own initiative, after notifying the director of the institute of their intention and obtaining his permission, so as to avoid any interference with the general programme. The higher staff should, for preference, be of Portuguese nationality ; they should be fully qualified and competent to carry out their duties. If, however, it is not possible to find a qualified Portuguese national to take charge of a certain section (such as that dealing with pathological anatomy, entomology, etc.), qualified foreigners may be engaged for a limited period, during which time facilities should be accorded to Portuguese nationals to enable them to qualify in those branches ; they should be given grants so that they can study at national or foreign institutes dealing specially with the subjects in question. In view of the fact that problems of human tropical pathology are frequently connected with identical problems relating to the pathology of animals, whether wild or domestic, living in the same areas, it would be advisable, when these matters are being investigated, for contact to be established with the Directorate of the Veterinary Department, so that the two institutes might collaborate in carrying out a joint programme. Animals may in some cases be regarded as the reservoir of the worst viruses causing human diseases, such as, for instance, the *Tripanosoma brucei* in the case of tripanosomiases ; a joint enquiry carried out with the co-operation of persons competent in each of the two branches of human and veterinary pathology is much more likely to be successful and to lead to definite results. I am also of opinion that a museum of parasitology, medical entomology, pathological anatomy, specimens of harmful animals and exotic plants, some of which are useful in therapy while others are dangerous, should be established in the institute for the guidance of any medical men desirous of exercising their profession in these districts. This museum should have a section for the information of the general public, and public lectures should be given, cinematograph films being shown at the same time. When, as the delegate of our School of Tropical Medicine, I visited Brighton for the purpose of attending a general session of the Royal Institute of Public Health of London, I was present at a lecture of this kind given by Dr. Andrew Balfour on malaria, with a film prepared, if I am not mistaken, by the Rockefeller Institute, showing the whole cycle : the evolution of the

mosquito from the larva, the manner in which it sucks human blood, its infection by malaria parasites, the development of these protozoa in the blood, the method of destroying these blood-sucking insects, protective measures against them, etc. Instruction in this form is of enormous value in applying the prophylactic measures necessary for combating diseases. As good prophylaxis is impossible unless the educated public is thoroughly convinced of the utility of the measures proposed, the institute should be in constant touch with the health delegations of the various districts in the province and should endeavour to obtain their co-operation in solving the problems in question ; I also consider that the co-operation of the medical services of independent companies, such as the Mozambique and Nyasa Companies, should be enlisted. For this purpose, health delegations and sub-delegations and, in general, all doctors carrying on their profession in the various localities, should be supplied with a Zeiss microscope with sliding stage, good slides and cover-glasses for preparing specimens, good staining materials — distilled water duly rectified with reference to its reaction, albums, bottles and other necessary materials for collecting blood-sucking insects and other arthropoda or animals which can be regarded as carriers. The Institute should send missions of enquiry to various parts of the province to study the different problems of tropical pathology, in conjunction with the doctors in those places, to prepare by degrees a reliable map showing the distribution of the diseases and their carriers, and to propose such protective measures as the results of their enquiries may suggest. These missions should, in the first place, be sent to frontier districts to make the necessary arrangements with the authorities on the other side of the frontier for combating endemic diseases ; this is in conformity with the recommendations of the International Congress of Loanda and the last international Conference held in London under the auspices of the League of Nations. The institute should have sufficient funds to enable it to carry out all its objects, and its Directorate should have the necessary autonomy combined with the maximum responsibility.

We would also recall the concluding remarks to the preliminary report of Dr. Duke, Chairman of the Entebbe International Commission, which was set up by the League of Nations on the proposal of the Second International Conference held in London in May 1925. Dr. Duke stated that :

> " The further investigations shown to be necessary as a result of the preliminary enquiries carried out in accordance with our programme greatly exceed the scope of the existing International Commission. Consequently, the establishment of an international commission of enquiry, whose action will not be restricted by local conditions, has become necessary. Much valuable work has been done in various parts of the world with reference to human trypanosomiasis and many problems have already been solved. Nevertheless, the problems still awaiting solution are of the utmost importance for combating sleeping-sickness ; they will never be solved by isolated workers and are only capable of solution through the patient and co-ordinated efforts of technical experts working in the infected regions in Africa. A judicious combination of all the various methods should be applied as far as possible in all the infected areas ; a systematic technique and a free exchange of views are also essential

Only by this means will it be possible gradually to collect reliable data from which definite conclusions can be drawn.

It is only natural, now that the International Commission has completed its work, that the same views should be expressed in its final reports ; all our laboratories in Africa need to be re-organised to enable our medical men in charge to co-operate effectively in future investigations, not only into sleeping-sickness, but into other tropical diseases as well ".

Ayres Kopke.

DIRECTORATE OF MEDICINE AND HYGIENE.

Minute No. 23. — Meeting held on February 2nd, 1928, at the Directorate of Medicine and Hygiene.

After exchanging views with Dr. Ayres Kopke, the Chairman drew up the following draft, which he submitted to the Council.

Draft Decree.

In view of the urgent necessity for enlarging and improving the existing laboratories attached to the Miguel Bombarda Hospital and for providing them with the necessary installations and staff to enable them to carry out all their objects, the whole of the scientific research and investigations undertaken in the laboratories being co-ordinated under one head, who shall also be responsible for any co-operation invited by other colonial Powers ;

In accordance with the proposal of the Chief of the Health Department and with the views of the Council of Medicine and Hygiene ;

And after hearing the opinion of the Council of State ;

I have decreed as follows :

1. The Medical Research Institute shall be created as an extension of the existing Central Laboratory attached to the Miguel Bombarda Hospital.

2. This Institute shall be divided into four sections : one dealing with parasitology, another with bacteriology, a third with pathological anatomy and a fourth with chemistry.

3. Each section shall have a head and the various sections shall have the following assistant heads : bacteriology, 2 ; parasitology, 2 ; pathological anatomy, 2 ; chemistry, 1.

4. The higher staff shall, for preference, consist of duly qualified Portuguese nationals. If, however, it is not possible to find suitable nationals to fill these posts in any section, others may be engaged for a limited number of years, or duly qualified foreigners, but for a short period only, during which time facilities shall be accorded

to Portuguese nationals to enable them to qualify in those branches, and they shall be given grants so that they can study at national or foreign institutes dealing specially with those subjects.

5. One of the medical men in charge of one of the sections shall, on the proposal of the Directorate of Health, be appointed Director of the Institute.

The first Director shall be the present Director of the Central Laboratory and the time served by the present Assistant Head shall count as part of his total period of service.

6. There shall be an adequate number of Laboratory assistants, clerks, typists and attendants for all the services.

7. The principal object of the Institute shall be to carry out scientific research and investigation, the scheme of work being drawn up by the Director.

8. The Institute shall collaborate with the technical experts of other nations in scientific research work or in combating tropical diseases, when the co-operation of our country is invited or when our country invites the co-operation of other nations.

9. The Institute shall carry out all clinical analyses required by hospital or other services and by the public.

Remuneration shall be received for such work as may be stipulated in the future regulations.

10. The Institute shall prepare anti-smallpox vaccine and other sera and vaccines in common use in this colony ; payment shall likewise be received for these products in accordance with the future regulations.

11. The Chemical Laboratory shall carry out chemical analyses of urine, gastric juices, fæces, etc., and shall prepare culture media in accordance with the instructions of the Director of the Institute.

12. The Institute shall send missions of enquiry to the various parts of this colony, including the territories of independent companies. These enquiries shall be carried out with the co-operation of the medical men in those districts.

13. The medical services in the various districts of the colony shall be supplied with a sufficient quantity of suitable laboratory material to enable them to co-operate effectively in any investigations which it may be considered necessary to carry out.

14. The Chief of the Health Department, the Director of the Miguel Bombarda Hospital and the Director of the future Institute, after consulting any experts whose opinion they may desire to obtain, shall draw up plans for sheds in which to house the small animals required by the laboratory and the large animals for the manufacture of sera and vaccines.

15. The following revenue shall be earmarked for the construction and upkeep of the Institute and for the adequate remuneration of its staff :

(a) An appropriation in the general estimates for the colony ;

(b) An appropriation in the Poor Relief Fund.

16. The Directorate of the Institute shall submit each year to the Chief of the Health Department and to the Directorate of the Miguel Bombarda Hospital

the programme of work to be carried out and an annual report on the investigations undertaken and their results. After submission to the Governor-General, with the views of the Chief of the Health Department, this report shall be published in Portuguese, with a summary in French or English.

17. The Directorate of the Institute shall be granted administrative autonomy, the expenditure incurred being for the account of the Administrative Board of the Health Department.

18. The future regulations of the Institute shall be drawn up on these lines by a Committee consisting of the Chief of the Health Department, the Director of the Miguel Bombarda Hospital and the Director of the Institute.

19. The annual salaries (in gold) of the staff appointed to the Medical Research Institute shall be as follows : one Director, Esc. 9,720 (nine thousand seven hundred and twenty escudos) ; three Heads of Section at Esc. 6,480 (six thousand four hundred and eighty escudos) — Esc. 19,440 (nineteen thousand four hundred and forty escudos) ; seven Assistant Heads at Esc. 4,320 (four thousand three hundred and twenty scudos) — Esc. 30,240 (thirty thousand two hundred and forty escudos).

The salaries of the Assistant Heads shall rise each year, subject to satisfactory service, by Esc. 45 (forty-five escudos) a month until they reach the maximum monthly salary of Esc. 450 (four hundred and fifty escudos).

(a) The whole of the higher technical staff appointed to the various sections of the Institute, whether they form part of the colony's health personnel or not, shall be considered to be engaged for periods of two years and their contracts shall only be renewed provided a good report is received from the Director of the Institute.

(b) The salaries of the higher technical staff shall be regarded as maximum salaries and they shall not be entitled to any other grants.

(c) As regards salaries, the higher technical staff shall be divided into the following grades :

Director of the Institute (equivalent to Assistant Head of the Health Department) ;

Heads of Section (equivalent to first-class medical officers) ;

Assistant Heads (equivalent to second-class medical officers).

20. The salaries (in gold) of the minor staff appointed to the Institute shall be as follows : seven laboratory assistants at Esc. 1,620 (one thousand six hundred and twenty Escudos) — Esc. 11,340 (eleven thousand three hundred and forty escudos) ; one secretary at Esc. 1,620 (one thousand six hundred and twenty escudos) ; one clerk at Esc. 864 (eight hundred and sixty-four escudos) ; two typists at Esc. 864 (eight hundred and sixty-four escudos) — Esc. 1,728 (one thousand seven hundred and twenty-eight escudos).

As regards the salaries of the minor staff 50 per cent (fifty per cent) of the scale shall be granted to laboratory assistants and the secretary, while the salaries of the clerk and typists shall be the same as in the equivalent grades in other departments.

The authorities and other persons responsible for noting and executing this Decree shall duly carry out this task.

As there were no further items on the agenda, the meeting to which the present Minute refers was closed.

The undersigned, as secretary, gave instructions for the report to be entered in the Minute-book and has affixed his signature.

Certified true copy.

Technical Section of the Directorate of Medicine and Hygiene at Lourenço Marques.

May 7th, 1928.

For the Chief Secretary :

(Signed) A. Ferreira.

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