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## FOREWORD

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The final six months of the Commission's stay in Uganda have passed all too rapidly.

Much of the time has been spent in the field. Professor KLEINE and Dr. VAN HOOF have been almost constantly on *safari*. The former has carried on his work at Ikoma in Tanganyika Territory ; the latter has in turn visited the Semliki Valley in company with Dr. LAVIER ; the Uelle district of the Congo with me ; and, finally, the Budama-Kavirondo area with Dr. PRATES.

In spite of all the travelling — and travelling in sleeping-sickness areas is generally anything but comfortable — the general health of the Commission has been excellent. During the Semliki visit, the most arduous and trying of all the *safaris* undertaken by the Commission, Dr. LAVIER fell ill, and it was some weeks before he finally shook off the after-effects of this journey. On the other two expeditions most of the journey was performed by car.

Professor KLEINE returned to Entebbe in the middle of June, and a conference was held to decide upon matters affecting the final Report. The last few weeks have been devoted to putting the finishing touches to various undertakings, writing up reports and winding up accounts.

An important event in this last phase of our labours was the arrival at Entebbe, on February 7th, of Dr. PERUZZI, the Italian delegate to the Commission. It is a great pity his stay has been so short, and I think it is little short of marvellous that he has been able in such a short time to accomplish so much.

Now that our term in Uganda has come to an end, on looking back over the last eighteen months, we realise how much we owe to the Governments of the various territories into which our work has led us. Our relations with the Uganda Government throughout our stay at Entebbe have been most cordial, and we have always received the greatest kindness and invaluable assistance from all the Departments to whom we have appealed.

The Governments of Kenya and Tanganyika Territory have borne the *safari* expenses of Professor KLEINE during his stay in Kavirondo and Ikoma respectively, and we are especially indebted to Dr. ENZER and Dr. CORSON, of these two services, for the help they have given to Professor KLEINE. The Government of the Belgian Congo has also borne very considerable expense in connection with the *safari* to the Semliki and Uelle districts, and has always helped the Commission in every possible way.

As to the future, I am glad to be able to state that arrangements are being made by the British Government to carry on in Uganda the work inaugurated by the Commission. It is to be hoped also that there will be co-ordination of research activities between the various African territories where human trypanosomiasis still takes its annual toll of human life.

In executing our programme we have adhered as much as possible to the terms of reference laid down by the Committee of Experts. The study of the treatment of the disease did not fall within our scope and has, in consequence, received but little attention.

We have not finally disposed of the problems we were asked to solve ; indeed, we never expected to do so in the time at our disposal. On the whole, our expectations have been more than realised. Fortune plays a part even in such prosaic enterprises

as scientific Commissions, and we have had our share of luck. Without it, no amount of hard work in research of this kind can win through to interesting results ; and we are fully conscious and grateful for many valuable opportunities that have come our way.

Before leaving Uganda, His Excellency Sir William GOWERS, K.C.M.G., Governor of the Uganda Protectorate, sent to the Commission the following message of farewell :

“I greatly regret my inability to be present in person to bid farewell to the members of the International Commission on Sleeping-Sickness. The work of the Commission will undoubtedly mark a new era of international co-operation among all the Powers possessing territory in Africa, and I trust that it will lead to standardisation of methods for controlling sleeping-sickness.

“In this respect the Governments of the British Territories in Africa have much to learn from the methods employed in those other colonies which have experienced the disease for many years past and have, perhaps, devoted more attention to the clinical and therapeutic aspects of this great problem. The results of the Commission's labours will be of the utmost value to African Administrations as well as to science ; and this Protectorate, as well as Kenya and Tanganyika Territory, have already derived material benefit from the indefatigable labours of Dr. KLEINE and Dr. VAN HOOF in the areas visited by them. I am glad that Uganda was chosen as the scene of operations of the distinguished scientists, to whom I now wish to convey an expression of my deep appreciation and my best wishes for the progress of their researches in other countries.”

May our achievement be found worthy of the sentiments here so gracefully expressed — an addition to our knowledge of the human trypanosomes and a step forward towards the ultimate solution of the administrative problems to which they give rise.

H. Lyndhurst DUKE.

ENTEBBE, *June 30th*, 1927.

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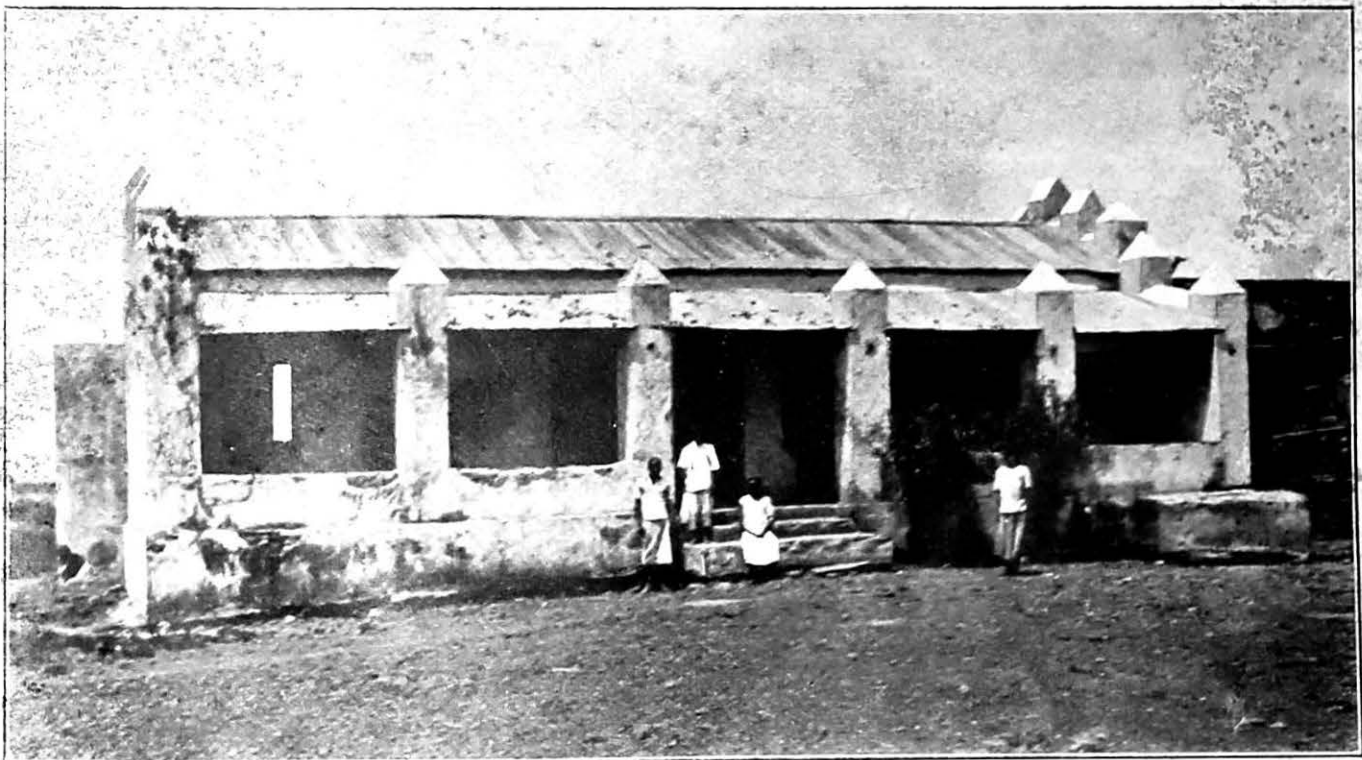
I.

**REPORT ON THE NEW SLEEPING-SICKNESS  
FOCUS AT IKOMA.**

BY F. K. KLEINE,  
Professor at the Robert Koch Institute,  
Honorary Professor at the University of Berlin.



The Hospital at Fort IKOMA



The Doctor's house at Fort IKOMA

## REPORT ON THE NEW SLEEPING-SICKNESS FOCUS AT IKOMA.

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The Medical Department of Tanganyika Territory had suggested as an appropriate starting-place for my further investigations the station of Musoma, situated on the Victoria Nyanza. The local Medical Officer, Dr. J. F. CORSON, had instructions to help me. On September 3rd, 1926, my wife and I arrived at Musoma. Dr. CORSON told me on my arrival that he had treated a number of sleeping-sickness patients in Musoma, but at the moment there were no more there. He proposed moving our quarters to the Ikoma district, a few days' march away. The causative agent of the epidemic there is *T. rhodesiense*, the insect-carrier *G. swynnertoni*.

At Ikoma we established ourselves in the old military post, now in ruins, which the Germans had formerly erected for the protection of the natives against the Masai. Dr. CORSON accompanied us. During the whole course of our stay (till June 1927) his help was of great value to us.

We saw by degrees 56 sleeping-sickness patients of both sexes from 4 to 50 years of age. Since our patients came, or in many cases were carried, for treatment, generally in rather advanced stages of their illness, it may with safety be assumed that there are also present in the native villages patients in an earlier stage of the disease. Infection occurs in the more thickly grown parts of the bush country where *G. swynnertoni* is fairly numerous. There are no grounds to suggest a mechanical transmission of the disease.

Considered *as a whole*, the cases are without any doubt clinically distinct from those I had the opportunity of observing previously. In the Sese Islands, on the River Mori, on Lake Tanganyika and in the Belgian Congo, patients with severe glandular swellings and with nervous symptoms of all degrees up to pronounced mental disturbance were in the majority. Patients in whom the disease in a few months led to complete physical collapse before any definite enlargement of the glands occurred, or infection of the central nervous system became clinically apparent, were scarce there. Here they are in the majority. In spite of this, I do not believe that the epidemic here can be clinically separated from the sleeping-sickness of those old foci. Not one of our patients would, in the *palpalis* regions on Lake Tanganyika, the Congo, etc., be considered as presenting any unusual symptom but would merely be regarded as a case in which the disease was running a particularly severe course.

### TRYPANOSOME FINDINGS.

The diagnosis was in all cases made on finding trypanosomes in the blood. While in the old foci of sleeping-sickness (*G. palpalis*, *T. gambiense*) the examination of only the fresh unstained blood preparation would in a *very* large percentage of cases lead to mistaken diagnoses, here we frequently found the parasites in the fresh preparation at the first glance. The blood was swarming with trypanosomes. At the same time, the examination of the living blood was by no means sufficient. Often the diagnosis could be made only from the stained preparation (see the technique in the earlier report) and sometimes only after long searching, exactly as in a *T. gambiense* area. In the case of one patient, three blood examinations at intervals of several days were necessary. There was no glandular swelling.

Through the kindness of Dr. J. L. GILKS, Director of Medical and Sanitary Services at Nairobi, and of Dr. W. H. KAUNTZE, Deputy Director of the Laboratory there, and through the foresight of Dr. CORSON, we always had at our disposal a sufficient number of experimental animals, so that we had the opportunity of studying the morphological characters of twelve *rhodesiense* strains inoculated directly from man to guinea-pig. Since the so-called posterior-nuclear forms have been found also in *T. gambiense* and this phenomenon can no longer be admitted as characteristic of *T. rhodesiense* (see Dr. LAVIER's report from Entebbe and mine from Homa Point), we tried to ascertain whether perhaps the percentage of nuclear displacements renders possible the differential diagnosis between *T. rhodesiense* and *gambiense*. To determine the percentage, we counted *every fourth day* 500 parasites in smear preparations stained by the Romanowsky-Giemsa method. The counts began on the seventh day after the first appearance of parasites in the blood. If only a few parasites were present in the preparations, we did not keep to the number 500 but stopped counting after half-an-hour. At first we reckoned only the blunt unflagellated forms which show a particular tendency to nuclear displacements. Not infrequently, especially in the later course of the infection, nuclear displacements are also encountered in the so-called transitional forms, *i.e.*, trypanosomes which are half way between short and long parasites. In such a case it is fairest and at the same time most convenient to count all the trypanosomes *without distinction* and then determine the percentage of posterior-nuclear forms.

In strains 1, 2 and 3, *T. rhodesiense* seemed to be marked off as a separate parasite easily distinguishable from *T. gambiense*. The incubation in the guinea-pig took 9 days. The parasites were very large and thick, and showed a pronounced dimorphism, though the plump forms with short flagella predominated. A week later appeared the first posterior nuclear forms. On different days the percentage varied, gradually increasing, between 3 and 20. Shortly before the death of the guinea-pig the posterior-nuclear forms were particularly numerous. We often saw several, up to as many as 8, in a single field. The microscopic appearance resembled exactly the drawings of *T. rhodesiense* we are accustomed to find in textbooks. The guinea-pigs died 23, 47, and 53 days after the inoculation.

Strain 4. — Predominantly thick trypanosomes with short flagella. Tendency to forward displacement of the nucleus. Eleven days after the occurrence of the parasites, the first posterior-nuclear forms appeared. Marked dimorphism. After a further 14 days, 1 per cent posterior-nuclear forms. This proportion remained practically unchanged. Death of the guinea-pig after 81 days.

Strain 5. — Predominantly thick trypanosomes without free flagella. Nineteen days after the occurrence of the parasites, the first posterior-nuclear forms appeared. They disappeared after 27 days and returned again after 43 days. Thenceforward there were on the average 2.3 per cent, but on two days 3.4 per cent were observed. Microscopic examination of the blood was continued for three months. Death of the guinea-pig after 107 days.

Strain 6. — Many thick trypanosomes without free flagella, a few thin ones with long flagella. Twenty-four days after the occurrence of the parasites, the first posterior-nuclear forms appeared. On some days they were wholly absent. Average 0.15 per cent, maximum 0.6 per cent. After the end of the three months' observation of the blood, I examined it once more shortly before the death of the guinea-pig and found 3 per cent posterior-nuclear forms. Death of the guinea-pig after 133 days.

Strain 7. — Predominantly thick trypanosomes with a tendency to nuclear displacement. Twenty days after the occurrence of the parasites, the first posterior-nuclear forms appeared. On some days they were wholly absent. Average 0.2 per cent; maximum 1.4 per cent. At the next count only thin trypanosomes with long flagella; no nuclear displacement. Later still 0.2 per cent. Death of the guinea-pig after 71 days.

Strain 8a. — Nearly always long slender trypanosomes with free flagella, but now and then also large thick ones. In counting 7,630 parasites, *no* posterior-nuclear forms were observed. Death of the guinea-pig after 78 days. Spleen small (2.8 cm. long, 2 cm. wide).

Strain 8b (Strain 8 was inoculated into two guinea-pigs). — Nearly always slender trypanosomes with free flagella, now and then large and small thick ones. In counting 9,300 parasites, *no* posterior-nuclear forms were observed. Death of the guinea-pig after 85 days. Spleen small (3 cm. long, 1 cm. wide).

Morphologically, strain 8 was indistinguishable from a *gambiense* strain during the whole period of observation. The number of trypanosomes in the blood, however, was generally greater than we are accustomed to see with *T. gambiense*, and at the same time their virulence was greater.

Strain 9. — A great many trypanosomes, mostly slender with long flagella. Four weeks after their occurrence the first posterior-nuclear forms appeared, the percentage of which rose slowly to 3.5. Then they disappeared altogether for a few days. Sudden death of the guinea-pig on the 61st day from rupture of the greatly enlarged spleen.

Strain 10. — Slender trypanosomes predominant. Eighteen days after their occurrence the first posterior-nuclear forms appeared. The percentage rose to 3; on several days there were none at all present. Shortly before the death of the guinea-pig, 7 per cent exhibited very marked backward and as many forward nuclear displacements. In addition, there were large slender parasites. Death after 68 days.

Strain 11. — Pronounced dimorphism, but small thick trypanosomes predominated. Eighteen days after their occurrence, the first posterior-nuclear forms appeared. Gradual increase to 6 per cent. Then a decrease to an average of 0.26 per cent; on some days no nuclear displacements. Three months' microscopic observation of the blood.

Strain 12. — Slender trypanosomes predominant, but now and then a good many thick ones with short flagella. Nineteen days after the occurrence of the parasites, the first posterior-nuclear forms appeared. On one day 0.8 per cent, otherwise ordinarily 0.2 per cent. Sudden death of the guinea-pig after 44 days. On the day of its death no nuclear displacements were observed.

Further transmission by inoculation of the blood into another guinea-pig. Mostly slender trypanosomes with long flagella. No posterior-nuclear forms. After two months the number of parasites fell considerably. Within half-an-hour there were never as many as 500 parasites counted.

This behaviour of the strain is noteworthy, for usually posterior-nuclear forms do not disappear throughout several passages and months. Their complete absence here as well as the small number of parasites — once, 84 days after the inoculation, only 18 were counted in half-an-hour! — remind one very much of the peculiarities of *T. gambiense*.

On looking over these strains, we are struck by the varying percentage of posterior-nuclear forms, which ranges from 0 to 20! If it were not for the presence of all degrees of transition between the individual strains, we might easily think we were dealing

with different species of trypanosomes. As a matter of fact, in comparing strains 1 to 3 with strain 8, I did for many weeks ponder whether this might not be the case. Moreover, within the individual strains great differences are to be noted at different times. It may happen that to-day parasites show the pronounced *rhodesiense* type and after a few weeks occasionally that of *gambiense*, i.e., in particular, few or no posterior-nuclear forms.

With very virulent strains the nuclear displacements as a rule increase gradually until the animal's death. If, however, the animal withstands the disease for some time, their number falls — probably under the influence of newly formed antibodies — to a considerable extent, and only later increases again. In one rat I saw the percentage of posterior-nuclear forms fall within a week from 32 to 1.8.

The suppression of posterior-nuclear forms can sometimes be brought about artificially if parasites which, under normal conditions, have a tendency to nuclear displacement are placed in a medium that is not suited to their development. If rats or guinea-pigs are first inoculated with a weak *gambiense* strain and afterwards with *T. rhodesiense*, the nuclear displacements occur *for a time* much more scantily than in the controls not previously inoculated with *T. gambiense*.

Experiment 1. — In the blood of a rat infected with *T. gambiense* on November 19th, 1926, and subsequently inoculated with *T. rhodesiense* on January 5th, 1927, we found in the period from January 15th to February 12th, 1927, in counting 4,000 parasites, only 2 posterior-nuclear forms. In the control not previously infected with *T. gambiense*, however, we found 53.

Experiment 2. — In the blood of a rat infected with *T. gambiense* on November 25th, 1926, and inoculated with *T. rhodesiense* on February 3rd, 1927, we found in the period from February 10th to 24th, 1927, in counting 2,000 parasites, 32 posterior-nuclear forms; in the uninfected control 186.

Experiment 3. — In the blood of a guinea-pig infected with *T. gambiense* on January 7th, 1927, and inoculated with *T. brucei* on January 25th, we found in the period from February 5th to 17th, 1927, in counting 2,000 parasites, no posterior-nuclear forms; in the infected control 98.

From Experiment 3 it is apparent that the antibodies formed against *T. gambiense* act also upon *T. brucei*. This is not surprising. Some years ago B. MÖLLERS and myself produced an antiserum with *T. brucei*, which protected mice from infection with *T. brucei*, but also exerted a certain influence upon *T. gambiense*. Suppression of the posterior-nuclear forms can be achieved only with difficulty by the employment of medicaments. Often the dose employed is too small to produce any effect or else it is so large that the trypanosomes disappear altogether.

The human trypanosome strains examined by us at Ikoma were considerably more virulent than those on the Kavirondo Coast. There, out of 16 strains, only 4 took in guinea-pigs; here, out of 20 strains, 15 took. The greater virulence can hardly be used for purposes of differential diagnosis. Even in sleeping-sickness foci that are admittedly caused by *T. gambiense*, the virulence of the parasites seems to change. When the R. Koch Expedition came to the Sese Islands in 1906, according to the statement of the natives, most of the dogs had died. Dr. VAN SOMEREN had demonstrated trypanosomes in a dog examined some time previously on the Buninga Peninsula. We ourselves, in the few dogs we were able to examine, have seen no parasites. Since nagana has not occurred on the Sese Islands, so far as I am informed, VAN SOMEREN'S view that the dogs died from an infection with *T. gambiense* is very important. Moreover, W. FISCHER (1913), on Lake Tanganyika, reported to me on the strikingly rapid death of monkeys which he had infected there by means of *G. morsitans* with *T. gambiense*. I regret that we did not pay more attention to these experiments.

Henceforward more attention will have to be devoted to the varying virulence of human trypanosomes in the different sleeping-sickness foci, as well as to the morphological study of the different strains. I include here the encountering of chronic sleeping-sickness patients and *T. gambiense* in *old* foci of a *morsitans* area, and of the *rhodesiense* type in freshly infected *palpalis* regions, on the assumption that the occurrence of many posterior-nuclear forms indicates, as a rule, a particularly strong propagative energy on the part of the parasites. The quantity and quality of the nuclear displacements seem to be dependent on the natural virulence of the strain. In the guinea-pig they generally occur much more strongly and in greater number in highly virulent strains than in weak ones ; in a preparation in which 100 parasites are found in a few fields, we have a greater prospect of seeing posterior-nuclear forms than in 100 trypanosomes that are spread out over several preparations. Strains cultivated for a long time in the mammal lose the nuclear displacements along with the dimorphism of the parasites.

Passing mention may be made here of an interesting morphological finding on the Uyoma Peninsula (Kavirondo Coast, *palpalis* area). During a period of observation of six months on a *gambiense* strain in a guinea-pig's blood, I always saw some parasites whose blepharoplast remained unstained with Romanowsky-Giemsa. On further inoculation of the strain, the parasites without blepharoplast did not disappear. It is well known that WERBITZKI, KUDICKE, LAVERAN and others were able to produce this phenomenon artificially by the action of certain substances upon living trypanosomes.

#### MUTUAL RELATIONS OF THE HUMAN TRYPANOSOMES AND "T. BRUCEI."

Where did the epidemic originate which, in its present distribution at Ikoma, was until lately unknown to the natives ? In order to answer this question it is desirable to discuss in a few words the relations of *T. rhodesiense* and *brucei* to each other. During their comparative investigations with the parasite of sleeping-sickness in Nyasaland and with other trypanosomes, BRUCE, HARVEY, HAMERTON, DAVEY and Lady BRUCE came to the conclusion that *T. rhodesiense* is nothing else than *T. brucei* and that the sleeping-sickness in Nyasaland is identical with nagana. I was unable to agree with this view, although it was supported by the authority of the man who discovered the causative agent of nagana and recognised in *T. gambiense* the mysterious cause of sleeping-sickness. In my opinion, there are too many reasons against the identity of *T. rhodesiense* and *brucei*. That both are very closely related genetically will be doubted by nobody. At present they are in one essential point distinct: the pathogenicity of the human parasite divides it sharply from its relative, just as the cholera vibrio is separated by its pathogenicity from similar water vibrios. Whether *T. rhodesiense* and *T. brucei* are reckoned as separate species, varieties or races is for the present unimportant. It is best to wait for the result of experimental investigations in this direction.

Even though *T. rhodesiense* — up to the present, at any rate — is found in *G. morsitans* regions which one is inclined to consider as the domain of *T. brucei*, yet sleeping-sickness — even that caused by *T. rhodesiense* — and tsetse disease of cattle are by no means confined to the same geographical limits. BEVAN was the first to point out that lack of coincidence. On the basis of my own experience, I share his opinion ; the numerous German officials, both civilian and military, who in the course of years have passed along the caravan road from Tabora to Ujiji, which teems with tsetse flies, have without any exception remained healthy. Exhaustive investigations by my



colleagues and myself into the occurrence of human trypanosomiasis in that region, where neither ox nor dog can live, led, before the war, to an absolutely negative result. During the construction of the railway from Tabora to Kigoma no case of infection was reported, although the Government had taken the precautionary measure of detailing for supervision work a health officer experienced in the control of sleeping-sickness, and the railway doctors too were familiar with the diagnosis. In 1903 (quoting from TODD), Dr. ASCENSO inoculated himself and two natives with blood from a cow suffering from nagana. In Portuguese East Africa, TAUTE, in 1913, injected into himself animal blood containing *T. brucei* and also allowed himself to be bitten by infected glossinæ. TAUTE and HUBER, during the East African campaign, inoculated themselves and 129 natives, many of whom were only in a moderately healthy condition, with *T. brucei*. W. A. COLLIER recently went still further. Since we know from LAVERAN and from LAVERAN AND MESNIL that *T. brucei* is killed by human serum, COLLIER gradually accustomed a strain of *T. brucei* in the animal to human serum and injected into himself and four other persons about 200 millions of serum-fast trypanosomes. None of these investigators became ill.

All this shows that man is resistant to the parasite of animals (*T. brucei*). H. L. DUKE and others suppose an *occasional* interruption of this resistance. When man is physically weakened by disease or starvation, when the glossinæ for some reason or other seek their nourishment from man instead of wild game and when an habituation of *T. brucei* to human blood thus gradually takes place, then the opportunity for the infection of man with *T. brucei* is said to occur. These conditions are certainly not operative in the Ikoma district. There is no famine or other debilitating condition of any importance. The natives uninfected with trypanosomes are in an *excellent* state of nutrition. Game, whose blood the glossinæ can suck, is present in abundance. They are now no longer driven to feed on human blood as they were formerly. It seems obvious, therefore, that the epidemic has been brought from the lake shore only a few days' march away, or from the River Mori (*palpalis* area), where there were formerly many sleeping-sickness patients, into the hitherto epidemic-free district of Ikoma (*swynnertoni* area) by infected natives. I see no essential difference between the trypanosome here and *T. gambiense*, and that *T. gambiense* can be carried by *G. swynnertoni* admits no doubt.

#### TRANSMISSION OF "T. GAMBIENSE" BY "G. SWYNNERTONI", ETC.

At the time when I succeeded in demonstrating the development of *T. brucei*, the cattle trypanosome, in the sleeping-sickness fly, *G. palpalis*, the conclusion seemed justified that *G. morsitans*, the ordinary host of the cattle trypanosome, might in its turn also become the host of the sleeping-sickness parasite, *T. gambiense*. Experiments which I undertook with Dr. TAUTE in Kirigu, not far from the Victoria Nyanza, in order to transmit *T. gambiense* with *G. swynnertoni* (which belongs to the *morsitans* group<sup>1</sup>), led to no positive result. Although the negative result agreed with the then current *opinio omnium*, I nevertheless — strengthened in my opinion by an article by A. G. BAGSHAW in the *Sleeping-Sickness Bulletin* — considered it inconclusive and caused Dr. TAUTE to repeat the experiment once more on Lake Tanganyika. Here, under the very favourable climatic conditions, the transmission of *T. gambiense*

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<sup>1</sup> At that time we took the flies used in our experiment for *G. morsitans*, Westwood. This fly, however, is not found in the Ikoma district from which we obtained our pupæ. The glossina in question was later described by AUSTEN as *G. swynnertoni*.



by *G. morsitans* was accomplished without difficulty. Later, W. FISCHER, on the Victoria Nyanza, also achieved the same result with *G. swynnertoni*, and Dr. FISCHER and myself were then able to show on Lake Tanganyika in thirteen parallel experiments on thirteen different strains of *T. gambiense* that, in every case where *G. palpalis* conveys the human parasite, *G. morsitans* does so too. RODHAIN and his collaborators infected 4.76 per cent of *G. morsitans* with *T. gambiense*, and BRUMPT considers it probable that in nature this fly, at least in the Katanga Province, plays an important part in the spread of sleeping-sickness caused by *T. gambiense*. In this connection it may be recalled that KINGHORN AND YORKE (1912) transmitted *T. rhodesiense* by means of *G. morsitans*, and ECKARD (1913) by means of *G. palpalis*.

In order to prevent the spread of sleeping-sickness, a strict control of native traffic was formerly exercised by the German Government. In particular, a thorough medical examination was made of the carriers of all those caravans that passed from infected regions, e.g., Lake Tanganyika, to the coast. Labour recruiting in infected districts was absolutely forbidden. During the war these sanitary measures could not be maintained. When and to what extent they have come into force again since the war in Tanganyika Territory, and in particular whether they can be strictly carried out in view of the rather small number of the European personnel familiar with the control of sleeping-sickness, is unknown to me. As I assume a connection between the epidemic in Ikoma and the old foci on the River Mori and Victoria Nyanza, I consider it very probable that the other outbreaks in Tanganyika Territory, in the Mwanza and Tabora district and in the Ufipa district are also to be traced back to the immigration of sick natives coming from the Victoria Nyanza or Lake Tanganyika. A study of the map and traffic routes of East Africa supports the assumption.

It may be reckoned that, unless the necessary measures are taken, the whole colony will be threatened in all too short a space of time. Sleeping-sickness is progressing from west to east. When it became more generally known at the beginning of the century, it had reached the lakes and was about to pass over from the domains of *G. palpalis* into those of *G. morsitans*. This has now happened. This point is of practical importance in combating the epidemic. I can subdue an epidemic of which I am convinced that it is spread only by infected individuals with more prospect of success than one which, upon purely hypothetical grounds, appears likely to break out in a number of unforeseen fresh places.

#### RISE AND FALL OF THE EPIDEMIC.

The question why sleeping-sickness occurs with such excessive violence in hitherto immune regions is difficult to answer and cannot quite be separated from the general question how epidemics occur. Probably the concurrence of a number of favouring external — perhaps even climatic — circumstances is necessary, the significance of which, taken singly, we cannot perceive. For the present, let us assume that an epidemic of sleeping-sickness can arise where numerous flies, a series of freshly infected cases and a dense population are present. I do not believe in the mechanical transmission of trypanosomes. It is unproved, and its great improbability was indeed the very reason that led to investigations on the development of trypanosomes in the tsetse-fly. Also, as BRUMPT has recently emphasised, the hypothesis of mechanical transmission is not even necessary to explain an increase of virulence,

We usually place the responsibility for the extinction of epidemics upon sanitary measures, a certain immunity of the infected population and also the decrease in virulence of the causative agent. By sanitary measures, for instance, sleeping-sickness

was formerly banished from the River Mori. The belt of woodland in which the flies were living was deforested, and on the cleared ground the natives were made to plant shambas. The epidemic disappeared with the flies.

Of the occurrence of immunity through the infection of the population I myself have as yet seen no evidence anywhere. The extinction of the epidemic at Homa Point — as I mentioned in the previous report — is traceable not to immunity of the natives but to slight infectivity of the flies. The want of immunity to sleeping-sickness on the part of man is not an extraordinary phenomenon. It has a parallel in the tsetse disease of cattle. We do not yet know any race of cattle that is really immune to *T. brucei*. Were it possible to breed such a race in a reasonable space of time, then cattle-breeding people, *e.g.*, the Zulus — as David BRUCE rightly remarks — would have done it long ago. Also the natives' dogs have not so far acquired any immunity to nagana.

ROUBAUD sees in the disappearance of sleeping-sickness an immunity of the flies rather than of the human beings. When glossinæ are continually ingesting blood containing trypanosomes, antibodies may be formed in the insect, and the progeny of such flies may become insusceptible to infection by trypanosomes. I tested this question experimentally on a former occasion together with Dr. B. ECKARD, but with a negative result. The progeny of flies that had been fed only with blood containing trypanosomes became infected in the same percentage as the progeny of glossinæ that had sucked pure blood exclusively.

It is probable that, in the subsidence of a sleeping-sickness epidemic, an important part is played by losses of virulence on the part of the parasites. The most serious cases are soon confined to their huts by their disease and die quickly, so that the flies have the opportunity of becoming infected with highly virulent strains only for a relatively short time. The more chronic the course of the disease, the more remote the time of infection, the more do the parasites lose the capacity of developing further in the carrying insect (KLEINE, FISCHER AND ECKARD, 1914; H. L. DUKE, 1923).

Moreover, the decrease of the population by death and migration compels the glossinæ not to confine themselves in their feeding to human blood, and passage to animals<sup>1</sup> helps to weaken the virulence of the parasites for man. This assumption is justified by analogy. We know — I recall R. KOCH's old experiments on the protective inoculation of cattle, and those of Claus SCHILLING — that *T. brucei*, in the course of passages through *different* species of animals, loses to a considerable extent, but not altogether, its pathogenicity for that species of animal from which it was first inoculated. Thus many reasons can be given for the decrease in virulence of the parasite and for the gradual weakening and occasional extinction of sleeping-sickness.

#### THE COMBATING OF SLEEPING-SICKNESS IN THE DISTRICT OF IKOMA.

The control measures against the epidemic are directed against the carrying insect and on the other hand against the parasite in the human being. Deforestations with subsequent cultivation of the cleared areas have proved very useful on the Victoria and Tanganyika Lakes in the destruction of flies. The deforestations fulfil

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<sup>1</sup> According to my earlier experimental researches, animals infected by *T. gambiense* scarcely count at all as a means of spreading the disease. Infection of the fly is produced at first through the medium of the sick man. On the other hand, we are still insufficiently informed as to the way in which *T. rhodesiense* behaves in the animal's body, and *experimental* researches on this point seem to be indicated.

their purpose, however, only when the population is numerous enough really to carry it out and maintain it. If fields are not at once laid out on the cleared spaces — they are best sown with low-growing cultivated plants like peanuts, potatoes, beans, etc. — the cleared brushwood soon grows up again luxuriantly and the glossinæ return. Time and money are then wasted.

If the population is not numerous enough for a successful campaign against the glossinæ, smaller settlements can with advantage be united into a larger one, or specially endangered districts can be evacuated altogether.

Besides the methods of fly destruction mentioned above which proved satisfactory, SWYNNERTON advises as a new measure for this purpose that the burning of the grass, which the natives usually begin towards the end of the dry season, should be carried out *simultaneously over a wide area*. Swynnerton's suggestion will achieve success where uniform dryness of the ground allows a simultaneous burning of the grass, and particularly where the Government authorities exercise so much influence upon the natives that each one does not light a fire on his own responsibility as soon as he himself considers time and place to be suitable.

Before any sanitary measures can be considered in Ikoma, it will be necessary to obtain more exact information on the distribution of sleeping-sickness. For this purpose it seems necessary to increase the medical personnel or at least to provide the doctor with a motor-car. It is not sufficient to provide medical treatment only for patients who report themselves, but a diligent search must be made for sick persons in the native villages. Upon the location and number of the cases depends the nature of the further measures to be taken. The hunting up of cases of the disease is the first step in combating the epidemic. Medicinal treatment, even when no final cure results, may sterilise the patients' blood for months and thereby deprive the "clean" glossinæ of the possibility of becoming fresh carriers of infection. It is by no means certain that in the bush country of Ikoma the treatment of patients may not be the most effective form of combating sleeping-sickness.

#### TREATMENT OF PATIENTS.

The treatment of patients in Ikoma has to contend with the difficulties that are usual in a land with an uncivilised population very little touched by European influence. The natives bring their sick not in the initial stage of the illness but only when they seem in a condition of complete exhaustion approaching death. If an improvement occurs as the result of the treatment, the patients readily withdraw from medical care and return to their villages, pleading some fictitious domestic circumstance or other as an urgent reason for their departure. To get a sight of former patients for the purpose of subsequent examination is often not easy. This behaviour of the natives spoils the therapeutic results to a quite extraordinary degree.

At Homa Point I noticed that, in order to escape the tiresome enquiries, the patients who were being sought got their friends to report them as having died. I had left ten sick persons behind on the Uyoma Peninsula. When, a few weeks later, the Medical Officer wanted to place them under treatment, some of them were not to be found and were quite unknown, although there was an exact list with names, villages, etc. This shows how little the statements of the natives can be trusted as soon as their comfort is disturbed by the European's questions. If the influence of the Government is used to its fullest extent in supporting the physicians, the treatment of the sick and with it the control of the epidemic becomes easier.

*Medicaments: Germanin ("Bayer 205").* — Our chief endeavour in the treatment was to cure the patients as quickly as possible. Chemotherapeutic experiments with drugs which were kindly sent to the International Commission from various sources for testing had to be adapted to this purpose. Each remedy was as far as possible tested on a small group of hitherto untreated patients. If the effect upon the parasites was not soon evident, the drug was discarded or used only in combination with "Bayer 205". This drug far surpasses all others in its beneficial effect and, as so many authors have ascertained, sterilises the blood for a long space of time (*cf.* also W. H. DYE, *Trop. Dis. Bull.*, 1926, No. 12, p. 894). Its employment is therefore indicated in any properly organised scheme, and is essential for a systematic campaign against the epidemic.

From earlier and recent experiences I cannot agree with the statement that "Bayer 205" does not influence the trypanosomes in the brain. I quote two specially clear examples :

1. Merana, a boy about 12 years old, who was said to have been ill only for five months but already slept continuously, was given, on October 26th, 1926, 1 gm. of "Bayer 205", dissolved in 5 c.c. of water, intragluteally<sup>1</sup>. From October 25th onwards he slept less, began to take a little notice of his surroundings and, with the help of his father, to walk a few paces. Further treatment led to rapid improvement. Lumbar puncture made on December 26th, 1926, revealed nothing pathological beyond a very high pressure of the fluid. This, of course, is not to say that the improvement will last.

2. Nyambura, a woman of 20, came for treatment on January 28th, 1927. Small cervical glands, swollen feet. Patient was very weak and could no longer walk without help. She was given 1 gm. of atoxyl subcutaneously. On the evening of January 28th sudden convulsions occurred. Morphine was administered. February 1st, unconsciousness continued, head retracted. 1 gm. of "Bayer 205" intragluteally. February 3rd slow return to consciousness, less rigidity of the neck. February 4th, 1 gm. of "Bayer 205" intragluteally. February 10th: 1 gm. of "Bayer 205" intravenously. February 14th: 1 gm. of atoxyl subcutaneously. February 17th: 1 gm. of "Bayer 205" intravenously. February 23rd: patient walked without help. Lumbar puncture refused. Treatment was continued.

Recently I saw a further case which in the essential points resembles the one last mentioned. But in order to prove the effect of "Bayer 205" upon the parasites in the cerebrospinal system it is not necessary to select only cases of this kind with quite severe cerebral symptoms. Repeatedly there came for treatment patients whose excitability and timidity made an intravenous injection impossible. After two intragluteal injections the excitable conditions disappeared and treatment was continued intravenously.

According to animal experiments by Dr. BEHNSEN in Kiel, the barrier between blood and cerebrospinal fluid even under normal conditions is by no means always of the same potency. The permeability of the limiting membrane diminishes *pari passu* with the age of the animal. In the healthy adult animal there are still a few small permeable places in the otherwise impermeable membrane, and with illnesses still more "weak" places become permeable. The same must be the case with sick human beings. How otherwise is one to explain the disappearance of the somnolent condition in the boy, or the cessation of cervical rigidity and unconsciousness in the woman ?

*Tryparsamide.* — This drug is specially noted for its effect upon the parasites that have already penetrated into the cerebrospinal system. We had no opportunity of collecting observations in this direction. Considering the long period which is

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<sup>1</sup> Following Dr. J. F. CORSON'S procedure, I employed intragluteal injections in very weak patients in whom the intravenous method of injection met with difficulties. No abscesses were observed and no special pain was noticed.

recommended for the therapeutic employment of tryparsamide, the rapid course of the disease may well mask any possible favourable effect. We therefore had to confine ourselves to studying only the immediate effect upon the blood parasites. This we regarded as the measure of the trypanocidal strength of a drug. After 2 gm. and also after 3 gm. of tryparsamide intravenously, parasites were found in the blood on the second day after the injection. On one occasion after 2 gm. they had even definitely increased on the second day. The injection had therefore exercised a stimulating effect. Even after a further 1.5 gm. the parasites did not disappear. This slight influence does not always seem to be confined to *T. rhodesiense*. At Homa Point, I examined a patient who, within seven weeks, had been given by someone else 12.5 gm. of tryparsamide; a fortnight after the last injection, he had *T. gambiense* in his blood. Attempts to infect monkeys with 5 and 10 c.c. of the blood were unsuccessful. This non-infectivity of the blood often lasting for a long time, in spite of the presence of living trypanosomes, we were able to demonstrate also after treatment with other drugs. Unfortunately, the effect on the parasites thus evidenced is not great enough to give any prospect of a favourable course of the disease. Tryparsamide exercises a marked tonic action upon the patients.

*Atoxyl and Soamin.* — After the subcutaneous injection of 1 gm. of atoxyl or Soamin, the parasites disappeared for 7 to 9 days. In one case, after 1 gm. of Soamin, they did not reappear in the blood until the fifteenth day. Transmission of these trypanosomes to rats was not successful. The tonic action of the preparations is very considerable.

*B. R. 34, Na. Salt.* — This arsenical compound produced by Professor A. BINZ had hitherto been used only by Professor DAHMEN in Berlin upon artificially infected animals. Experiences on the dosage in man were non-existent. Accordingly, we proceeded very cautiously with our patients. With the small dose of 0.2 gm. intravenously, the blood remained free from parasites for 8 to 9 days. In view of this result; the further testing of the drug seems indicated.

*Preparation 2754* (I. G. Farbenindustrie; works at Höchst-a-M.); a drug containing arsenic for internal use in the form of tablets. — In two hitherto untreated patients the trypanosomes remained constantly demonstrable in the blood during a daily administration of six tablets continued for 5 days and 8 days respectively. A relapse case was given six tablets daily for 8 days; on the fourth day the parasites disappeared from the blood for one month. The drug was also given to some seriously ill patients in whom relapses were to be feared. Whether their non-occurrence is to be put down to the credit of the remedy must be proved by further experiences. Its tonic action is definite.

“*Sb 212*”. — This complex antimonial compound has the advantage over all other antimonial preparations that a subcutaneous administration is possible. 4 c.c. of the 2% solution caused the parasites to disappear for a day. If this was followed on the next day by an injection of 5 c.c., the blood remained free from parasites for 2 more days. In one patient the injections (2 c.c., 4 c.c., 6 c.c., 8 c.c.) were continued until symptoms of intoxication occurred. The trypanosomes returned 6 days after the last injection.

*Antimosan* (“*Heyden 661*”). — After 2 c.c. and 4 c.c. of the 5% solution administered intragluteally, the trypanosomes did not disappear from the patient's blood. After a dose of 5 c.c. they were absent for 2 days. In one patient who received intragluteal injections one day after the other, of 3 c.c., 4 c.c., 4 c.c., 4 c.c., 4 c.c., respectively,

the parasites were always demonstrable in the blood throughout the treatment. Transmission of the trypanosomes to rats was not successful.

*Stibosan* ("Heyden 471"). — In patients who had a relapse after the conclusion of their treatment with "Bayer 205", the parasites disappeared from the blood for about 12 days after an injection of 0.3 gm. intravenously. Experiences with continued injections will be reported later. Of the antimonial preparations tested, *Stibosan* is the most efficacious, though a single dose of 0.3 gm. intravenously is without effect upon previously untreated trypanosomes.

The foundation of our therapy was in all cases Germanin ("Bayer 205"). After four or more injections we followed them up in case of need by arsenical preparations in consideration of their tonic effect. If, after the treatment, parasites again appeared in the peripheral blood or if any other symptoms suggested the continuance of the infection, we gave a few more injections of "Bayer 205". We were also glad to use *Stibosan* in relapses on account of its high antimonial content. According to W. FISCHER's and my own experience and that of DU TOIT and of THEILER, antimony exercises a special influence upon trypanosomes in animals which have been previously treated with "Bayer 205". The reasons for this have been convincingly explained by J. MORGENROTH. The idea therefore suggests itself of applying this combined therapy to human beings. By microscopic blood examinations, I was actually able to convince myself of the difference in the action of *Stibosan* upon untreated trypanosomes and those under the influence of "Bayer 205" in our patients.

Although most of our patients at the time of their arrival were already in a very advanced stage of their illness, the therapeutic results seem so far satisfactory. To give case histories at present would be premature. After a few months, Dr. J. F. CORSON will be better able to report definite results. Perhaps it will then appear desirable to distribute the "Bayer 205" treatment over a longer period, *i.e.*, to give the injections at greater intervals than we have done hitherto. Unfortunately, it is to be feared that many patients will withdraw from permanent medical supervision and thereby make the trouble expended upon their treatment of no avail as regards the result.

#### CONCLUSIONS.

1. The two trypanosomes of man, *T. gambiense* and *rhodesiense*, are probably identical.
  2. The various fresh outbreaks of the epidemic in Tanganyika Territory are probably to be traced to immigration of sick natives from the old sleeping-sickness foci on Victoria Nyanza and Lake Tanganyika.
  3. The results of sleeping-sickness therapy will improve in so far as the infected natives can be kept under permanent medical control.
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**II.**

**STUDIES ON THE BIONOMICS OF THE POLYMORPHIC  
TRYPANOSOMES OF MAN AND RUMINANTS**

**BY H. LYNDHURST DUKE, M.D., Sc.D. (CANTAB).**

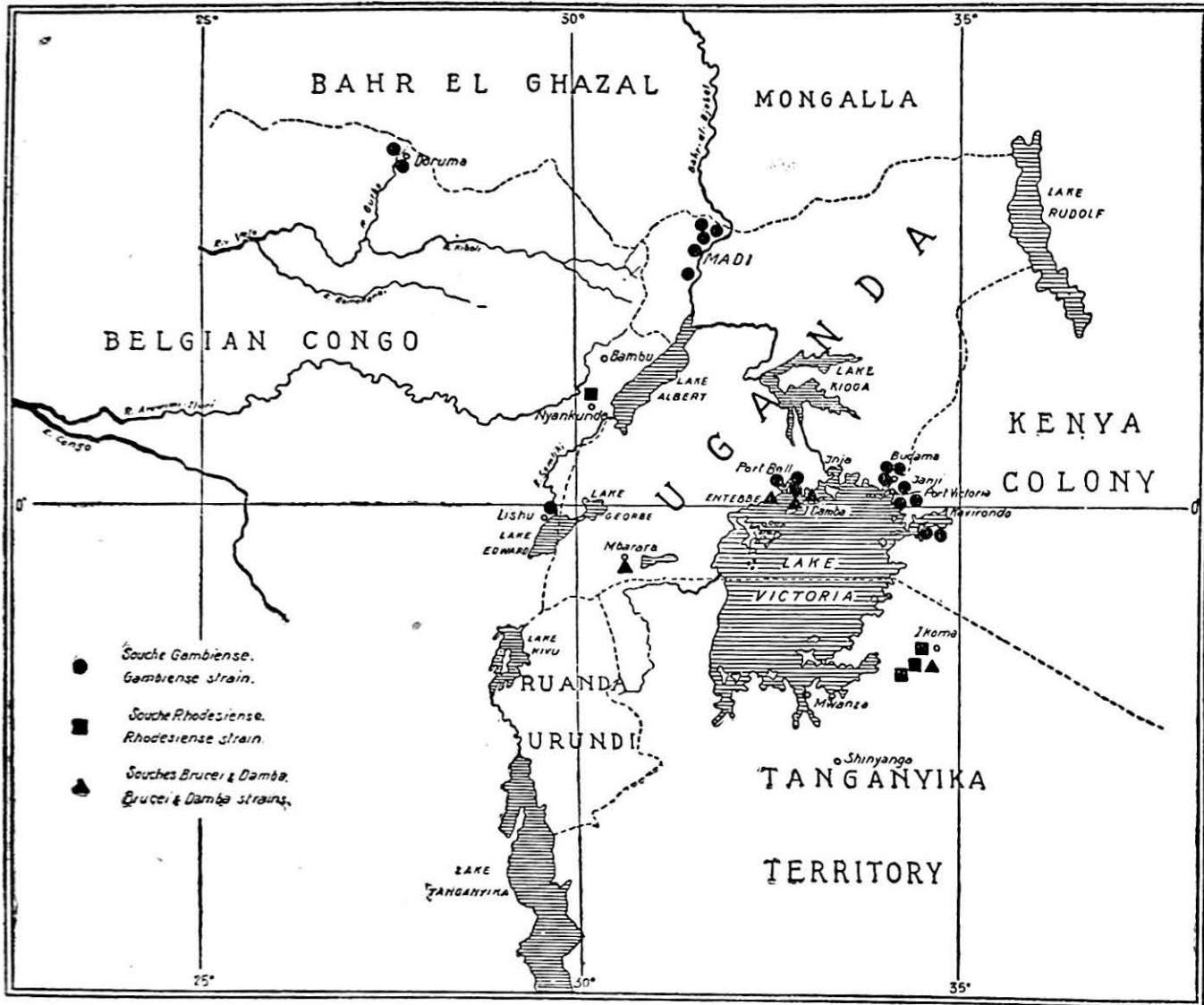
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## INTRODUCTION.

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Two of the items in the original terms of reference of the Commission are : "The Relationship between *Trypanosomiasis gambiensis* and *Trypanosomiasis rhodesiensis*", and "The Rôle of Ruminants in the Spread of Human Trypanosomes". In the group of papers presented below, an attempt is made to throw some light on these two questions.

From the outset it was realised that the inquiry would last a long time ; and the prospects of a definite issue without recourse to experiment with man were not very bright. Within the limited time at our disposal, final conclusions could not be expected. It must therefore be understood that these studies, if not actually premature in their appearance, are incomplete. The conclusions drawn are provisional and are useful mainly in determining the trend of further investigations.

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At the first meeting of the Commission, held at Entebbe in February 1926, it was unanimously decided to commence our labours by a comprehensive study of *T. gambiense*.

The programme was divided into two parts. Professor KLEINE and Dr. VAN HOOF undertook the epidemiological work in the field and the collection of strains for study at the Laboratory. The study of the morphology of the trypanosome was assigned to Dr. LAVIER, and the transmissibility and animal reactions were my special concern.

Dr. PRATES, on his arrival, began investigating the value of the precipitin test in determining the diet of wild tsetse ; he also undertook a series of culture experiments, the examination of the application of the formol-gel test in trypanosomiasis, and rendered me valuable assistance in carrying out my own experiments.

Histological investigations found little or no place in our programme until the arrival of Dr. PERUZZI, in February 1927. Judging by the astonishing progress he has made in the short time at his disposal, it is a matter of infinite regret that he did not join us sooner.

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For many years past the human trypanosomes have been studied in various parts of the world by a number of different workers. On the whole, the results, from the point of view of the sanitarian, are disappointing. One important point that has been clearly demonstrated by these studies is the extreme instability of these trypanosomes once they are removed from their natural environment.

The method commonly adopted in studying these parasites is, of necessity, bad. A strain is isolated in a laboratory animal — generally a rat, guinea-pig or monkey — from a patient who, as often as not, has already relapsed after inadequate treatment. The trypanosome is then maintained at a laboratory by direct passage, often for years, in an environment entirely artificial and abnormal. From time to time the pathogenicity and morphology of the strain are investigated, and the findings are chronicled as characters of the original strain. The results of this system are very confusing. Ancient laboratory strains which, in the course of years in rats or guinea-pigs, have

developed all kinds of strange proclivities are cited as typical of this or that human trypanosome. In reality, such strains bear no resemblance to their wild forbears. For years they have been exposed to none of the influences that determined their distribution in nature. From an academic point of view, it is interesting to follow the career of the prisoner; but, in so doing, we see very little of those attributes of the trypanosome that determine its survival in native communities in Africa.

Of a very different nature are the researches of the various Commissions that have from time to time studied, on the spot, outbreaks of human trypanosomiasis. The Royal Society's Commissions in Uganda and Nyasaland; the French and Belgian in the Congo; the Portuguese in Principe; the German, and the Commissions of the Chartered Company in Southern Africa — these have all done valuable work in determining the habits, prevalence and means of spread of the trypanosomes of man.

The development in *glossinæ* of several species of mammalian trypanosomes has also been worked out, their morphology studied biometrically, and their pathogenicity to laboratory animals carefully examined; immunity reactions have been investigated, and attempts have been made to study the behaviour of the human trypanosomes in ruminants. Most important of all, this work has been done with strains recently isolated from man, and in surroundings approaching very nearly the natural environment of the trypanosome. But in every case there has been an inexorable time-limit set to the activities of these workers. After one, two or three years, just when the experience of many months of patient study is ripening and a valuable and irreplaceable material has accumulated, the curtain drops and the Commission disbands.

Circumstances have changed, even since the days of these Commissions. Their labours have cleared up all but a few broad biological problems. The only way of solving these is by uninterrupted research over a long period of time, in the course of which every gain is consolidated and every indication followed as it appears.

And so it should be with the work of this Commission, and particularly with the studies dealt with below. There are now, at Entebbe, strains that have been under observation for a little more than one year, strains that have been carefully transmitted from ruminant to ruminant by cyclical passage through tsetse. The present is not the moment of choice for publishing a report on these experiments. They will, I hope, continue for years, unless the ever-dreaded accident of an inter-current epidemic intervene. Certain definite indications have already developed, but nothing final.

If the work is interrupted, it will mean starting all over again in the uncertain hope of recovering the lost ground. Meantime, the experiments are still in progress.

\* \* \*

The relation of the trypanosomes to man is in some respects difficult to understand. In many localities, the distribution of human trypanosomiasis suggests that the adaptation of the parasite to its human host is by no means perfect. Often the very survival of the trypanosome seems precarious.

To elucidate these phenomena, it is necessary to study both the epidemiology of the disease and the causative parasites under conditions as near as possible to those obtaining in actual infected areas. It is of prime importance that the strains to be studied be freshly isolated from man, and examined immediately, before they reflect their altered circumstances. In this way Dr. LAVIER has already been able to throw an interesting light on the nature and significance of posterior-nuclear forms, and of other morphological variations. At the same time, the trypanosome must be studied in relation to the normal insect vector. Strains maintained at the Laboratory by

cyclical passage through tsetse may be studied with confidence and a reasonable hope that something will be learnt of what occurs in nature.

Guided by these considerations, we have collected strains from the following districts : the Budama-Busitema area in the Eastern Province of Uganda, and from the adjoining Kenya infected area in Kavirondo ; from near Kampala, in the Buganda Lake area ; from the Madi area, near the Nile, in the Northern Province of Uganda ; from the vicinity of Lake Edward ; and from Doruma, at the western end of the Haut Uelle district of the Belgian Congo near the French and Sudanese border.

In this way we have studied strains of *T. gambiense* from widely distant sources, as may be seen from the map on page 22.

Of the *T. rhodesiense* type of human trypanosome we have received at the Laboratory altogether four strains—one from Dr. CORSON, two from Professor KLEINE from the Mwanza-Ikoma infected area in Tanganyika Territory, and one recovered by Dr. VAN HOOFF and myself during our tour in the Congo. This last strain possesses a peculiar interest. Not only is it the first case of *T. rhodesiense* discovered in the Congo, but the patient's history included no visit to a known *G. morsitans* area.

With one exception — this last case of *T. rhodesiense* — only untreated human cases have been selected. Whenever it was possible, the patient himself was brought to the Laboratory and clean flies infected directly from him. By this means the possible effects of an unnaturally abrupt change to an abnormal host are avoided, and really first-hand information is secured. These patients were housed, fed, studied and treated at the Laboratory, and in due course sent back healthy and happy to their distant homes. Reports subsequently received about these travellers have shown that their visit served as excellent propaganda in encouraging their fellows to apply for treatment.

In addition to human trypanosomes, certain other organisms of the polymorphic group have also been studied in a similar manner. They are described in Paper No 3.

As already stated, it was decided to concentrate first on *T. gambiense*, the human trypanosome of *G. palpalis* regions. We had to begin somewhere, and *T. gambiense* offered greater facilities for study. Our acquaintance with *T. rhodesiense* is less extensive, owing to limitations of time and personnel. Much work remains to be done with this latter organism, of which hitherto only four strains have been investigated at the laboratory.

The rôle of ruminants has been examined during these studies in transmission. In the case of certain strains it was necessary to start the ruminant series by means of direct inoculation of infected blood. Generally, however, it was possible to employ cyclically infected flies ; and in every case subsequent passages from animal to animal were effected by this method.

In setting forth these experiments I have not hesitated to give them in considerable detail. I find that seemingly irrelevant information is often of great importance in assimilating experiments of this kind. Many times one finds data suppressed, for reasons of economy or from the conviction that no one will read them, which are essential to the student who, perhaps years after, wants to make a comparison or check a dawning surmise.

Dates of experiments, details of dissections, information about the origin of the strains employed, evidence affecting diagnosis, etc., are all of interest, and should not only be recorded at the time but also published. In looking up my own work of some fifteen years ago, in connection with the preparation of these papers, I realise how many gaps there are that could easily have been filled from the routine books of those days.

Before proceeding to the actual experiments, I will describe a new method that will be adopted in these papers to indicate the degree of transmissibility, or as Dr. VAN HOOF has styled it "la contagiosité", of a strain of trypanosomes. (VAN HOOF, 1926.)

It is already known that certain strains of trypanosomes are not cyclically transmissible by tsetse. ROUBAUD's strains of *T. gambiense* and *T. brucei* from the Pasteur Institute in Paris are examples of this. (ROUBAUD, 1910.)

KLEINE and REICHENOW also speak of certain *T. gambiense* strains that were, at certain stages of their career, non-transmissible. (KLEINE, 1914; REICHENOW, 1921.) This inability to pass cyclically through tsetse may be complete, or it may extend only to the final and all-essential invasion of the salivary glands; for, to be cyclically transmissible, a strain must be able to invade the salivary glands of the fly. Some strains are very much more readily transmissible than others, and it is important to recognise these differences in comparing different strains.

Now, the percentage of infected flies in any experiment or series of experiments gives no reliable information about the transmissibility of the strain by tsetse. A strain may be entirely non-transmissible and yet give a high percentage of intestinal infections in the fly (cf. Strain VIII). How, then, can we indicate the aptitude of a strain to invade the salivary glands?

At first glance it seems possible to do this by determining the percentage of the flies, dissected during the experiment, that shows salivary-gland infections. But this calculation leaves out of account infected flies that die before a sufficient time has elapsed for the glands to become infected. Twenty-five days may be taken as the time required in the development of an average strain of polymorphic trypanosomes in *G. palpalis* before the glands are invaded; before this date the flagellates are confined to the alimentary tract. In some strains the gland invasion occurs earlier, but it is improbable that it is ever delayed much after the twenty-fifth day.

To correct, or at any rate reduce, this error we must concentrate attention on the infected flies that have lived long enough for gland infection to develop, *i.e.*, those of twenty-five days and upwards, or whatever be the earliest date for the particular strain at which gland invasion occurs. Note the proportion of these mature infected flies that shows salivary-gland infections; this figure we will call the *metacyclic index* (M) of the strain. Then calculate the percentage of the total number of flies of all ages, dissected during the experiment, that contains flagellates, including both gut-only and gut-and-gland infections: we will call this percentage the *infectivity index* (i) of the strain.

Now, if we multiply the infectivity index by the metacyclic index, we obtain the percentage of infected flies which would have developed salivary-gland infections *if all had lived long enough*.

This product we will call the *Transmissibility Index* (T) of the strain.

Thus :  $T = M \times i$ ;

$$\text{otherwise expressed : } T = \frac{s}{p} \times \frac{n \times 100}{N}$$

Where N = total number of flies dissected; *n* = total number of flagellate-containing flies; *p* = number of infected flies of twenty-five days old or over; and *s* = the number of these mature infected flies that shows gland infections.

It will be shown below that this Transmissibility Index is a valuable basis on which to compare different strains. This index can also be calculated for the *T. congolense* group of trypanosomes, whose "anterior station" in the tsetse is the proboscis;

and, according to LLOYD AND JOHNSTON'S work, this should be possible, though more difficult, in the case of the *T. vivax* group. (LLOYD AND JOHNSTON, 1924.)<sup>1</sup>

Before proceeding to the actual experiments, I wish to express my gratitude to my colleague Dr. PRATES for the invaluable assistance he has given me during the progress of these transmission experiments. During my two months' absence on safari with Dr. VAN HOOFF, he carried on this work alone; and ever since his arrival at Entebbe he has always helped in every way in his power in the dissection of flies and the examination of animals. All the clinical examinations of the patients studied at the Laboratory were done by Dr. PRATES. I must also thank Dr. VAN HOOFF for frequent assistance, Dr. LAVIER for advice about the Transmissibility Index, and Dr. PERUZZI for information on the post-mortem appearances of a number of the experimental animals.

#### TECHNIQUE.

The following is the method employed in examining the transmissibility of a trypanosome strain by *G. palpalis*.

Boxes containing about fifty laboratory-bred flies are fed on an animal infected with the strain under investigation.

Each examination of the transmissibility of a strain entails the use of about five boxes of flies. These boxes are so manipulated that at least one box is fed on the animal each day during a period of seven to eight days, this period corresponding roughly to a single revolution of what ROBERTSON calls the endogenous cycle in the mammal's blood. (ROBERTSON, 1912.)

By this arrangement, one, at all events, of the boxes will have a chance of taking up the fly-infecting forms of the parasite, if such exist, of that particular strain.

As will be seen below, it is very improbable that the peculiarities of the individual tsetses play any important part in influencing the percentage of infected flies in any experiment. We may therefore employ the insect much as we should, if it were possible, employ a culture-pipette, *i.e.*, if the appropriate form of trypanosome is taken up during the feed, it will develop. Experience has shown that this method yields reasonably reliable information about the transmissibility of a strain.

For each strain two Tables are prepared, the first showing the details of the transmission experiments, the second giving the Transmissibility Index and the data from which it is calculated, according to the explanation given above.

Most of the headings of the columns of the Tables are self-explanatory; one point does, however, need explaining. In the last column but one of the Transmission Tables is a column headed "Ages of the infected flies dissected during the experiment". The figures given in this column are preceded either by commas or by semicolons. The semicolon indicates that the number following it refers to a fly with an infection of the salivary glands; the gut-only infections are preceded by commas. The

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<sup>1</sup> It will be seen from the above that, when a strain is being examined by this method it is essential that some, at any rate, of the positive flies should have lived until the 25th day of the experiment. When no infected flies are found in any of the test boxes then we are in all probability dealing with a non-transmissible strain. But where the strain produces gut-infections, it is obvious that no opinion on its transmissibility can be reached until some flies have been examined that have lived long enough to develop salivary gland infection. Obviously, the greater the number of such flies in any test the greater the accuracy of the result: when only one is obtained nothing definite can be said. For example on p. 62, the second series of tests on Sheep No. 161 gave only one 25th day positive fly with no gland infection, but this does not necessarily mean that the strain had lost its transmissibility.

respective numbers of gut-only and gut-and-gland infections in the experiment are shown in the two preceding columns. The column headed "Date on which dissection began" indicates that all flies dying before that date were rejected. Flies dying in the early days of an experiment have either not fed at all on the infecting animal or, if they have fed, the trypanosomes they contain may be merely passive survivors still living in the undigested blood of the infecting animal.

In the lists of animals infected with each strain, a single line indicates a passage made by subinoculation of blood by a syringe; a double line means that the infection was brought about by cyclically infected laboratory-bred *G. palpalis*. In this section, m = monkey; p = guinea-pig; s = sheep; g = goat; c = calf; d = dog.

The figures in italic represent the duration in days of the disease in the infected animal; the number that follows—if any—shows the day of the disease in the animal of the preceding passage on which subinoculation was performed; heavy block figures represent the Transmissibility Index.

ENTEBBE, July 16th, 1927.

H. LYNDBURST DUKE.

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1.

STUDIES OF NINETEEN RECENTLY ISOLATED STRAINS  
OF *T. GAMBIENSE* FROM NINETEEN DIFFERENT  
UNTREATED AFRICAN NATIVES IN *G. PALPALIS* AREAS.

Part I.

AN ACCOUNT OF THE ORIGIN, UPKEEP AT THE LABORATORY AND THE ANIMAL REACTIONS OF THESE STRAINS; AND AN EXAMINATION OF THEIR CYCLICAL TRANSMISSIBILITY BY *G. palpalis*, WHEN FIRST ISOLATED FROM MAN.

In the literature available at Entebbe there are two references to transmission experiments with *T. gambiense* in the human host.

BRUCE and his collaborators performed a number of experiments with sleeping-sickness patients, but unfortunately the details of the fly-dissections are too incomplete to warrant any conclusions about the transmissibility of the strains examined. (BRUCE, 1911.)

RODHAIN, in the Lualaba region of the Belgian Congo, carried out some experiments with laboratory-bred *G. morsitans* and *T. gambiense*, which are given in more detail. From the negative experiments there is not much to learn. But the "virus Sindano", with which positive transmissions were obtained, was subjected to three experiments from which we can obtain an idea of the transmissibility of this strain.

STRAIN SINDANO. (RODHAIN, 1912.)

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
Sindano		81	9	12.3	2	9	2.4	2.7

\* \* \*

We will now consider the nineteen strains of *T. gambiense* that form the subject of this paper. Each strain will be taken in turn, and the bare details given of the manipulations to which it was subjected.



Dr. LAVIER has already referred to some of these strains, and the numbering followed here is the same as that used in his paper. (LAVIER, 1927.) All these strains were recovered from untreated natives, and in this Part only the transmission experiments performed immediately after the arrival of the strains at the Laboratory are given.

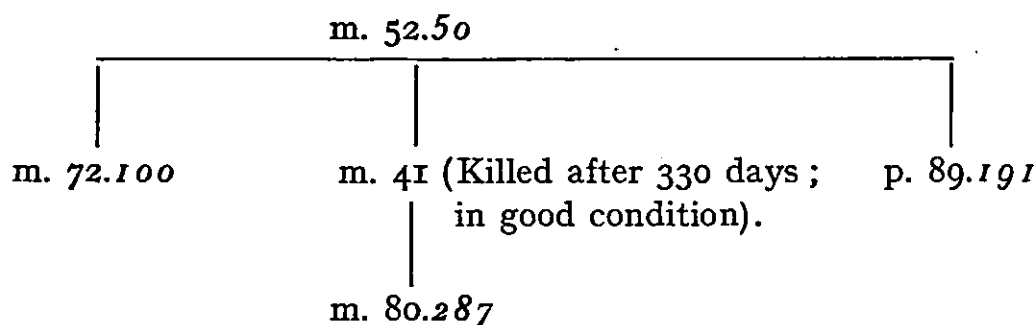
STRAIN No. I. (Case No. 8 of VAN HOOF.)

*Walubani*, ♂ 15 years, from Dadira village near Majanji: glands negative from 17. XII. 25 till 10. I. 26; + 1. III. 26, when the juice was subinoculated into monkey No. 52, which was sent to the Laboratory. This patient's mother supplied strain No. III, possibly the same fly infected both mother and son!

*Clinically* : showed enlarged glands, and was depressed and anæmic.

*Cerebro-spinal fluid*: 10 cells per cubic mm. ; no muriform cells or large mononuclears or trypanosomes; albumin 0.20‰.

*Animal Inoculations* :



A guinea-pig inoculated from monkey No. 41 failed to take. Two monkeys and a guinea-pig inoculated from monkey No. 52 also failed to take.

*Transmission Experiments* :

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
41	m. 52	3	37	28	19	47	52	1	3	0	,13 ,23 ,29	29 & 30. III. 26.
51	"	6	25	16	20	36	56	1	0	1	,56	29 & 30. III. 26.
3	"	7	37	30	11	41	54	1	1	0	,19	3. IV. 26.
4	"	7	20	15	9	24	54	1	0	0		3. IV. 26.
7	"	5	27	19	16	35	49	1	0	1	,30	8. IV. 26.
8	"	10	27	16	17	33	48	1	0	0		8. IV. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 52	Mar. & Apr. '26	216	6	2.7	1	3	0.9	0.9

The feebly transmissible strain from this lad may be compared with the non-transmissible strain No. III, which came from another inmate of the same hut — his mother.

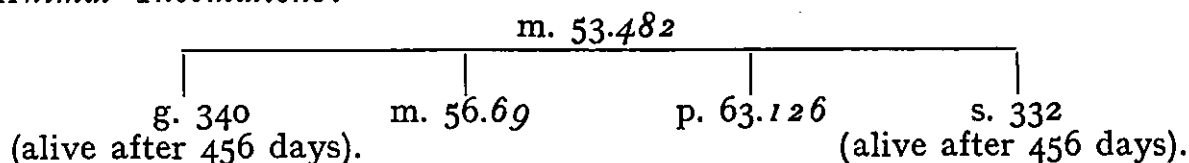
STRAIN No. II. (Case No. 34 of VAN HOOFF.)

*Agaimbo*, age 29-30, of Butenge, sub-village of Lunyo, near Majanji; very advanced case, showing paresis and wasting. First symptoms noticed by relatives a year before. Diagnosed 17. III. 26.

*Cerebro-spinal fluid*: 1,900 cells per cubic mm.; mulberry cells, large mononuclears and trypanosomes all present; Weichbrodt's reaction + : albumin 1.10‰.

Monkey No. 53 was inoculated with 1 cc. cerebro-spinal fluid on 17. III. 27 and sent to Entebbe.

*Animal Inoculations:*



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
1	m. 53	8	23	21	8	29	48	1	5	0	.7, .29, .30, .46, .46°	2. IV. 26.
2	"	5	11	6	11	17	48	1	0	0		2. IV. 26.
5	"	7	24	13	20	33	54	1	0	0		4. IV. 26.
6	"	3	22	22	44	52	52	1	0	0		4. IV. 26.
52	"	7	16	21	11	32	53	1	0	0		19 & 20. IV. 26.
54	"	6	22	13	31	44	53	1	2	0	.10, .46	19 & 20. IV. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 53	Mar. '26	199	7	3.5	0	5	0	0

*Note:* This strain, which proved at first non-transmissible in monkey No. 53, afterwards in the same monkey proved infective to fly. (*Cf.* Part II for the Transmissibility Index.)

STRAIN No. III. (Case No. 9 of VAN HOOFF.)

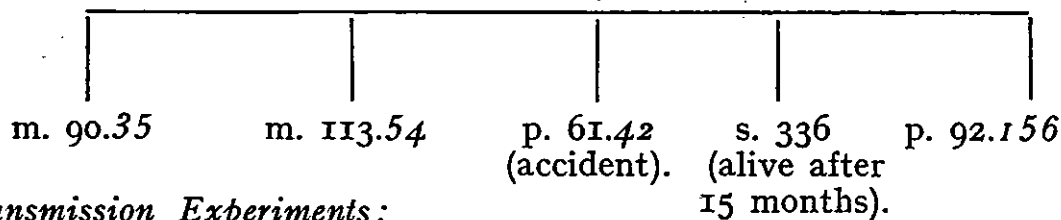
*Taka:* ♀ age 45, mother of case No. 8, which gave Strain No. I; probably infected at the same place as her son.

Glands + I. III. 26, when subinoculated into guinea-pig No. 54.

*Cerebro-spinal fluid:* 2.5 cells per cubic mm.; albumin 0.27‰; no muriform or large mononuclear cells; clinically looks fit, but glands enlarged.

*Animal Inoculations:*

P. 54.173



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
80	m. 90	12	37	21	19	40	50	2	0	0		
84	"	6	28	23	17	40	44	2	0	0		26 & 27. v. 26.
88	"	6	25	21	26	47	40	2	1	0	40	1 & 2. VI. 26. 16. VI. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 90	May & June '26	127	1	0.7	0	1	0	0

This strain has never succeeded in infecting the salivary glands of tsetse since its arrival at the Laboratory.

STRAIN No. IV.

This patient, *Blasio Uma*, was studied and treated at the Laboratory. From Masaba in Samia; most probably infected at Port Bell, near Kampala. ♂ age 25-30; glands enlarged; diagnosed by Dr. CARPENTER in April; gland juice + 22. IV. 26, subinoculated into monkey No. 73.

*Cerebro-spinal fluid:* 52.5 cells per cubic mm. : large mononuclears present; no muriform cells or trypanosomes; Weichbrodt's reaction +, slow; albumin 0.29<sup>0</sup>/<sub>100</sub>.

*Animal Inoculations :*

*Blasio.*

m. 73.153	m. 74.206	m. 96.259	p. 91.173
	g. 339 (died accident, no symptoms trypanosomiasis, after 60 days).		
	g. 342 (alive after 316 days).		
	m. 256 (alive in good condition after 208 days).		

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
57	<i>Blasio</i>	7	20	20	17	37	42		2	1	34, 42, 35	26. IV. 26.
58	"	4	25	15	24	39	46		1	0	46	27. IV. 26.
60	"	2	32	28	12	40	47		0	2	33, 39	28. IV. 26.
61	"	4	23	21	18	39	46		0	0		29. IV. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
<i>Blasio</i>	Mar. '26	155	5	3.8	5	5	1.9	<b>2.2</b>

STRAIN No. V. (Professor KLEINE.)

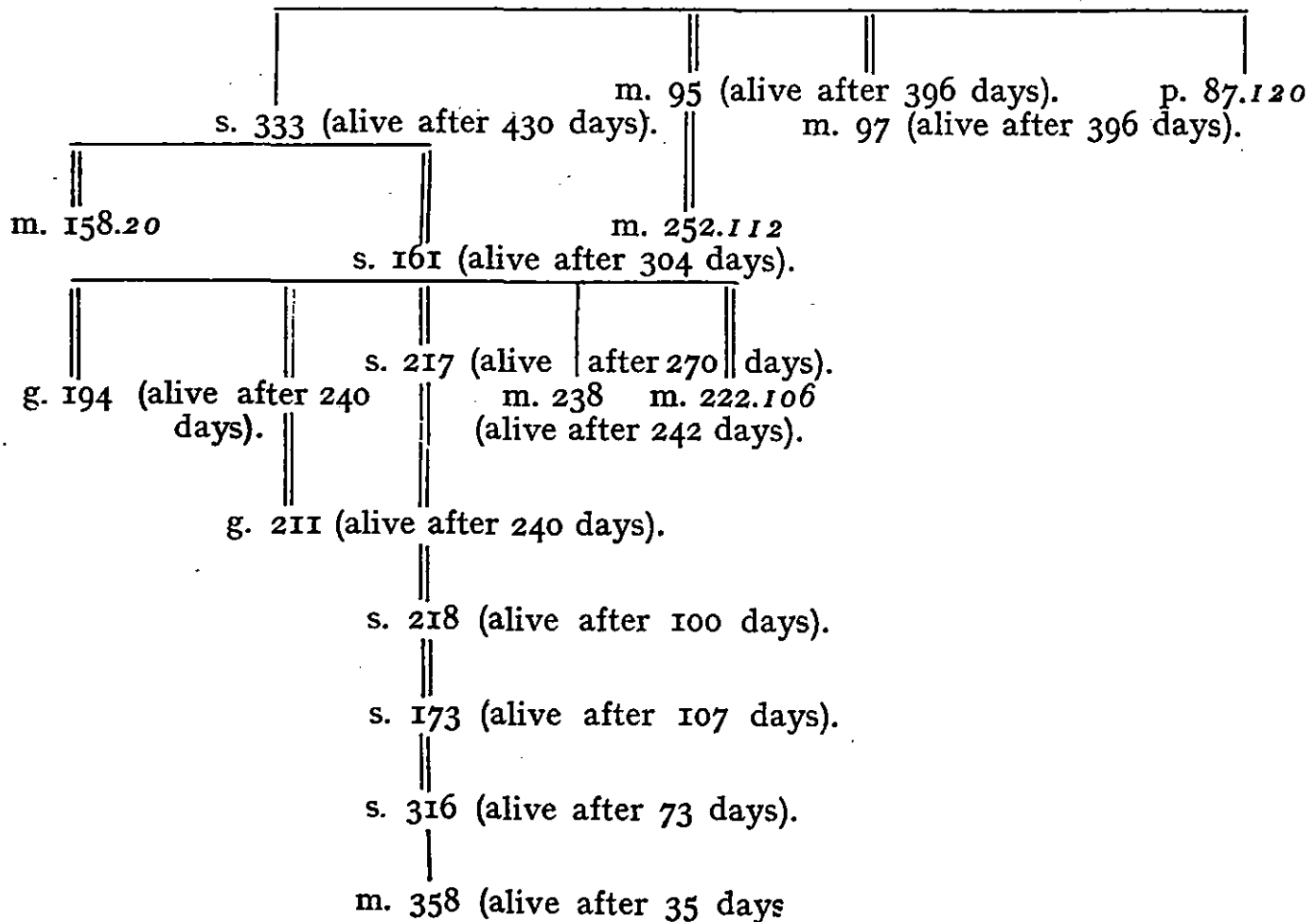
From *Bwana*, ♂ 22 years of age ; of Ogol village, Homa Point, South Kavirondo. Very little, if any, glandular enlargement ; in first stage of disease.

*Cerebro-spinal fluid*: not examined.

Inoculated into monkey No. 64 on 16. III. 26.

*Animal Inoculations:*

m. 64.



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
53	m. 64	17	32	25	12	37	52	1	0	0		20 & 21. IV. 26.
56	"	6	24	21	11	32	48	1	1	2	:8 ;31 ;39	24 & 25. II. 26.
59	"	5	34	17	21	38	47	1	0	3	:24 ;46 ;47	27 & 28. IV. 26.
66	"	4	42	26	21	47	37	1	0	2	:35 ;37	6 & 7. V. 26.

*Transmissibility Index:*

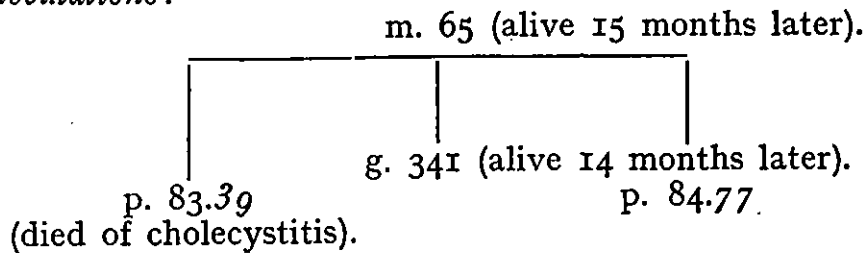
Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 64	Apr. & May '26	154	8	5.0	7	7	4.5	5.0

This strain gave the second highest Transmissibility Index of all the *T. gambiense* strains examined, the highest being strain No. IX. It will be seen in Part II that it was readily transmissible in a number of different animals. It will be noted that the patient, Bwana, was in a very early stage of the disease.

STRAIN No. VI. (Professor KLEINE.)

From ♀, aged 35 years, of Homa Point, South Kavirondo.  
 Large cervical glands on both sides ; second stage of disease.  
*Cerebro-spinal fluid*: not examined.

*Animal Inoculations:*



*Transmission Experiments :*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
76	m. 65	16	29	18	20	38	54	1	2	0	23, 23	20 & 21. v. 26.
78	"	12	24	23	13	36	50	1	2	0	31, 47	23 & 24. v. 26.
82	"	13	26	15	20	35	46	1	0	0		30 & 31. v. 26.
85	"	6	34	12	29	41	42	1	0	0		11 & 13. v. 26.

*Transmissibility Index :*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 65	May '26	150	4	2.6	3	4	0	0

Another non-transmissible strain.

STRAIN No. VII.

*Tekisoka*, ♀ 30 years of age ; diagnosed by Dr. GRIFFIN at Port Bell, near Kampala. Suffered from headaches and febrile attacks ; somewhat ill-nourished, but going about her daily occupations without difficulty. Cervical and axillary glands enlarged. Gland-puncture and centrifugation of blood + on 25. VI. 26.

*Cerebro-spinal fluid*: 3. VII. 26 : cells 104 per cubic mm., no other abnormalities ; albumin 0.30‰. She then ran away from the Entebbe Hospital, and on her return after two months the cerebro-spinal fluid was re-examined on 13. IX. 26 and showed cells 208 per cubic mm. ; muriform cells and large mononuclears +, no trypanosomes ; albumin 0.3 ‰.

*Animal Inoculations*: Subinoculations of this patient's gland-juice into a monkey failed to infect ; an inoculation of an infected gland of a fly of Exp. 95 (*cf.* hereafter) into a guinea-pig also failed to take.

Patient

s. 335.51  
(cause of death doubtful).

g. 337.221

m. 112.268

m. 117.64

g. 330  
(alive after 228 days).

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
95	<i>Tekisoka</i>	5	40	22	23	45	57		2	1	,36 ;57 ;57	24. vi. 26.
96	"	4	34	23	22	45	55		0	1	,44	25. vi. 26.
99	"	4	26	25	20	45	55		2	1	,13 ;40 ;55	28. vi. 26.
101	"	6	35	11	34	45	53		3	3	,9 ;31 ;41 ;41 ;51 ;52	29. vi. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
<i>Tekisoka</i>	June '26	180	13	7.2	6	11	3.3	<b>3.9</b>

This strain, in the patient, showed a fairly high degree of transmissibility.

STRAIN No. VIII.

*Zakayo*, ♂ 25 years of age ; diagnosed by Dr. GRIFFIN at Port Bell, near Kampala ; in good health save for occasional headaches ; not wasted ; glands enlarged. On 27. vi. 26, gland juice and centrifuged blood +.

*Cerebro-spinal fluid*: cells 11.5 per cubic mm. ; no muriform or large mononuclear cells or trypanosomes ; albumin 0.15 %.



*Animal Inoculations:*

Zakayo

m. 120.144

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
97	Zakayo	17	40	25	21	46	52		0	0		27. vi. 26.
98	"	7	41	19	37	56	52		0	0		28. vi. 26.
100	"	3	26	15	21	36	52		0	0		29. vi. 26.
102	"	3	31	19	26	45	50		3	0	,31 ,50 ,50	30. vi. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
Zakayo	June '26	183	3	1.6	0	3	0	0

STRAIN No. IX.

This patient was studied at the Laboratory and treated in Entebbe Hospital by kind permission of Dr. S. FORREST.

*Mangasi*, ♂ 25 years old ; very ill indeed ; fever, headache, mental disturbances ; asthenic ; gland juice and blood + on 23. vii. 26.

*Cerebro-spinal fluid*: on 24. vii. 26, 560 cells per cubic mm., muriform cells, large mononuclears and trypanosomes present ; albumin 1.25 ‰.

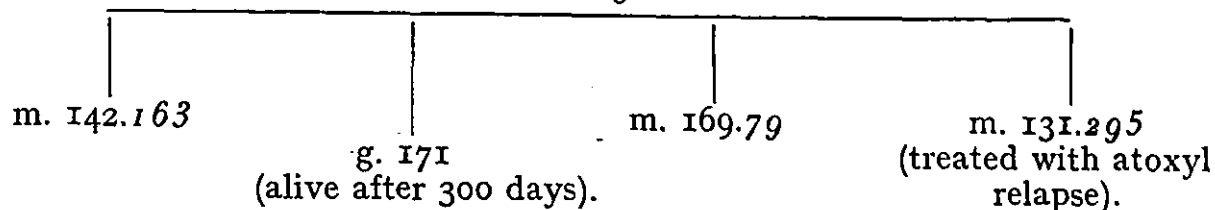
On 15. x. 26, after a course of treatment with 24 grammes of tryparsamide, the cerebro-spinal fluid was re-examined, with the following results : cells 15 per cubic mm. ; muriform cells, large mononuclears and trypanosomes absent ; albumin 0.2 ‰.

Monkey No. 142 was inoculated with the gland juice on 23. VII. 26 and on the following day with the cerebro-spinal fluid.

A guinea-pig inoculated with the positive cerebro-spinal fluid of the patient, and subsequently with the infected salivary glands of four positive flies of the transmission experiments, never became infected.

*Animal Inoculations :*

*Mangasi*



*Transmission Experiments :*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
141	<i>Mangasi</i>	3	32	28	21	49	49	0	5	;39 ;42 ;49 ;49	23. VII. 26.	
142	"	3	41	28	33	61	48	2	3	;11 ;48 ;34 ;41 ;48	24. VII. 26.	
143	"	4	33	27	21	48	47	3	3	;14 ;27 ;27 ;36 ;40 ;47	25. VII. 26.	

*Transmissibility Index :*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility n
					With glands infected.	Total.		
<i>Mangasi</i>	July '26	158	16	10.1	11	14	6.9	<b>7.9</b>

This strain gave the highest transmissibility of all these strains of *T. gambiense*. Its subsequent behaviour, shown in Part II, is all the more interesting.

STRAIN No. X. (Case No. 141 of VAN HOOFF.)

*Mulongo*, ♂ 30 years old; diagnosed 17. VII. 26; gland puncture and centrifugation of blood +; thick film examined 20 minutes, nil; mentally depressed, very thin, febrile, but no mental symptoms.

*Cerebro-spinal fluid*: 17. VII. 26: 2,320 cells per cubic mm.; large mononuclears and muriform cells +; no trypanosomes seen.

Gland juice and blood inoculated into monkey No. 149 on 18. VII. 26.

A white rat inoculated intraperitoneally from monkey No. 149 never became infected.

*Animal Inoculations:*

m. 149.102

g. 338 (alive after 280 days). (treated with atoxyl: relapse).

m. 187.296

p. 154.200

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			It gut only.	In gut and salivary glands.		
163	m. 149	8	43	18	40	58	45	1	0	1	45	11 & 13. VIII. 26.
169	"	6	35	23	26	49	40	1	2	0	29, 40	16 & 18. VIII. 26.
176	"	8	34	25	26	51	38	1	1	0	26	19 & 21. VIII. 26.

*Transmissibility Index:*

Animal.	Date	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 149	Aug. '26	158	4	2.5	1	4	0.6	0.6

A virulent strain (*cf.* VAN HOOFF, Interim Report), with a low Transmissibility Index.

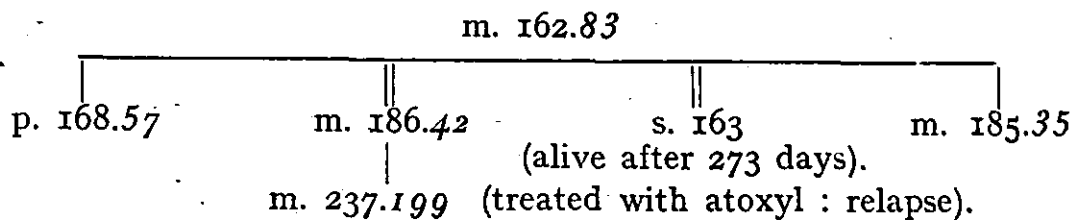
STRAIN No. XI. (Case No. 155 of VAN HOOFF.)

*Awunu*, ♂ 10 years old, son of case No. 149 (VAN HOOFF) from Sissenie, near Port Victoria, in the Samia county of Kavirondo. Clinical condition good, save that he was thin; no clinical signs of the disease. Diagnosed 29. VII. 26: gland juice +, thick film and centrifuged blood +.

*Cerebro-spinal fluid*: 15 cells per cubic mm.; grand mononuclears +; no muriform cells or trypanosomes.

Monkey No. 162 was inoculated intraperitoneally with the gland juice of the patient on 29. VII. 26.

*Animal Inoculations:*



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
170	m. 162	6	39	30	29	59	43	1	2	1	,24 ,28 ,34	16 & 18. VIII. 26.
171	"	8	47	29	24	53	37	1	3	1	,18 ,28 ,37 ,37	17 & 19. VIII. 26.
173	"	15	41	23	26	49	40	1	2	1	,26 ,26 ,41	19 & 21. VIII. 26.
180	"	7	33	31	26	57	35	1	4	2	,15 ,16 ,16 ,35 ,18 ,34	20 & 22. VIII. 26

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 162	Aug '26	218	16	7.3	5	11	2.2	3.3

STRAIN No. XII.

This and the following three cases were studied and treated at the Laboratory. *Acholi*, ♂ aged 20, native of Madi country near the Nile, and near the Sudan frontier. This and cases Nos. XIII, XIV, and XV were sent to Entebbe by Dr. Louw, who was engaged in investigating an epidemic of trypanosomiasis in this area.

Thin; suffered from headaches; glands enlarged; intelligence good.

i. i. 27: gland juice, centrifuged blood and thick film +.

*Cerebro-spinal fluid*: cells 1,030 per cubic mm.: muriform cells and large mononuclears and trypanosomes +; albumin 0.80‰.

Re-examined on 13.ii. 27, after receiving 24 grammes of tryparsamide, the cerebro-spinal fluid gave 22.4 cells per cubic mm.; no trypanosomes, muriform cells or large mononuclears; albumin 0.40‰.

Animal Inoculations:

*Acholi*

m. 258.98

Another monkey, No. 273, was fed on by a fly showing a very early gland-infection. The animal never became infected.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates		Age of the infected ies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
321	<i>Acholi</i>	3	22	30	28	58	30		2	1	,17 ,20 ;23	27. XII. 26.
325	"	7	33	35	18	53	29		0	0		28 & 29. XII 26.
329	"	5	30	16	30	46	28		0	0		30 & 31. XII. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
<i>Acholi</i>	Dec. '26	157	3	1.9	1	1	0.6	1.9

STRAIN No. XIII.

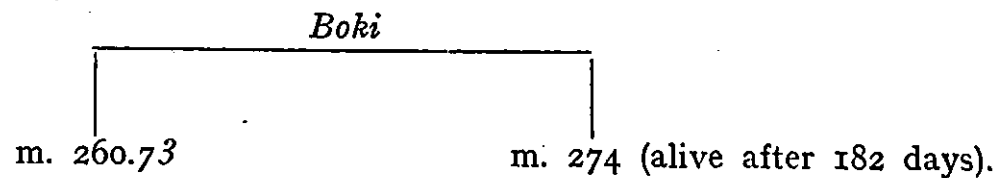
*Boki*, ♂ 30 years of age ; also from Madi area ; a little thin, otherwise apparently in fairly good health ; large glands.

I. I. 27 : gland juice and blood (thick film and centrifuged) + .

*Cerebro-spinal fluid*: cells 41 per cubic mm. ; rare muriform cells and large mononuclears, no trypanosomes ; albumin 0.25‰.

Re-examined on 13. II. 27, after receiving 24 grammes of tryparsamide, the cerebro-spinal fluid contained 7.7 cells per cubic mm. ; no trypanosomes, large mononuclears or mulberry cells ; albumin 0.20‰.

*Animal Inoculations:*



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
322	<i>Boki</i>	2	33	22	27	49	29		0	0		27. XII. 26.
326	"	16	36	23	24	47	29		0	0		28 & 29. XII. 26.
331	"	5	25	30	13	43	28		0	3	;21 ;28 ;28	30 & 31. XII. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
<i>Boki</i>	Dec. '26	139	3	2.1	3	3	1.1	2.1

STRAIN No. XIV.

*Falajala*, ♂ 30 years old ; also a Madi case ; apparently in good health ; glands a little enlarged.

Gland juice + ; blood + (thick film and centrifuged).

*Cerebro-spinal fluid*: I. I. 27 : cells 15 per cubic mm. ; no muriforms, large mononuclears or trypanosomes ; albumin 0.2<sup>o</sup>/<sub>100</sub>.

Re-examined later on 13. II. 27, after 24 grammes of tryparsamide : cells 5 per cubic mm. ; no other abnormalities ; albumin 0.2<sup>o</sup>/<sub>100</sub>.

*Animal Inoculations:*

*Falajala*

m. 275 (alive after 182 days).

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
323	<i>Falajala</i>	7	27	21	22	43	29		1	2	;29 ;29 ;29	27. XII. 26.
328	"	7	37	23	23	46	30		1	0	;29	28 & 29. XII. 26.
332	"	5	29	20	26	46	28		1	2	;22 ;28 ;28	31. XII. 26 & 1. I. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
<i>Falajala</i>	Dec. '26 Jan. '27	135	7	5.1	4	6	2.9	3.4

STRAIN No. XV.

*Awasi*, ♂ 25 years of age ; also a Madi case ; thin, rather weak at times ; has headache and fever ; active ; intelligence good ; gland juice + ; blood + (thick film and centrifuged).

*Cerebro-spinal fluid*: I. I. 27 : cells 106 per cubic mm. ; muriform cells and large mononuclears + ; no trypanosomes ; albumin 0.5‰.

Re-examined on 13. II. 27, after 24 grammes of tryparsamide, showed 10 cells per cubic mm. ; no other abnormalities ; albumin 0.2‰.

*Animal Inoculations :*

*Awasi*

m. 276 (killed after 178 days ;  
trypanosomes in pericardium).

*Transmission Experiments :*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
324	<i>Awasi</i>	2	45	20	33	53	29		0	3	:29 ;:29 ;:29	27. XII. 26.
327	"	7	26	34	11	45	29		2	1	:17 ;:23 ;:28	28 & 29. XII. 26.
330	"	3	31	28	23	51	28		0	1	:24	30 & 31. XII. 26.

*Transmissibility Index :*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
<i>Awasi</i>	Dec. '26	149	7	4.6	5	5	3.3	4.6



STRAIN No. XVI.

Sent by VAN HOOFF from Lake Edward region. Studied and treated at the Laboratory.

*Kakowa*, ♂ aged 25; in good condition; glands enlarged; gland juice + ; blood centrifuged +.

*Cerebro-spinal fluid*: I. II. 27: 13 cells per cubic mm.; no other abnormalities; albumin 0.2 ‰.

Re-examined on 13. II. 27, after 30 grammes of tryparsamide, showed 3 cells per cubic mm.; albumin 0.12 ‰.

*Animal Inoculations*: nil.

*Transmission Experiments*:

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males	Females.	Total.			It gut only.	In gut and salivary glands.		
400	<i>Kakowa</i>	4	30	33	26	59	36	0	0		10. II. 27.	
401	"	4	29	15	19	34	35	2	0	,30 ,35	11 & 13. II. 27.	
403	"	6	22	26	20	46	34	2	1	,15 ,18 ;34	12 & 14. II. 27.	
406	"	4	31	27	21	48	35	0	1	,24	15 & 17. II. 27.	

*Transmissibility Index*:

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
<i>Kakowa</i>	Feb. '27	187	6	3.2	2	4	1.0	1.6

STRAIN No. XVII.

Brought back by VAN HOOFF and myself from Doruma, western end of Upper-Uelle district, Belgian Congo ; studied and treated at the Laboratory.

*Duzungu Pio*, ♂ aged 8, Azande tribe ; diagnosed 10. IV 27 ; glands very slightly enlarged, juice + ; some fever, inclined to somnolence.

*Cerebro-spinal fluid*: 9. v. 27 : 415 cells per cubic mm. ; large mononuclears and muriform cells + ; no trypanosomes.

*Animal Inoculations*: nil.

*Transmission Experiments*:

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			It gut only.	In gut and salivary glands.		
478	<i>Duzungu Pio</i>	7	37	30	18	48	32		0	0		6 & 9. v. 27.
480	"	4	28	20	23	43	32		0	0		8 & 10. v. 27.
481	"	5	31	30	14	44	31		0	0		11 & 13. v. 27.
484	"	6	30	25	17	42	31		1	0	15th day : only,	15 & 16. v. 27.

*Transmissibility Index*:

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
<i>Duzungu Pio</i>	May '27	177	1	0.5	0	0	0	0

A non-transmissible strain that has all but lost all power of development in *G. palpalis*.

STRAIN No. XVIII.

*Mayani*, ♂ aged 20-25 ; Azande tribe ; also from Doruma, Upper-Uelle ; diagnosed 4. IV. 27 ; glands enlarged and juice + ; no clinical signs of disease save typically enlarged glands.

*Cerebro-spinal fluid*: 6 cells per cubic mm. ; no other abnormality.

*Animal Inoculations*: nil.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
495	Mayani	12	31	25	17	42	31		0	0		24 & 26. v. 27.
496	"	4	35	13	31	44	33		0	0		25 & 27. v. 27.
503	"	13	38	29	21	50	32		0	0		28 & 30. v. 27.
505	"	12	45	18	28	46	32		0	0		29 & 31. v. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index
					With glands infected.	Total.		
Mayani	May '27	182	0	0	0	0	0	0

It is instructive to note that both these strains from the Upper-Uelle district of the Belgian Congo, where human trypanosomiasis occurs very sporadically, are non-transmissible by *G. palpalis*.

STRAIN No. XIX.

*Labilo*, 13 years ; of Nyajowok village, Alego. Diagnosed 12. IX. 26 by VAN HOOF ; gland juice + .

*Cerebro-spinal fluid*: 12. IX. 26 : 1,610 cells per cubic mm. ; large mononuclears and muriform cells + ; no trypanosomes.

*Animal Inoculations*: nil.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
211	<i>Labilo</i>	8	33	8	34	42	36		0	0		28. IX. 26.
219	"	22	40	23	23	46	36		0	0		29. IX. 26.
222	"	14	43	25	22	47	36		0	0		1. X. 26.
223	"	8	27	25	15	40	36		0	0		2. X. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
<i>Labilo</i>	Sept. & Oct. '26	175	0	0	0	0	0	0

This strain was completely non-transmissible.

SUMMARY.

It is clear from these experiments that, on their first isolation from man, or actually in man himself, different strains of *T. gambiense* show great differences in cyclical transmissibility by *G. palpalis*.

Of the nineteen strains examined above, twelve were studied in the human patient. Of these twelve strains, four were non-transmissible cyclically by laboratory-bred *G. palpalis*, i.e. 33.3 per cent.

The transmissibility of a strain seems to bear no constant relation to the virulence of that strain in man. Strains Nos. VII, IX and X all appeared to be virulent in their effects on the patient; their Transmissibility Indices were respectively **3.9**, **7.9** and **0.6**.

As a rule, it is very difficult to determine the duration of an infection in a native. Consequently, it is very difficult to determine to what extent prolonged sojourn in

man affects the transmissibility of a trypanosome. The condition of the cerebro-spinal fluid indicates the stage of the disease, but it does not tell us much about its duration ; a virulent strain may produce big changes in a comparatively short time. The cell-counts of the cerebro-spinal fluid from five of the six patients who supplied the non-transmissible strains were as follows :

Strain.	Per cubic mm.
III . . . . .	2.5
VIII . . . . .	11.5
XVII . . . . .	415
XVIII . . . . .	6
XIX . . . . .	1,610

and these figures teach us nothing definite. Taking now the cases showing the greatest alteration of the cerebro-spinal fluid :

Strain.	Cells per cubic mm.	Index.
XV. . . . .	106	4.6
VII. . . . .	208	3.9
XVII. . . . .	415	0.0
IX . . . . .	560	7.9
XII. . . . .	1,030	1.9
XIX . . . . .	1,610	0.0
II. . . . .	1,900	1.2 ( <i>cf.</i> Part II)
X. . . . .	2,320	0.6

and again there is little to learn.

Possibly there is a slight indication that a low degree of transmissibility is associated with a much-changed cerebro-spinal fluid, and this with an old-standing infection ; but this is little more than speculation.

More justifiable would seem the inference that, at any stage of an infection of man by *T. gambiense*, the transmissibility of the trypanosome by *G. palpalis* may be lost.

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## Part II.

### THE BEHAVIOUR OF SOME OF THESE STRAINS IN RUMINANTS, AND THE CHANGES THEY UNDERGO DURING A SOJOURN IN ANIMALS OTHER THAN MAN.

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We will now follow up the history of some of the strains of *T. gambiense* described in Part I. Not all of these nineteen strains were introduced into ruminants, and, of those that were, some have been much more carefully studied than others.

The sheep and goats used in these experiments were bought in a fly-free area, and were all carefully examined at the Laboratory before being used. The method included a daily examination of stained thick films of the blood, as recommended by the German workers in Tanganyika (KLEINE, 1911), and by the inoculation of 8 to 10 c.c. of defibrinated blood into clean monkeys. In addition, some of the animals were examined by feeding clean flies on them and dissecting the flies fifteen days later.

In no sheep or goat were any trypanosomes ever found save after exposure to a known source of infection, *i.e.*, subinoculation of infected blood or exposure to infected laboratory-bred flies.

As a general rule, it was fairly easy to demonstrate trypanosomes in the sheep and goats once they had become infected. The thick-film method gives excellent results with these animals. On the other hand, centrifugation of the blood, so useful in the diagnosis in human trypanosomiasis, is much less reliable in the case of ruminants. For one thing, goat's blood is relatively difficult to centrifugalise, and the physical qualities of the blood in all these animals are such that the separation of trypanosomes does not seem to take place in the same manner or with the same facility as in the case of man.

We abandoned this latter method, therefore, and relied on subinoculations, the fly-test and examination of thick films for the demonstration of trypanosomes in sheep, goats or calves. So far, I have made three subinoculations into monkeys from sheep or goats known to be infected, and in each case the monkey has become infected.

There appears to be little difference in the susceptibility of sheep and goats to *T. gambiense*. Probably the goat is a little more resistant to trypanosomes, and this may explain, partially at all events, the reason why in tsetse regions goats are more often seen than sheep. In carrying out experimental feedings with laboratory-bred flies, it is generally easier to make the flies feed well on sheep than on goats. Otherwise both animals serve equally well for trypanosome experiments.

In the experiments given below, there are several instances of these animals being bitten by flies infective with *T. gambiense* and yet not becoming infected. On two occasions sheep failed to become infected and twice the same thing happened with goats bitten by infective flies. In the case of one goat, one of the flies that fed upon it had bitten a clean monkey before, and another after feeding on the goat. Both the monkeys became infected and the goat did not — an experience similar to that recorded by KLEINE (KLEINE, 1911). Sheep Nos. 326 and 327 both resisted several attempts to infect them with the metacyclical forms of *T. gambiense*. Both these animals were subsequently infected with *T. rhodesiense* by cyclically infected flies, and both died of the disease in a few weeks. They were both fine adult rams, and of very powerful build.

These examples of resistance to cyclically carried trypanosomes tend to show that certain individual goats and sheep may be extremely resistant to *T. gambiense*, a conclusion that agrees with KLEINE'S experience. We shall see, also, that calves are exceedingly resistant to this trypanosome, though dying rapidly when infected with certain strains of *T. rhodesiense*.

The possibility of domestic ruminants acting as reservoirs for *T. gambiense* has been recognised for a long time. The principal investigator in this field is undoubtedly Professor KLEINE. His experiments with sheep and goats, carried out in the Tanganyika region, bear directly on my own work and will now be briefly considered. (KLEINE, 1911 and 1913.)

KLEINE worked with strains of *T. gambiense* and laboratory-bred *G. palpalis*. Unfortunately, he says nothing about the condition of the salivary glands in the flies he dissected, speaking only of examinations of the intestine. Also, there are no details from which to correlate the various experiments with the different strains of trypanosomes employed.

In studying this work, we must therefore presume that, in experiments where no transmission occurred, none of the flies had infected salivary glands; in positive experiments we cannot tell how many flies had infected glands.

KLEINE groups his experiments in four tables. (KLEINE, 1911.)

Table I consists of 17 experiments, in which 484 flies lived 18 days or more after the infecting feeding ; after several feeds on the infecting animal, the flies were fed for 30 days on a healthy sheep or goat. No animals became infected, and 13 flies were found to contain flagellates. In case this failure to transmit the trypanosomes were due to the insusceptibility of the sheep and goats, in the experiments of the other tables the flies were tested on one or more clean monkeys as well as on the ruminants.

Table II shows that, in 39 experiments, 966 flies lived at least 18 days after the infecting feeds ; that no transmission occurred ; and that only one fly was found to contain trypanosomes, *i.e.*, presumably a gut-only infection.

Table III shows 9 positive transmission experiments, in which only the monkey and not the goat or sheep was infected. There are here 233 18-day flies, of which 16 contained flagellates. Of these 16 positive flies, at least 9 must have shown gland-infections.

Table IV gives 4 positive transmission experiments, in which both a monkey and either a sheep or a goat became infected. There were 89 18-day flies, of which 8 were infected, with at least 4 gland infections.

Now, I am sure, from recent experience at Entebbe, that some of KLEINE'S strains must have been non-transmissible strains of *T. gambiense*. As already stated, it is not clear how the different fly-experiments were apportioned among the different strains used for the investigation. One must presume also that the division into the four tables is arbitrary and has no relation to the particular strains used to infect the flies.

We cannot, therefore, make any direct comparison with the experiments set forth in the present paper. One interesting point is, however, quite definite, namely, that KLEINE'S monkeys were, in certain circumstances, more susceptible to *T. gambiense* than were his sheep and goats ; in other words, flies that could infect a monkey failed to infect a sheep or a goat. Whether this phenomenon depended on the strain or on the mammal it is impossible to determine. The fact is of interest and suggestive. As mentioned above, one instance of a similar kind occurred in my experiments. KLEINE discusses the susceptibility of sheep and goats to *T. gambiense*. He found that, though the majority of the animals were susceptible, infection died out after a few months. The longest duration of infectivity to tsetse in any of his animals was 100 days.

In sharp contrast with KLEINE'S results are the experiments performed with the reedbuck and bushbuck at Mpumu. These animals remained infective to tsetse for a much longer time ; two of them still carried transmissible trypanosomes respectively fifteen and twenty-two months after their original infection. (Duke, 1912 *b*.) From the records of these experiments it is possible to calculate the Transmissibility Index of one of these strains, recovered by subinoculation into a monkey some twenty-two months after the original infection of the buck.

*Transmissibility Index :*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 199	Sept. '11.	188	15	7.9	4	6	2.6	5.2
"	Dec. '12	318	19	5.5	8	9	2.5	4.8
"	Jan. '12							

We shall refer to these antelope again. (Cf pp. 71-80).

\* \* \*

Having now briefly reviewed the work that has already been done on the behaviour of *T. gambiense* in ruminants, we will proceed with the later history of some of the strains already dealt with in Part I.

The transmission experiments and the Index Tables relating to these later passages will be presented in the same way, and, to bring out more clearly the changes undergone by the trypanosome, the Transmissibility Index Tables of Part I will be reproduced for each strain. All the passages depicted in these Tables were made by cyclically infected laboratory-bred *G. palpalis*, except where the contrary is expressly stated.

STRAIN NO. I.

*Transmission Experiments :*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
107	m. 80	7	31	28	15	43	49	3	0	0		2 & 4. VII. 26.
110	"	7	39	22	23	45	49	3	1	0	34	5 & 7. VII. 26.



*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 52	Mar. '26	216	6	2.7	1	3	0.9	0.9
m. 80	Apr. '26 July '26	88	1	1.1	0	1	0	0

Only two experiments were performed.

STRAIN NO. II.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
238	m. 53	6	24	28	11	39	36	1	7	0	,13 ,24 ,24 ,30 ,35 ,36 ,36	6 & 8. x. 26.
243	"	8	34	24	22	46	36	1	3	1	,15 ,17 ,26 ,36	7 & 9. x. 26.
250	"	10	21	29	8	37	40	1	4	0	,27 ,32 ,32 ,34	10 & 12. x. 26.
514	"	9	42	29	24	53	33	1	2	0	,25 ,33	2, 3 & 5. vi. 27.
523	"	10	28	21	21	42	34	1	1	0	,34	6, 8 & 9. vi. 27.
526	"	12	30	21	22	43	34	1	0	0		7 & 9. vi. 27.
531	"	7	40	37	23	60	33	1	0	0		11, 13 & 14. vi. 27.
62	s. 332	7	23	20	11	31	45	2	3	0	,22 ,32 ,32	1 & 2. v. 26.
81	"	5	27	22	17	39	50	2	0	0		27, 29 & 30. v. 26.
113	"	5	34	28	18	46	56	2	2	0	,53 ,56	5 & 6. vii. 26.
121	"	8	45	36	13	49	53	2	1	0	,49	9, 10 & 12. vii. 26.
136	"	6	32	14	31	45	48	2	0	0		20 & 22. vii. 27.
159	"	6	42	32	20	52	43	2	0	0		5 & 7. viii. 26.
462	"	13	28	25	15	40	33	2	2	0	,33 ,33	17, 19, & 20. iv. 27.
463	"	9	35	24	23	47	33	2	2	0	,25 ,33	18, 20, & 21. iv. 27.
468	"	13	42	17	27	44	34	2	0	0		22, 24 & 25. iv. 27.
472	"	9	30	14	27	41	32	2	0	0		26, 28 & 29. iv. 27.
473	"	7	40	26	25	51	32	2	0	0		27, 29 & 30. iv. 27.
123	g. 340	6	26	30	13	43	44	2	6	1	,28 ,42 ,44 ,44 ,44 ,44 ,42	10 & 11. vii. 26.
131	"	8	32	16	23	39	47	2	1	0	,47	16 & 18. vii. 26.
135	"	5	39	18	27	45	49	2	3	0	,31 ,49 ,49	19 & 21. vii. 26.
153	"	6	42	32	19	51	44	2	2	0	,16 ,40	31. vii. & 1. viii. 26.
464	"	9	33	26	19	45	32	2	0	0		19, 21 & 22. iv. 27.
470	"	8	27	20	22	42	33	2	0	0		24, 26 & 27. iv. 27.
471	"	15	37	23	23	46	33	2	0	0		25, 27 & 28. iv. 27.
475	"	6	34	29	17	46	31	2	0	0		29. iv. & 1. v. 27.
476	"	5	29	23	23	46	31	2	0	0		30. iv. & 2. 3. v. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 53.	Apr. '26	199	7	3.5	0	5	0	0
"	Oct. '26	122	15	12.2	1	10	0.8	1.2
"	June '27	198	3	1.5	0	0	0	0
s. 332	May '26	70	3	4.2	0	2	0	0
"	July & Aug. '26	192	3	1.5	0	3	0	0
"	Mar. '27							
g. 340	July & Aug. '26	178	13	7.7	1	12	0.5	0.6
"	Apr. & May '27	225	0	0	0	0	0	0

*Note.* — Sheep No. 332 and goat No. 340 were infected from monkey No. 53 by subinoculation.

The interesting point about this strain is that it is the only one of which the first examination gave a zero Index, whereas subsequent experiments with the same strain showed it to be transmissible. The explanation of this is to be found in the manner in which the feeds of these early negative experiments were arranged. Four out of five of these boxes of flies were used to investigate the effects of Bayer 205, and were fed in pairs each only once on the monkey — as subsequent events proved, on days when the transmissible forms of the trypanosome were absent from the blood. Haphazard feedings such as this may, of course, succeed; but, if the experiments are negative, they have little or no value in the assessment of the transmissibility of a strain.

Note the loss of transmissibility after a nine months' sojourn in goat No. 340. Flies fed on sheep No. 332 never developed salivary-gland infections, though gut-infections still occurred some thirteen months after the original infection of this animal. In monkey No. 53, fifteen months after its infection from man, the trypanosome, apparently, had become non-transmissible, and its infectivity for *G. palpalis* had greatly diminished.

STRAIN No. III.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			It gut only.	In gut and salivary glands.		
120	m. 113	7	32	27	19	46	53	2	8	0	,15 ,22 ,42 ,46 ,51 ,51 ,52 ,53	9 & 10. VII. 26.
126	"	8	44	35	17	52	50	2	3	0	,36 ,36 ,50	11 & 12. VII. 26.
138	"	6	50	31	26	57	46	2	12	0	,29 ,31 ,36 ,37 ,38 ,43 ,44 ,45 ,46 ,46 ,46 ,46	22 & 23. VII. 26.
148	"	8	33	22	19	41	39	2	6	0	,18 ,28 ,35 ,36 ,39 ,39	29 & 31. VII. 26.
124	s. 336	9	41	26	20	46	50	2	8	0	,19 ,34 ,41 ,42 ,43 ,43 ,43 ,50	10 & 13. VII. 26.
127	"	7	38	23	19	42	46		9	0	,28 ,28 ,29 ,34 ,36 ,36 ,38 ,41 ,46	12 & 13. VII. 26.
137	"	7	34	18	26	44	49		1	0	,39	20, 22 & 23. VII. 26.
155	"	7	37	30	20	50	49		0	0		2 & 8. VIII. 26.
160	"	7	38	22	25	47	43		0	0		7 & 8. VIII. 26.
241	"	8	27	22	15	37	37		6	0	,30 ,31 ,37 ,37 ,37 ,37	6, 7 & 9. X. 26.
256	"	9	42	31	21	52	38		3	0	,38 ,38 ,38	12 & 14. X. 26.
272	"	7	16	26	7	33	38		0	0		18 & 19. X. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 113	July '26	196	29	14.7	0	26	0	0
s. 336	July & Aug. '26	229	18	7.8	0	17	0	0
"	Oct. '26	122	9	7.3	0	9	0	0
m. 90	May & June '26	127	1	0.7	0	1	0	0

*N.B.* — Sheep No. 336 and monkey No. 90 were infected by guinea-pig No. 54 by subinoculation. This strain has never been transmitted since its first isolation from man. At one period in its career the percentage of positive flies was very high, both in monkey No. 113 and in sheep No. 336.

STRAIN NO. IV.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
87	g. 339	25	37	12	26	38	51	1	0	0		14 & 16. vi. 26.
92	"	9	41	22	27	49	56	1	1	1	;47 ;56	21 & 23. vi. 26.
117	"	8	36	24	23	47	54	1	8	1	,37 ,42 ,42 ,42 ,48 ,52 ,54 ,54 ;52	7, 9, 11 & 13 vii. 26.
266	"	8	21	33	23	56	36	1	0	0		14 & 16. x. 26.
198	g. 342	7	51	32	32	64	41	2	2	0	,19 ,38	21 & 23. ix. 26.
200	"	7	52	29	34	63	42	2	1	0	,41	22 & 24. ix. 26.
209	"	16	41	27	25	52	39	2	3	0	,32 ,33 ,33	26, 27 & 29. ix. 26.
225	"	6	41	25	25	50	38	2	1	0	,35	2 & 4. x. 26.
229	"	6	28	28	20	48	39	2	4	0	,22 ,24 ,28 ,33	3 & 5. x. 26.
251	"	10	30	19	20	39	38	2	0	0		10 & 12. x. 26.
305	"	12	28	22	21	43	33	2	2	0	,33 ,33	11, 13 & 14. xii. 26.
308	"	8	40	21	23	44	32	2	1	0	,32	14 & 16. xii. 26.
316	"	7	31	16	23	39	34	2	0	0		20, 21 & 24. xii. 26.
433	m. 256	9	19	14	13	27	37	3	0	0		8, 10 & 11. iii. 27.
450	"	8	49	34	19	53	41	3	0	0		27, 29 & 30. iii. 27.
453	"	10	16	13	4	17	34	3	0	0		2, 4 & 5. iv. 27.
456	"	8	45	23	27	50	34	3	0	0		3, 5 & 6. iv. 27.
458	"	18	28	20	10	30	38	3	0	0		6, 8 & 9. iv. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
<i>Blasio</i> g. 339	Apr. '26	155	5	3.8	3	5	1.9	<b>2.2</b>
	June & July '26	134	11	5.7	2	11	1.0	<b>1.0</b>
g. 342	Sept. & Oct. '26	316	11	3.4	0	0	0	<b>0</b>
	Dec. '26	126	3	2.3	0	0	0	<b>0</b>
m. 256	March & Apr. '27	177	0	0	0	0	0	<b>0</b>

Goat No. 339 died three months after its infection by flies fed on *Blasio*; the post-mortem suggested that it died of trypanosomiasis. Note the loss of transmissibility by this strain in goat No. 342, and the complete inability to develop in tsetse in monkey No. 256, which was infected by inoculation with the blood of goat No. 342.

STRAIN NO. V.

This strain has been studied more exhaustively than any other. It has proved very readily transmissible through a number of passages.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 26th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
244	m. 64	8	26	25	18	43	40	1	4	1	,27 ,36 ,36 ,40 ;40	7 & 9. x. 26.
247	"	8	18	24	17	41	40	1	2	1	,17 ,21 ,25	8 & 9. x. 26.
249	"	9	33	25	29	54	40	1	7	0	,9 ,10 ,11 ,14 ,40 ,40	10 & 12. x. 26.
264	"	8	17	21	20	41	39	1	1	0	,40 ,23	14 & 16. x. 26.
263	m. 95	8	23	27	18	45	44	2	4	1	,10 ,14 ,14 ,19 ;42	14 & 16. x. 26.
267	"	9	26	28	16	44	42	2	9	1	,9 ,10 ,11 ,11 ,13 ,17	15 & 17. x. 26.
412	"	7	27	21	21	42	37	2	0	3	,42 ,42 ,42 ;42	20, 22 & 23. II. 27.
415	"	10	22	15	20	35	36	2	0	0	,37 ;37 ;37	21, 23 & 24. II. 27.
508	"	6	35	19	21	40	35	2	0	0		30, 31. v. & 1. VI. 27.
509	"	7	37	22	26	48	35	2	1	0	,35	31. v. & 2, 3. VI. 27.
518	"	7	37	23	21	44	34	2	1	0	,34	4, 6 & 7. VI. 27.
521	"	7	37	31	20	51	34	2	1	0	,11	5, 6 & 8. VI. 27.
529	"	7	29	31	14	45	34	2	2	0	,33 ,34	9, 11 & 12. VI. 27.
116	s. 333	7	33	15	27	42	55	2	2	2	,21 ,38 ,46 ;49	7 & 9. VII. 26.
139	"	11	41	28	21	49	48	2	0	1	,48	20 & 22. VII. 26.
144	"	5	44	23	36	59	46	2	2	2	,14 ,43 ;43 ;46	26 & 28. VII. 26.
344	"	7	26	20	27	47	31	2	0	0		10, 12 & 13. I. 27.
352	"	5	27	22	20	42	31	2	0	0		17, 18 & 19. I. 27.
380	"	6	20	15	19	34	32	2	1	0	,32	31. I. & 3, 4. II. 27-
382	"	7	26	19	34	53	31	2	0	0		2, 4 & 5. II. 27.
391	"	6	25	26	18	44	37	2	0	0		7, 9 & 10. II. 27.
397	"	6	17	20	12	32	38	2	1	0	,38	8, 9 & 10. II. 27.
218	s. 161	8	48	29	32	61	39	3	3	1	,28 ,31 ,35 ;34	29. IX. & 1. x. 26.
220	"	8	41	22	35	57	39	3	7	8	,16 ,22 ,27 ,31 ,31 ,39	30. IX & 2. x. 26.
											,39 ;24 ,24 ;29 ;29 ;30	
											,32 ;39	
227	"	8	21	20	16	36	41	3	1	1	,28 ;41	3 & 5. x. 26.
248	"	7	27	26	24	50	46	3	3	3	,14 ,14 ,24 ,34 ;37 ;38	8 & 9. x. 26.
257	"	8	31	28	20	48	46	3	0	0		12 & 14. x. 26.
345	"	10	36	26	21	47	32	3	0	0		10, 12 & 13. I. 27.
351	"	6	20	13	20	33	31	3	0	0		16, 18 & 19. I. 27.
379	"	8	37	22	29	51	31	3	1	0	,26	31. I. & 3, 4. II. 27.
381	"	6	30	20	29	49	32	3	2	0	,9 ,15	1, 3 & 4. II. 27.
389	"	5	23	30	14	44	30	3	0	0		6, 8 & 9. II. 27.

STRAIN No. V (continued).

Transmission Experiments:

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
276	s. 217	8	30	20	21	41	41	4	4	2	,14 ,21 ,30 ,30 ;32 ;41	19 & 20. XI. 26
282	"	5	28	22	16	38	38	4	2	0	,12 ,18	23 & 25. XI. 26.
287	"	6	14	16	12	28	34	4	2	0	,13 ,19	27, 28 & 30. XI. 26.
294	"	5	26	19	24	43	30	4	0	0		4 & 5. XII. 26.
295	"	19	27	21	16	37	30	4	1	1	;23 ;30	4 & 5. XII. 26.
302	"	10	24	21	16	37	31	4	0	0		10 & 11. XII. 26.
435	"	10	28	21	13	34	38	4	0	0		9, 10 & 12. III. 27.
437	"	17	32	20	22	42	36	4	0	0		12, 14 & 15. III. 27.
442	"	6	28	20	14	34	36	4	0	0		19, 21 & 22. III. 27.
279	g. 211	12	23	19	10	29	40	4	0	0		20, 22, & 24. XI. 26.
283	"	8	35	23	22	45	36	4	0	0		24 & 25. XI. 26.
288	"	8	24	19	8	27	31	4	0	1	;29	30. XI. & 1, 3. XII. 26
281	g. 194	8	24	14	13	27	38	4	0	1	;38	22 & 24. XI. 26.
284	"	4	18	19	16	35	35	4	1	1	;25 ;35	25, 27 & 28. XI. 26.
292	m. 222	6	41	25	20	45	32	4	2	0	,6 ,27	2 & 3. XII. 26.
293	"	6	35	22	25	47	32	4	2	1	,6 ,8 ;32	2 & 3. XII. 26.
326	s. 218	8	23	17	22	39	34	5	0	0		4 & 6. I. 27.
339	"	11	26	23	24	47	33	5	0	0		7 & 9. I. 27.
341	"	5	20	22	25	47	31	5	0	0		9 & 11. I. 27.
346	"	7	29	20	24	44	35	5	0	0		12, 14 & 15. I. 27.
350	"	5	22	25	23	48	31	5	1	1	;25 ;26	16, 18 & 19. I. 27.
407	"	6	27	15	28	43	38	5	1	3	;17 ;35 ;38 ;38	16, 18 & 19. II. 27.
409	"	6	25	28	17	45	39	5	0	0		17, 19 & 20. II. 27.
416	"	6	24	11	28	39	36	5	0	0		22, 24 & 25. II. 27.
432	"	6	17	20	5	25	36	5	1	0	,36	8, 10 & 11. III. 27.
398	m. 252	5	19	23	16	39	37	5	3	3	,6 ,14 ,37 ;36 ;37 ;37	9, 11 & 12. II. 27.
405	"	5	21	31	16	47	35	5	1	0	,18	14, 16 & 17. II. 27.
410	"	8	33	30	15	45	38	5	0	0		18, 20 & 21. II. 27.
355	g. 214	6	6	14	14	28	28	5	0	0		17, 19 & 20. II. 27.
361	"	4	12	19	9	28	32	5	0	0		20, 22 & 23. II. 27.
364	"	5	13	13	6	19	31	5	0	0		21, 22 & 24. II. 27.
424	s. 173	9	44	27	33	60	42	6	0	0		28. II. & 2, 3. III. 27.
443	"	17	25	27	4	31	38	6	1	1	;17 ;38	24, 26 & 27. III. 27.
448	"	11	24	17	13	30	39	6	0	0		25, 27 & 28. III. 27.
449	"	22	29	17	13	30	39	6	0	3	;39 ;39 ;39	26, 28 & 29. III. 27.
478	"	19	36	22	17	39	34	6	0	0		4, 5 & 7. v. 27.
489	s. 316	9	38	21	24	45	34	7	0	0		20, 21 & 23. v. 27.
493	"	14	37	12	31	43	38	7	1	0	,38	22, 24 & 25. v. 27.
499	"	11	24	9	27	36	33	7	1	0	,22	26, 27 & 29. v. 27.
506	"	8	45	34	23	57	37	7	1	0	,37	30 & 31. v. & 1. VI. 27.
511	"	7	35	29	19	48	36	7	3	2	,23 ,23 ,36 ;36 ;36	1, 2 & 4. VI. 27.
520	"	7	35	25	26	51	34	7	0	0		5, 6 & 8. VI. 27.
536	"	11	29	20	21	41	37	7	0	0		13, 14 & 16. VI. 27.
525	m. 358	8	39	27	23	50	34	8	0	0		8, 10 & 11. VI. 27.
527	"	7	30	24	18	42	33	8	0	0		9, 11 & 12. VI. 27.
532	"	6	40	28	26	54	34	8	1	1	;34 ;34	12, 14 & 15. VI. 27.
533	"	11	22	21	13	34	37	8	3	0	,13 ,15 ,24	13, 15 & 16. VI. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 64	Apr. '26	154	8	5.0	7	7	4.5	5.0
"	May '26							
"	Oct. '26	179	16	8.8	2	9	1.1	1.9
m. 95	Oct. '26	89	15	16.8	2	5	2.2	6.7
"	Feb. '27	77	3	3.8	3	3	3.8	3.8
"	May '27	228	5	2.1	0	4	0	0
s. 333	July '26	150	9	6.0	5	7	3.3	4.2
"	Jan. & Feb. '27	252	2	0.7	0	2	0	0
s. 161	Oct. '26	252	27	10.1	12	22	5.1	5.5
"	Jan. & Feb. '27	224	3	1.3	0	1	0	0
s. 217	Nov. & Dec. '26	224	12	5.3	3	5	1.3	3.1
"	March '27	110	0	0	0	0	0	0
g. 211	Nov. & Dec. '26	101	1	0.9	1	1	0.9	0.9
g. 194	Nov. '26	62	3	4.8	2	3	3.2	3.2
m. 222	Dec. '26	92	5	5.4	1	2	1.0	2.7
s. 218	Jan. '27	225	2	0.8	1	2	0.4	0.4
"	Feb. & March '27	152	5	3.8	3	4	1.9	2.8
m. 252	Feb. '27	131	7	5.4	3	4	2.2	4.0
s. 173	March '27	151	5	3.3	4	4	2.6	3.3
s. 316	May & June '27	321	8	2.4	2	5	0.6	0.9
m. 358	June '27	180	5	2.7	1	2	0.5	1.3

*N.B.* — Sheep No. 333 was infected from monkey No. 64 by subinoculation.

After some six months in monkey No. 64, the infectivity of the strain to fly showed a definite increase, whereas its transmissibility diminished. The highest Index and the greatest infectivity for fly in this strain occurred in monkey No. 95 ; after four months, the infectivity had dropped from 16.8 to 3.8 and the Index from 6.7 to 3.8, but the examination was restricted to two experiments ; three months later, the infectivity had dropped still further to 2.1, and the Transmissibility Index to zero. Thus, after a year's sojourn in monkey No. 95, the strain was still capable of producing infection in *G. palpalis*, though the power to infect the glands had apparently disappeared. Similar behaviour was observed with strain No. II in monkey No. 53, gut-infection being obtained in flies fed on this monkey some fourteen months after its first infection.

These results illustrate the importance of distinguishing transmissible from non-transmissible strains. A positive result from a blood inoculation of a man or animal

suffering from trypanosomiasis has little value from an epidemiological point of view unless we have at the same time some information about the transmissibility of the strain by tsetse. The behaviour of this strain in sheep Nos. 333, 161 and 217 is very instructive, the infectivity to fly being greatly reduced and the Index falling apparently to **zero** after a few months in these animals.

STRAIN NO. VI.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
122	g. 341	10	48	39	13	52	51	2	2	0	,34 ,46	10 & 12. VII. 26.
125	"	10	18	20	9	29	45	2	2	0	,34 ,42	10, 12 & 13. VII. 26.
133	"	12	45	23	27	50	46	2	0	0		18 & 20. VII. 26.
145	"	10	33	26	20	46	45	2	2	0	,15 ,33	26 & 27. VII. 26.
158	"	10	45	44	14	58	45	2	0	0		4 & 6. VIII. 26.
516	"	7	39	33	18	51	34	2	0	0		2, 3 & 5. VI. 27.
519	"	7	43	29	27	56	35	2	0	0		4, 6 & 7. VI. 27.
528	"	8	37	26	22	48	33	2	0	0		9, 10 & 12. VI. 27
534	"	8	36	22	23	45	35	2	1	0	,26	13, 14 & 16. VI. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total		
m. 65	May & June '26	150	4	2.6	0	3	0	0
g. 341	July '26	235	6	2.5	0	5	0	0
	June '27	200	1	0.5	0	1	0	0

*N.B.* — Goat No. 341 was infected from monkey No. 65 by subinoculation. Never transmissible from the time of its arrival at the Laboratory, this strain had apparently lost most of its power of development in tsetse after ten months in goat No. 341.



STRAIN NO. VII.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In 'gut only.	In gut and salivary glands.		
199	s. 335	7	48	27	32	59	42	1	0	0		22 & 24. IX. 26.
206	"	12	42	25	32	57	40	1	3	1	,33 ,35 ,39 ,38	25 & 26. IX. 26.
207	"	7	44	29	28	57	40	1	3	0	,8 ,26 ,38	26 & 28. IX. 26.
202	g. 337	9	37	26	21	47	42	1	2	0	,29 ,29	23 & 25. IX. 26.
215	"	7	52	30	30	60	37	1	4	0	,26 ,34 ,36 ,37	1. X. 26.
226	"	7	47	25	36	61	39	1	4	0	,33 ,34 ,34 ,36	2 & 4. X. 26.
239	"	8	24	24	13	37	40	1	3	1	,13 ,38 ,40 ,40	6, 7 & 8. X. 26.
252	"	9	19	18	10	28	36	1	0	0		11 & 13. X. 26.
423	"	6	26	18	24	42	38	1	0	0		28. II. & 2, 3. III. 27.
426	"	7	27	23	22	45	38	1	0	0		2, 4 & 5. III. 27.
430	"	4	30	22	22	44	37	1	0	0		5, 7 & 8. III. 27.
434	"	10	17	13	10	23	36	1	0	0		9, 11 & 12. III. 27.
440	"	11	30	28	10	38	36	1	0	0		15, 17 & 18. III. 27.
280	g. 330	7	31	20	21	41	39	1	0	0		22 & 24. XI. 26.
286	"	8	37	18	22	40	37	1	0	0		26, 27 & 29. XI. 26.
298	"	6	52	33	28	61	33	1	2	0	,33 ,33	6, 7, 9 & 10. XII. 26.
354	"	5	21	19	20	39	32	1	1	0	,21	17, 19 & 20. I. 27.
360	"	6	30	26	17	43	33	1	0	0		19, 21 & 22. I. 27.
335	g. 174	12	25	21	25	46	31	2	0	0		4, 5 & 7. I. 27.
340	"	6	27	21	24	45	32	2	0	0		7, 9 & 10. I. 27.
342	"	6	29	15	31	46	31	2	0	0		10, 12 & 13. I. 27.
353	"	3	22	24	23	47	31	2	0	0		17, 19 & 20. I. 27.
357	"	7	29	17	24	41	31	2	0	0		19, 20 & 21. I. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index
					With glands infected.	Total.		
<i>Tekisoka</i>	June '26	180	13	7.2	6	11	3.3	3.9
s. 335	Sept. '26	173	7	4.0	1	6	0.5	0.6
g. 337	Sept. & Oct. '26	233	14	6.0	1	13	0.4	0.4
"	March '27	192	0	0	0	0	0	0
g. 330	Nov. & Dec. '26	224	3	1.3	0	2	0	0
"	Jan. '27	82	1	1.2	0	0	0	0

*N.B.* — Sheep No. 335 died of acute diarrhoea and not from trypanosomiasis. Note the complete loss of transmissibility in goat No. 337 after five months. Goat No. 174 was never infected, though fed upon several times by a fly with heavily infected glands ; it was also inoculated with the glands of this fly.

STRAIN No. VIII.

*Transmission Experiments :*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
195	m. 120	8	41	23	30	53	38	1	1	0	,37	20 & 22. IX. 26.
203	"	10	40	26	23	49	38	1	2	0	,23 ,35	23 & 26. IX. 26.
214	"	12	40	21	24	45	35	1	1	0	,31	28 & 30. IX. 26.
224	"	7	39	32	25	57	37	1	5	0	,16 ,20 ,21 ,37 ,37	2 & 4. X. 26.

*Transmissibility Index :*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
<i>Zakayo</i> m. 120	June '26 Sept. & Oct. '26	183	3	1.6	0	3	0	0
		204	9	4.4	0	5	0	0

*N.B.* — Monkey No. 120 was infected from *Zakayo* by subinoculation. A good example of a strain infective to fly but not transmissible.

STRAIN No. IX.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
194	g. 171	9	44	25	25	50	53	1	0	0		20 & 22. IX. 26.
217	"	8	37	16	34	50	39	1	1	0	.30	29. IX. & 1. X. 26.
228	"	6	37	23	31	54	39	1	1	1	.31 .28	3 & 5. X. 26.
235	"	8	25	17	21	38	39	1	0	1	.34	5 & 7. X. 26.
513	"	8	24	18	23	41	33	1	2	0	.31 .33	2, 3 & 5. VI. 27.
517	"	11	35	26	29	55	34	1	2	0	.14 .34	4, 6 & 7. VI. 27.
524	"	11	32	20	26	46	34	1	0	0		6, 8 & 9. VI. 27.
530	"	8	35	25	20	45	34	1	0	0		11, 13 & 14. VI. 27.
289	s. 195	7	32	18	18	36	35	0	0	0		29. XI. & 1, 3. XII. 26.
299	"	8	39	25	23	48	33	0	0	0		8 & 9. XII. 26.
304	"	9	31	15	26	41	33	0	0	0		10, 13 & 14. XII. 26.
309	"	10	35	30	19	49	32	0	0	0		14, 16 & 17. XII. 26.
343	"	10	21	28	25	53	31	0	0	0		10, 12 & 13. I. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
<i>Mangasi</i>	July '26	158	16	10.1	11	14	6.9	<b>7.9</b>
g. 171	Oct. '26	192	4	2.0	2	4	1.0	<b>1.0</b>
"	June '27	187	4	2.1	0	3	0	<b>0</b>

Sheep No. 195 never became infected, but it is doubtful if the only infective fly to which it was exposed bit this sheep. The glands from two other infective flies were also injected into this animal, but experience has shown that the injection of an infected gland is less effective than is the actual bite of an infective fly. Note the great difference between the Index in the patient himself and in goat No. 171, which was infected from him by laboratory-bed flies; also the loss of transmissibility and the diminution in the infectivity in this goat after nine months had elapsed.

STRAIN No. X.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
269	♂. 338	5	33	25	18	43	38	2	3	1	.38 .38 .38 .38	16, 18 & 19. x. 26.
296	"	4	36	35	27	62	31	2	2	0	.31 .31	6 & 7. XII. 26.
297	"	7	16	10	14	24	31	2	1	0	.7	6 & 7. XII. 26.
303	"	5	35	23	22	45	33	2	0	1	.33	10, 11 & 13. XII. 26.
314	"	10	37	19	33	52	32	2	0	0		19, 20 & 22. XII. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 149	Aug. '26	158	4	2.5	1	4	0.6	0.6
♂. 338	Oct. '26	43	4	9.3	1	4	2.3	2.3
"	Dec. '26	183	4	2.1	1	3	0.5	0.7

An interesting departure from the usual is shown in goat No. 338, in which both the infectivity and the Index are greater than in the first-passage monkey. Two months later, the strain in this goat was still infective, but the examinations were too irregular to supply much valid information.

STRAIN No. XI.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
268	s. 163	8	31	28	23	51	39	2	4	0	,19 ,39 ,39 ,39	15 & 17. x. 26.
270	"	8	17	15	14	29	37	2	0	0		16 & 18. x. 26.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 162	Aug. '26	218	16	7.3	5	11	2.2	3.3
s. 163	Oct. '26	80	4	5.0	0	3	0	0

The remaining strains of Part I were not examined once their transmissibility had been established by feeding flies on the patients themselves.

\* \* \*

Hitherto, the only ruminants we have used in these experiments are sheep and goats. A few experiments will now be given in which the behaviour of *T. gambiense* in calves was investigated.

It proved difficult to obtain satisfactory results with these animals at Entebbe. The only calves available were those that had served for smallpox-lymph production, and they were almost all in poor condition. All were infected with East Coast fever and their hold on life was very precarious. All that could be done was to select the best-conditioned beasts which had lived for some months at the Laboratory, and the supply of these was very limited.

The animals were tested by inoculation of their blood into clean monkeys, and then they were fed upon by flies infected with certain strains of *T. gambiense*. After sufficient time had elapsed, clean flies were fed upon them to see if they had become infected.

It is not worth while to give full details of these experiments ; a brief summary will suffice.

*Calf No. 13.*

Fed on for two days by a fly with heavily infected salivary glands from strain No. X. This fly infected a monkey before it bit the calf, and a goat afterwards. Sixteen days later, clean flies were put on the animal, and the feedings continued over a period of eight days. In these experiments 161 flies were dissected, of which 107 lived till the twenty-fifth day ; all were negative. Six weeks and two months later, 8 c.c. of defibrinated blood of the calf were inoculated into monkeys, which remained negative.

This animal therefore failed to become infected with *T. gambiense*. As will be seen in Paper No. 2, it succumbed rapidly to *T. rhodesiense*.

*Calf No. 76.*

Fed on for two consecutive days by three flies with glands heavily infected with strain No. VII. Also inoculated with gland-contents of another positive fly of this strain. These flies infected a goat, a monkey and a sheep before they bit calf No. 76. One month later, experiments with clean flies, in which 169 were dissected and 132 lived till the twenty-fifth day, were all negative. Two blood inoculations into monkeys some three months later were negative.

This animal also succumbed subsequently to *T. rhodesiense*.

*Calf No. 19.*

Fed on for two days by five flies with infected salivary glands from strain No. IX. These flies had infected two monkeys and a goat before feeding on the calf.

Some three weeks later, clean flies fed on the calf ; 211 were dissected and 146 reached the twenty-fifth day ; two of these flies were infected. This animal was thus infected by *T. gambiense*, but showed no symptoms whatever. It was eventually infected with *T. rhodesiense*.

*Calf No. 77.*

Inoculated with blood containing strain No. VI. Fed on for two days by two flies with glands infected with this strain ; also inoculated with infected gland of a third fly. These flies had infected two monkeys and then failed to infect a goat, a sheep and the calf. The sheep and the goat had resisted infection by another strain of *T. gambiense* ; the goat eventually became infected by strain No. X, and the sheep succumbed to *T. rhodesiense*.

The blood of this calf was examined nine times by centrifugation and found negative. Clean flies were fed on it, of which 183 were dissected and 120 lived till the twenty-fifth day ; all were negative. Stained thick films of blood were examined on 37 days, and no trypanosomes were seen.

This animal also eventually succumbed to *T. rhodesiense*.

It is evident from these experiments that calves of two or three years old are exceedingly resistant to *T. gambiense*. Even when they became infected with this trypanosome by the agency of cyclically infected flies, the organism appears to lose its transmissibility.

It is therefore exceedingly improbable that they can serve as a reservoir of *T. gambiense*.

SUMMARY.

These experiments must speak for themselves. The inferences we are tempted to draw from them at this stage are :

1. That, as a general rule, the transmissibility of a strain of *T. gambiense* diminishes when the strain is introduced into a sheep or goat.
  2. That after some months' sojourn in these animals, *T. gambiense* loses its transmissibility altogether.
  3. That a strain of *T. gambiense* may lose its transmissibility quite suddenly on being transferred from one host to another by cyclically infected *G. palpalis*.
  4. That, in the three patients whose strains were transmitted by tsetse fed directly on the patient, the trypanosome had a higher Transmissibility Index in man himself than in the animals infected from him by cyclical transmission.
  5. That it is improbable that sheep and goats play any important part as a reservoir of this trypanosome, but a final opinion on this important point must be reserved. According to these experiments, calves are a negligible factor in this respect.
-





An interesting feature of this strain was its pathogenicity for domestic ruminants. Some of the post-mortem examinations on the sheep, goats and calves killed by this trypanosome were kindly made by Captain HALL, Veterinary Pathologist, Uganda; the rest by Dr. PERUZZI. The detailed reports of these examinations will be found in Dr. PERUZZI's paper in this volume.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
164	g. 143	9	41	24	26	50	42	2	0	0		13. VIII. 26.
165	"	7	42	18	27	45	42	2	2	1	,17,42 ;37	14 & 16. VIII. 26.
175	"	22	36	25	14	39	35	2	2	4	,22,32 ;35 ;35 ;35 ;35	19 & 21. VIII. 26.
166	s. 144	7	50	28	30	58	42	2	2	5	,42,42 ;36 ;36 ;40 ;42 ;42	14 & 16. VIII. 26.
169	"	7	31	24	27	51	42	2	1	0	,34	14 & 16. VIII. 26.
174	"	15	40	22	25	47	36	2	1	5	,36 ;25 ;36 ;36 ;36 ;36	19 & 21. VIII. 26.
246	g. 176	7	23	21	16	37	33	3	4	0	,8,12,28,28	8 & 10. x. 26.
254	"	8	30	22	24	46	50	3	5	1	,8,12,25,50,50 ;35	11 & 13. x. 26.
260	"	10	26	19	21	40	41	3	4	3	,18,19,34,40 ;40 ;41 ;41	12. x. 26.
261	"	7	20	18	22	40	48	3	4	1	,15,18,19,39	13. x. 26.
291	g. 212	8	24	18	14	32	37	4	2	2	,9,18 ;37 ;37	1 & 3. XII. 26.
310	c. 77	5	34	24	21	45	33	4	0	0		15, 16 & 18. XII. 26.
358	"	5	22	25	23	48	31	4	1	0	,31	19, 21 & 22. I. 27.
363	"	5	20	26	13	39	31	4	3	0	,7,10,31	21, 22 & 24. I. 27.
367	"	5	27	24	22	46	31	4	1	0	,27	22, 24 & 25. I. 27.
373	"	6	22	15	18	33	33	4	0	0		26, 28 & 29. I. 27.
313	c. 76	7	32	21	22	43	31	4	0	0		17, 18, 19 & 20. I. 27.
356	c. 13	9	32	25	18	43	33	5	1	0	,28	17, 19 & 20. I. 27.
359	"	8	28	29	21	50	32	5	1	0	,32	19, 21 & 22. I. 27.
362	"	5	14	21	18	29	32	5	1	1	,15 ;32	20, 22 & 23. I. 27.
368	"	4	26	23	20	43	32	5	1	1	,19 ;32	23, 25 & 26. I. 27.
374	"	10	34	24	17	41	36	5	0	0		27, 29 & 30. I. 27.
451	c. 59	12	28	26	10	36	42	6	0	0		2, 4 & 5. IV. 27.
454	"	7	31	20	19	39	44	6	0	0		3, 5 & 6. IV. 27.
459	"	28	30	19	11	30	39	6	0	0		7, 9 & 10. IV. 27.
461	"	13	37	25	19	44	34	6	0	0		15, 17 & 18. IV. 27.
465	c. 19	15	39	28	16	44	34	7	0	0		19, 21 & 22. IV. 27.
466	"	9	32	26	14	40	34	7	1	0	,31	20, 22 & 23. IV. 27.
474	"	15	28	23	23	46	31	7	0	0		28 & 30. IV. & 1. v. 27.
477	"	8	34	21	20	41	33	7	0	0		2, 4 & 5. v. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total		
c. 143	Aug. '26	134	9	6.7	5	6	3.7	5.5
s. 144	"	156	14	8.9	10	14	6.4	6.3
c. 176	Oct. '26	163	23	13.4	5	13	3.0	5.1
c. 212	Dec. '26	32	4	12.5	2	2	6.2	12.5
c. 77	Dec. '26	213	5	2.3	0	3	0	0
c. 13	Jan. '27	216	6	2.7	2	4	0.9	1.3
c. 59	Apr. '27	149	0	0	0	0	0	0
c. 19	Apr. '27							
	May '27	171	1	0.5	0		0	0

It will be seen that the strain possessed a high Transmissibility Index during the first three passages, but when introduced into calves the Index diminished suddenly, soon becoming zero.

We have already noted the pathogenicity of this strain for the calves Nos. 13 and 77. Calves Nos. 59 and 19 are still alive and in good condition, but sufficient time has not yet elapsed to judge of their fate.

STRAIN No. XXI.

From the Ikoma area, Tanganyika ; isolated by professor KLEINE from a native.

*Animal Inoculations:*

Rat y  
|  
m. 281.14.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
384	m. 281	7	26	23	25	48	41	2	2	1	,16 ,17 ;41	2, 4 & 5. II. 27.
390	"	15	33	12	25	37	37	2	1	0	,37	6, 8 & 9. II. 27.
393	"	6	17	12	13	25	37	2	1	0	,24	7, 8 & 10. II. 27.
399	"	5	14	20	12	32	37	2	6	0	,5 ,6 ,7 ,9 ,10 ,16	9 & 11. II. 27.

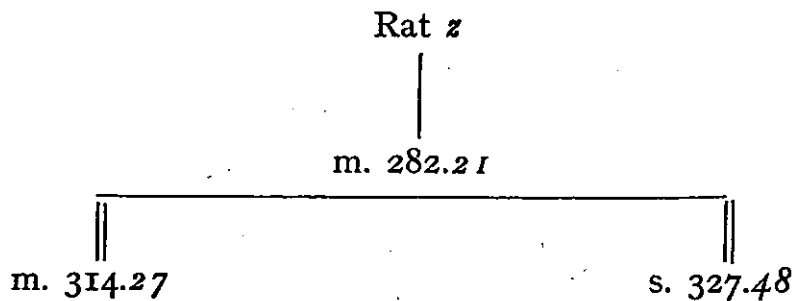
*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
281	Feb. '27	142	11	7.7	1	2	0.7	3.8

STRAIN No. XXII.

Isolated by Professor KLEINE, in the Ikoma area, from a native.

*Animal Inoculations:*



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates		Age s of the infected ies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
394	m. 282	9	26	14	18	32	53	2	0	0		7, 9 & 10. II. 27.
395	"	5	17	22	28	50	52	2	3	1	,5,7,52 ;51	8, 10 & 11. II. 27.
404	"	6	27	25	17	42	44	2	3	0	,7,9,9	14, 16 & 18. II. 27.
452	s. 327	8	27	21	15	36	34	2	0	0		2, 4 & 5. IV. 27.
455	"	12	51	27	25	52	34	3	0	0		3, 5 & 6. IV. 27.
457	"	7	26	20	19	39	39	3	0	0		6, 8 & 9. IV. 27.
460	m. 314	32	19	0	19	19	34	3	1	0	(only one or two flagellates seen),34	12, 14 & 15. IV. 27.

*Transmissibility Index:*

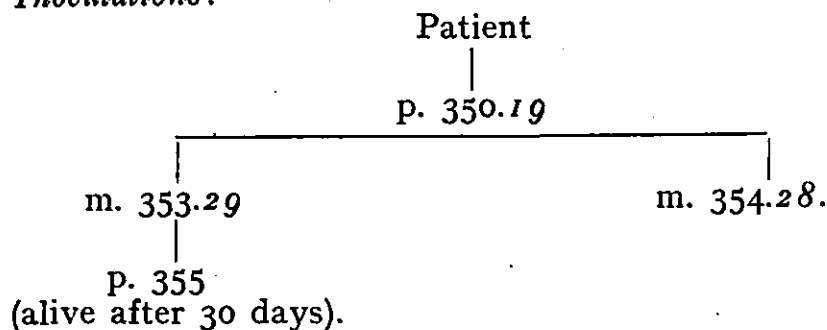
Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 282	Feb. '27	124	7	5.6	1	2	0.8	2.8

Both these strains proved very pathogenic to sheep ; the animals inoculated were big healthy beasts that had resisted all attempts to infect them by *T. gambiense*. The Transmissibility Index in these two strains appears to be low for *T. rhodesiense*.

STRAIN No. XXIII.

Isolated by Dr. VAN HOOFF and myself, at the end of our tour in the Belgian Congo. Chance decreed that we should find a case of human trypanosomiasis at Bambu, near Kilo, to the west of Lake Albert, on our journey south from the Uelle district. The patient was a woman of 40, in an advanced stage of the disease. The diagnosis was made on 4. VI. 26 by Dr. MONCAREY ; trypanosomes were found in the fresh blood and in the gland juice, and there was œdema of the feet. The patient received the following treatment : four injections of 0.50 gr. Bayer 205 ; fourteen injections of 0.50 gr. Soamin ; thirteen injections of 0.06 gr. Stibyl, and a course of Stovarsol. At the end of the treatment, on 16. IX. 26, the cerebro-spinal fluid contained four lymphocytes per cubic mm., but definite nervous symptoms had developed and she suffered from irregular fever. She was re-diagnosed by Dr. DACO at Bambu on 26. IV. 27, when trypanosomes were found in the blood, the lymph and the cerebro-spinal fluid, the latter containing a very large number of abnormal cells. On 27. IV. 27 we inoculated a guinea-pig which was taken to Entebbe, and showed trypanosomes in the blood on 12. V. 27. This woman moved in native mining circles, and never left the neighbourhood of Kilo and its mining camps and the country of the Babira and the region around Mombasa and Irumu, where *G. morsitans* has never yet been reported. In this region, rare sporadic cases of *T. gambiense* infection have been found near the Chari and its tributaries, where *G. palpalis* and a big tsetse of the *fusca-brevipalpis* type are found. The place where she was most probably infected is Nyankunde, her birthplace, which is included in the sleeping-sickness area of Geti-Boga, to the north of the Semliki Valley.

*Animal Inoculations:*



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			It gut only.	In gut and salivary glands.		
488	m. 353	10	38	27	19	46	33	3	2	0	,15 ;18	20, 21 & 23. v. 27.
497	"	5	38	16	31	47	34		1	1	;5 ;33	25, 26 & 28. v. 27.
501	"	6	31	18	29	47	34		1	3	;34 ;27 ;34 ;34	27, 29 & 30. v. 27.
504	"	8	32	18	22	40	33		0	0		28, 30 & 31. v. 27.
510	"	5	32	23	17	40	32		0	2	;31 ;32	31. v. 27 & 2, 3. III. 27.
491	m. 354	6	34	26	29	55	35	3	0	0		21, 22 & 24. v. 27.
494	"	12	42	18	31	49	35		0	1	;27	23, 25 & 26. v. 27.
498	"	5	39	26	23	49	34		1	1	;5 ;34	25, 26 & 28. v. 27.
502	"	6	37	22	24	46	33		1	0	;10	27, 29 & 30. v. 27.
507	"	9	41	25	26	51	33		1	2	;13 ;33 ;33	30. v. 27 & 1 & 2. VI. 27.
572	"	7	37	28	23	51	33		1	1	;32 ;32	1, 2 & 4. VI. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 353 and m. 354	May June '27	521	19	3.6	11	13	2.1	3.0

It is practically certain that this patient had relapsed after treatment and was not a cured case infected a second time. It is interesting to see that, in spite of all the drugs she received, her trypanosomes were still readily transmissible.

STRAINS "S" AND No. 483.

Isolated in 1922 in the Mwanza area. (DUKE, 1924a.)

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index
					With glands infected.	Total.		
Strain 483	Sept. '22	421	31	7.3	22	28	5.2	5.7
Strain "S"	"	120	9	7.6	8	9	6.6	6.7

Indices which resemble very closely that of strain No. XX.

From a study of these five strains it would appear that *T. rhodesiense* is better adapted to cyclical development in *G. palpalis* than is *T. gambiense*. It may well be that its transmissibility by the normal vector *G. swynnertoni* or *G. morsitans* is still higher.

\* \* \*

The pathogenicity of *T. rhodesiense* for domestic animals is remarkable. An attempt will be made to subject strains of this trypanosome to a series of cyclical passages through *G. palpalis*, to see whether the trypanosome alters in any way in consequence of the change of vector. This has already been done, for four passages, with strain No. XX; but the unsuitability of calves invalidates these experiments, and they must be repeated on different lines.

The behaviour of the calves used in these experiments is remarkable. Calves Nos. 76, 77 and 13 apparently died of trypanosomiasis, after having been infected on the second or third passage of the strain by cyclically infected flies. The animals all showed numerous trypanosomes in the peripheral blood on many days of the disease, and suffered acutely. The pathogenicity of the parasites in these three animals is remarkable, the more so as calves Nos. 59 and 19, also infected with the same trypanosome, are still alive at the time of writing and apparently in fairly good condition.

In considering the rapid demise of these calves, there is, however, a possible though very improbable fallacy. During the period that the flies were being fed on the calves, and until the animals were found to be infected, they were herded with the cattle belonging to the Veterinary Pathologist, two of whose animals were discovered, at about the same time as calf No. 77, to be infected with a polymorphic trypanosome of which strain No. XXV of Paper No. 3 is an example. These animals had arrived at

Entebbe three weeks before from a considerable distance. Careful examination of the remainder of the herd by Captain HALL revealed no more or subsequent cases; but the possibility must be admitted that calves Nos. 76, 77 and 13 might have contracted a double infection. Strain No. XXV was completely non-transmissible by tsetse, and there is no doubt, from the details of my experiments, that all these three calves were infected with *T. rhodesiense*. Moreover, this particular strain of *T. rhodesiense* had already proved very fatal to sheep and goats, all of which were kept entirely apart from the cattle, and were under constant and careful supervision<sup>1</sup>.

Another point to be considered is that all these calves, Nos. 13, 76 and 77, had been bitten by flies infected with *T. gambiense*; and although every test employed had failed to demonstrate the presence of this trypanosome in their blood, yet it may be that there was a cryptic infection that, on being augmented by *T. rhodesiense*, lit up and exerted an effect on the animal.

On the other hand, strains Nos. XXI and XXII proved very pathogenic to sheep Nos. 326 and 327, after both these animals had resisted frequent attempts at infection with *T. gambiense*; and sheep No. 144 and goats Nos. 143, 176 and 212 all succumbed very rapidly to an uncomplicated *T. rhodesiense* infection.

Everything points, then, to the conclusion that the animals died of *T. rhodesiense*, and strain No. XX, in its earlier passages at all events, must be regarded as very pathogenic for domestic ruminants. Apparently in the sixth and seventh passages strain No. XX had lost its virulence for calves. Why this happened is not clear; but, in conjunction with other observations made during the study of *T. rhodesiense* at Entebbe, it is evidently desirable to study this trypanosome further in relation to *G. palpalis*.

#### DISCUSSION.

“The relation between *T. gambiense* and *T. rhodesiense*” is one of the questions that this Commission was intended to investigate.

As is evident from the account already given of the activities of the different members, the majority of our time has been devoted to the study of *T. gambiense*. Professor KLEINE is the only one who has been able to concentrate his attention on the other human trypanosome.

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<sup>1</sup> Lest it should be thought that the transmission experiments at Entebbe are open to the charge of casual contamination by other trypanosomes than those actually under observation, the following facts must be realised.

1. A constant guard was kept against this contingency. Every precaution was taken to protect the sheep and goats, which were the animals especially under investigation, from contact with cattle or with biting flies. No case of casual infection was ever observed, despite frequent search.

Captain G. Norman HALL, Veterinary Pathologist, most kindly aided me in every way in his power in this matter, and the strain above referred to (No. XXV) is the only instance of any trypanosome unexpectedly appearing in his cattle.

2. In case this trypanosome (No. XXV) was in fact *T. rhodesiense* and derived from my infected calves, Captain HALL put two clean animals and calf No. 77 in a fly-proof stable and introduced daily for thirty days 200 wild *Stomoxys*; the clean animals never became infected.

3. The experimental sheep and goats were herded entirely separately from the cattle, and never met tsetse. Large biting flies are rare near the Laboratory.

4. No case of casual infection has ever occurred in the numerous monkeys kept behind the Laboratory in the open air.

5. In his experiments on direct transmission of trypanosomes, as yet unpublished, Captain HALL also tried to transmit the Gomba-cattle organism (of which strain No. XXV is an example) by *Stomoxys* — also with negative results.

Professor KLEINE's conclusions about the two human trypanosomes are interesting and far-reaching. Among other indications, they drive home the urgent necessity for experimental work with tsetse of the *G. morsitans* type, to see whether *T. gambiense*, when transmitted by game-tsetses over a long period of time, acquires the characters of *T. brucei*.

For several years past I have been writing about the relationships of these two human trypanosomes, and have advanced the view that the differences that exist between *T. gambiense* and *T. rhodesiense* are, to a great extent, to be explained by the difference that exists between man, the host of the one, and the game which serves the other. In other words, the type of vertebrate host on which the trypanosome mainly relies determines, broadly speaking, its biological characters.

As stated below, in Paper No. 5, experience has lately convinced me that, to test this point, it will be necessary to have recourse to certain species of antelope, as domestic ruminants are unsuitable.

But the fly aspect of the problem also demands an essay. The polymorphic group of trypanosomes is represented in both *palpalis* and game-tsetse areas, but the type is different in the two areas. Typical *T. brucei* is, as far as I know, rarely, if ever, found in wild *G. palpalis*; the type carried by this fly is what has been described by French workers as *T. pecaudi*, or what I call the Damba trypanosome, which differs in some respects from the typical *T. brucei* of game-tsetse areas. LAVIER's work on this Commission is interesting in this connection, for he finds certain slight morphological differences between the Damba trypanosome and *T. brucei* or *T. rhodesiense*; the two last he finds practically indistinguishable in their morphology.

Evidently, it is possible that the fly exerts an influence on the trypanosome it carries, though we know already from the experiments of this Paper that this influence need not assert itself for, at any rate, four generations. Naturally, in KLEINE's conclusions I see welcome support to my views. Where we differ is in regard to *T. brucei*. KLEINE believes that species to be entirely different from the two human trypanosomes, which he considers essentially identical. I, too, believe that the differences between *T. gambiense* and *T. rhodesiense* are differences due to environment — and by environment I mean the habits and species of tsetse and the nature of the principal host animals, man or game. But I believe, with BRUCE and YORKE, that *T. rhodesiense* and *T. brucei* are even more nearly related to one another.

In discussing the origin and nature of *T. rhodesiense* I have hitherto laid great stress on direct transmission as an important factor in the spread of this type of trypanosomiasis. (DUKE, 1923 and 1924.) Experience acquired during the last eighteen months has led me to modify my views. The experiments described in the Preliminary Report of the Commission, on the transmission of arsenic-fast strains, inconclusive though they are, have shaken my faith in the stabilising effect of cyclical passages through fly. If, as these experiments indicate, acquired arsenic-fastness survives two passages through tsetse, there is no reason to suppose that a strain of *T. brucei* that has established itself in man would lose its adaptation to man when it passes cyclically through tsetse. The epidemiological studies of VAN HOOFF and a long series of transmission experiments with *T. gambiense* strains have convinced me that the disease generally spreads from man to man by means of cyclically infected tsetse, be they *G. palpalis* or *G. morsitans*.

In view of Professor KLEINE's conclusions, I wish to make my present attitude towards *T. brucei* and *T. rhodesiense* quite clear. My view is that *T. brucei* and *T. rhodesiense* are the same species. *T. rhodesiense* is the name given to strains of *T. brucei* that can utilise man as a host. Wherever game-tsetse are thrown in close



biological contact with man, there *T. rhodesiense* will be found — if looked for. The majority of the strains of *T. brucei* are incapable of utilising man ; in the same way, a number of strains of *T. congolense* cannot infect dogs or monkeys. In the former case, the more enterprising strains have earned the distinctive name of *T. rhodesiense* ; in the latter, the more conservative variety is known as *T. nanum*.

The essential point of this view is that the *selection is exercised by the trypanosome rather than by the mammalian host*. Viewed from this angle, the much-discussed experiment of TAUTE takes on a new significance. The inoculation of those 129 natives does not show that these individuals were resistant to the species of trypanosome known as *T. brucei*, nor does it show that this species of trypanosome is characterised by the inability to infect man. It merely shows that the six particular strains of *T. brucei* that TAUTE took from the horses and mules could not infect man.

If we take all the published experiments in which *T. brucei* has been inoculated into man, we see that, in all, some ten different strains have been investigated, all of which have been found unable to infect man.

There remains the contention that a number of people, black and white, have been bitten by wild game-tsetse without becoming infected. To avoid interminable repetition, all that need be said here about this class of evidence is that it is vague in the extreme, and that it is improbable that human strains of *T. brucei* do reveal themselves in nature unless there is a fairly close contact between man and fly.

To prove the contention of the German workers that *T. brucei* is, for all practical purposes, distinct from *T. rhodesiense*, I consider that what is needed is an experimental inoculation of say 129 strains of *T. brucei* from game and wild fly into one man or into a number of different men. And even then the experiment will be open to the objection that the positive result, if such occur, is attributable to the unsuspected presence of *T. rhodesiense*!

\* \* \*

There is one other point that ought to receive more attention than it does in discussions on the pathogenicity of *T. brucei* for domestic ruminants. The experience of workers in Uganda, I think, goes to show that an uncomplicated infection of *T. brucei* in cattle does not often, and certainly does not always, prove fatal. The deadly trypanosome for cattle is *T. congolense*, and it is often difficult to detect its presence. I feel sure that a great deal of the mortality caused by trypanosomes in cattle that has hitherto been referred to *T. brucei* is in reality due to *T. congolense*.

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3.

AN INVESTIGATION OF THE TRANSMISSIBILITY,  
BY LABORATORY-BRED *G. PALPALIS*, OF CERTAIN STRAINS  
OF POLYMORPHIC TRYPANOSOMES FROM MAMMALS  
OTHER THAN MAN.

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In this Paper we will consider the transmissibility of several strains of polymorphic trypanosomes of diverse origin. Some of them have already been described elsewhere and others have been isolated during the Commission's stay at Entebbe (Nos. XXIII, XXIV, XXV and XXVI).

These strains include :

(a) A trypanosome from East Africa sent to me from Kenya by Mr. MONTGOMERY, Veterinary Pathologist, Nairobi, and described in 1912.

(b) A strain of the Lake Victoria polymorphic organism recovered from wild-fly on the mainland shore in 1920.

(c) A similar strain isolated from a situtunga antelope shot on Damba Island in 1920.

(d) A wild-fly strain from the *G. swynnertoni* zone near Mwanza, isolated in 1922.

XXIII. A strain isolated from a Damba antelope in September 1926.

XXIV. A strain of *T. brucei* sent to the Laboratory in February 1926 by Professor KLEINE.

XXV. A polymorphic organism found by Captain HALL, Veterinary Pathologist, Uganda, in cattle from the Gomba county, in Uganda.

XXVI. A strain of *T. brucei* from a dog infected in a *G. morsitans* area near Mbarara, in the Western Province of Uganda.

Our object is to study the transmissibility of these strains ; we are not concerned with their animal reactions.

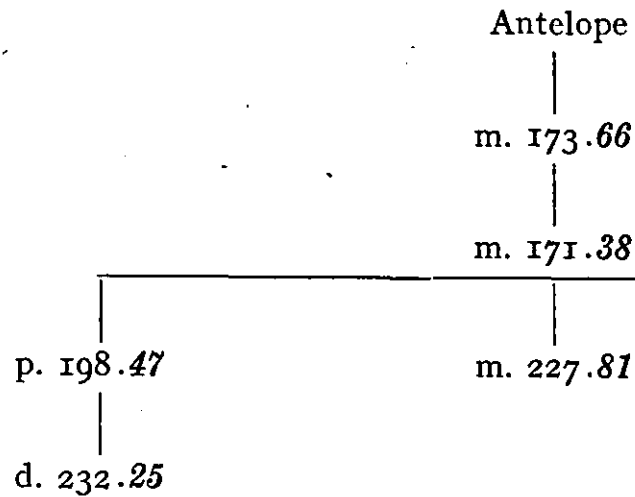
(a) A polymorphic trypanosome from the northern part of Kenya Colony, where the prevailing tsetse species are *G. longipennis*, *G. pallidipes*, and *G. brevipalpis*.

This organism has been fully described elsewhere. (DUKE, 1913*d*). The figures given hereafter are extracted from that Paper.



(c) Damba antelope strain, 1920. (DUKE, 1921.)

*Animal Inoculations:*



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
70	m. 173	11		22	18	40	36	1	2	0	,24 ,36	5 & 9. x. 20.
71	"	10		16	18	34	38	1	2	0	,10 ,10	5 & 9. x. 20.
77	"	11		21	27	48	30	1	0	1	,27	13 & 16. x. 20.
78	"	11		26	33	59	32	1	0	1	,32	13 & 16. x. 20.
81	"	10		21	20	41	24	1	0	0		17 & 19. x. 20.
82	"	11		28	22	50	39	1	1	0	,29	17 & 19. x. 20.
83	m. 171	8		31	22	53	41	2	1	0	,10	19 & 21. x. 20.
84	"	8		24	27	51	38	2	2	0	,34 ,36	19 & 21. x. 20.
112	m. 227	8		40	23	63	44	3	1	0	,18	21 & 24. i. 21.
114	"	10		27	35	62	52	3	0	1	,52	24 & 26. i. 21.
115	"	12		27	26	53	51	3	1	0	,51	26 & 27. i. 21.
118	"	8		36	28	64	61	3	1	0	,20	26 & 27. i. 21.
11	d. 232	7		18	39	57	32	3	1	0	,11	24 & 26. i. 21.
113	"	10		21	37	58	31	3	0	2	,31 ,31	24 & 26. i. 21.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 173	Oct. '20	272	7	2.5	2	4	0.7	1.2
m. 171	"	104	3	2.8	0	2	0	0
d. 232	"	357	7	1.9	3	4	0.8	1.4
m. 227	"							

(d) A wild-fly strain of *T. brucei* (or *T. rhodesiense*) from *G. swynnertoni* zone, near Mwanza, 1922. (DUKE, 1924a).

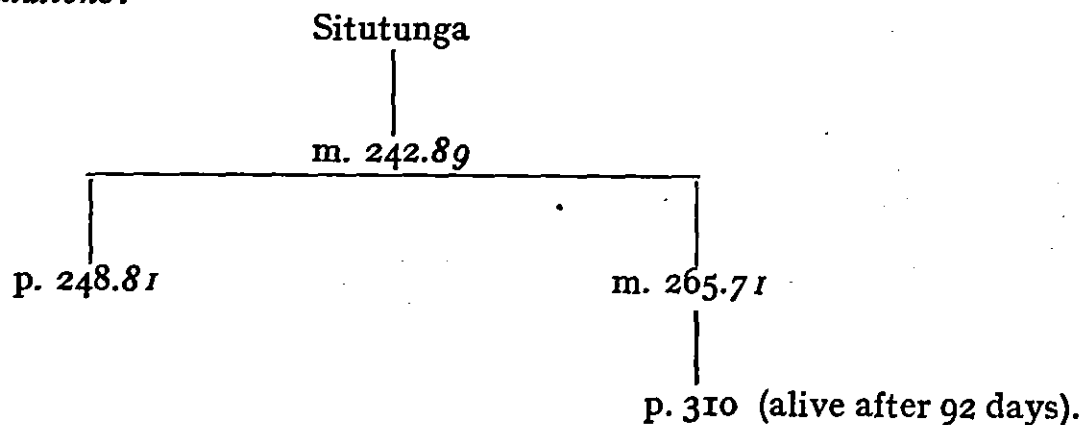
*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
	Sept. '22	535	25	4.6	14	18	2.6	3.5

STRAIN NO. XXIII.

Isolated from a Damba situtunga in September 1926.

*Animal Inoculations:*



*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			It gut only.	In gut and salivary glands.		
277	m. 242	9	23	25	18	43	34	0	0		19 & 21. XI. 26.	
278	"	10	35	27	22	49	32	0	0		20 & 22. XI. 26.	
285	"	9	32	15	22	37	33	0	0		25 & 27. XI. 26.	
290	"	8	41	25	25	50	32	0	0		29. XI. & 1. XII. 26.	
307	"	8	37	27	19	46	32	0	0		14 & 16. XII. 26.	
311	"	5	32	20	25	45	32	1	0	,6 (only a few trypanosomes seen)	16, 17 & 18. XII. 26.	
318	"	10	30	23	17	40	34	1	0	,24 (only 3 trypanosomes seen)	22, 23 & 24. XII. 26.	
365	g. 215	6	25	14	24	38	31	0	0		22 & 24. I. 27.	
369	"	4	23	16	32	48	31	0	0		24, 25 & 27. I. 27.	
372	"	11	30	35	14	49	33	0	0		26, 28 & 29. I. 27.	
377	"	5	31	26	27	53	32	0	0		29 & 31. I. &	
385	"	6	29	25	18	43	30	0	0		1. II. 27.	
365	m. 265	5	29	21	21	42	31	0	0		3, 5 & 6. II. 27.	
370	"	7	28	20	26	46	31	0	0		21 & 24. I. 27.	
371	"	5	32	18	21	39	32	0	0		24, 26 & 27. I. 27.	
375	"	6	35	20	28	48	31	0	0		25, 27 & 28. I. 27.	
376	"	6	35	20	28	48	31	0	0		28, 30 & 31. I. 27.	
383	"	7	24	29	22	51	32	0	0		29 & 31. I. & 1. II. 27.	
417	d. 297	4	22	28	23	51	30	0	0		2, 4 & 5. II. 27.	
420	"	7	18	14	15	29	35	0	0		24, 25 & 27. II. 27.	
428	"	9	25	19	16	35	36	0	0		25, 27 & 28. II. 27.	
428	"	6	25	27	13	40	39	0	0		3, 5 & 6. III. 27.	
436	"	9	30	17	19	36	36	0	0		11, 13, & 14. III. 27.	

*Transmissibility Index:*

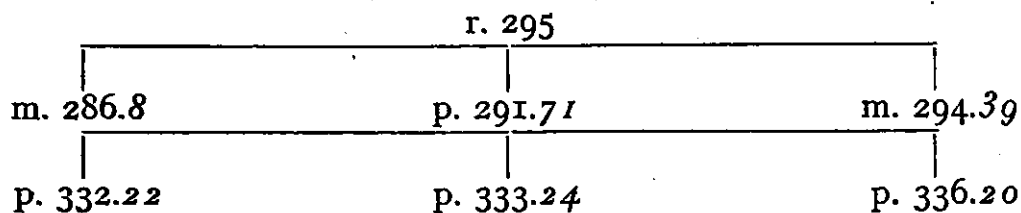
Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
	Nov. '26 March '27	958	2	0.2	0	0	0	0

This strain has been fairly thoroughly examined. In order to see whether changing the species of host affected the transmissibility, the strain was introduced into a goat and a dog ; but the trypanosome remained throughout entirely non-transmissible. This strain will be referred to again in Paper No. 6.

**STRAIN NO. XXIV.**

A *T. brucei* strain sent to the Laboratory by Professor KLEINE from an ox in the Ikoma area.

*Animal Inoculations:*



In Paper No. 2 we have seen that the Transmissibility Index of *T. rhodesiense* in calves is unaccountably low. The fact that strain No. XXIV was derived from an ox must therefore be kept in mind in considering this Index of 2.8. On the other hand, it corresponds closely to the Index of *T. rhodesiense*, strain No. XXII, isolated from man in the same area.

STRAIN NO. XXV.

A polymorphic organism in an ox belonging to Captain HALL, probably infected in Gomba county; nothing definite is known of the origin of this strain. (Cf. Paper No. 2.)

*Animal Inoculations:* nil.

*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
300	ox	7	21	26	9	35	32		0	0		8 & 12. XII. 26.
301	"	5	29	19	20	39	32		0	0		9 & 10. XII. 26.
306	"	12	28	22	14	36	32		0	0		13 & 16. XII. 26.
312	"	5	32	21	25	46	31		0	0		17 & 18. XII. 26.

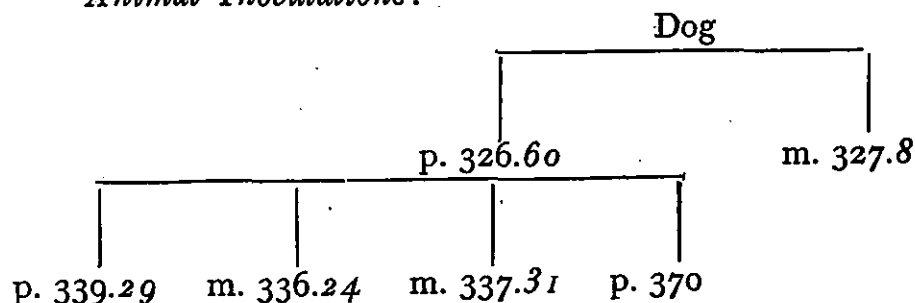
*Transmissibility Index:*

The strain gave no positive flies.

STRAIN NO. XXVI.

A *T. brucei* strain from a dog infected by *G. morsitans* in the Western Province of Uganda.

*Animal Inoculations:*





*Transmission Experiments:*

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 26th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			In gut only.	In gut and salivary glands.		
336	m. 336	6	33	23	24	47	32	2	0	1	:32	11, 13 & 14. v. 27.
		12	33	29	22	51	32	2	0	0		17, 19 & 20. v. 27.
337	m. 337	7	49	26	27	53	33	2	1	0	:29	18 & 19. v. 27.
		6	32	24	27	51	32	2	0	0		13, 15 & 16. v. 27.
338	"	5	24	22	23	45	33	2	0	1	:33	18, 20 & 21. v. 27.
		7	30	19	23	42	35	2	0	0		21, 23 & 24. v. 27.
500	"	11	31	18	28	46	36	2	2	0	:19, 25	22, 24 & 25. v. 27.
		7	36	22	21	43	35	2	2	0	:23, 35	26 & 27. v. 27.

*Transmissibility Index:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility index.
					With glands infected.	Total.		
m. 336 & 337	May '27	378	7	1.8	2	5	0.5	<b>0.7</b>

This Index is very low for a strain from a *G. morsitans* area. Dogs cannot, however, be considered as normal hosts for these trypanosomes, and it is possible that the extreme pathogenicity of the strain in this animal has influenced the transmissibility.

\* \* \*

These experiments convey an impression — it amounts to nothing more — that the game trypanosomes from *G. palpalis* areas have, on the whole, a low Transmissibility Index; lower, indeed, than that possessed by strains derived from game-tsetse areas.

4.

FURTHER EXPERIMENTS ON THE EFFECT  
OF DIRECT TRANSMISSION ON AN ANTELOPE TRYPANOSOME  
OF THE POLYMORPHIC GROUP.

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The following experiments were performed with a strain of polymorphic trypanosomes isolated from the blood of a situtunga antelope shot on Damba Island early in February 1924. The blood was inoculated into monkey No. 839, and the strain passed from monkey to monkey by the syringe until the 34th passage, when a guinea-pig was introduced. By this time the strain had lost its transmissibility.

The technique is thus the same as in previous experiments of the same kind, and the species of monkey used was the same green *Cercopithecus* with the black face.

From time to time in the series of passages the transmissibility of the strain was examined by feeding clean laboratory-bred *G. palpalis* on the monkeys.

Details of these transmission experiments will not be given; they would cover several pages, and their presentation can serve no useful purpose.

The essential data needed to calculate Transmissibility Indices of the different passages are given in Table I below, and from this a good idea can be obtained of the behaviour of the strain in this respect.

For carrying on these experiments during my absence on leave, and thus ensuring the continuity of this somewhat wearisome investigation, I have to thank Dr. M. MARTIN, then Acting Deputy Director of Laboratory, Uganda.

The series began in February 1924 and terminated in January 1927. As in previous papers, the figures in italic represent the duration, in days, of the disease in the infected animal; the number that follows shows the day of the disease in the animal of the preceding passage on which subinoculation was performed.

Thus

“m. 839

|  
m. 872. 215. 61 ”

tells that monkey No. 872 was inoculated from monkey No. 839 on the 61st day of the disease in No. 839, and then lived 215 days.

*Plan showing the Upkeep of the Strain by Direct Passage from Monkey to Monkey.*

	m. 839 inoculated	from situtunga shot on Damba Island;
Passage.		positive 21. 11. 24.
1.	m. 872. 215. 61.	
2.	m. 890. 53. 44.	
3.	m. 892. (?) . 46.	
4.	m. 902. 310. 22.	

5.	m. 903.39.1	
6.	m. 913.19.1	
7.	m. 916.44.9	
8.	m. 923.32.2	
9.	m. 938.58.2	
10.	m. 952.113.	
11.	m. 953.10.1	
12.	m. 955.(?)1	
13.	m. 958.255.	
14.	m. 959.108.	
15.	m. 972.115.	
16.	m. 978.85.6	
17.	m. 980.34.5	
18.	m. 993.20.2	
19.	m. 996.57.1	
20.	m. 999.33.2	
21.	m. 9.19.22.	
22.	m. 13.69.19.	
23.	m. 16.62. 8.	
24.	m. 31.78.22.	m. 26.74.20.
25.	m. 42.15.20.	m. 39.(?).18.
26.	m. 46.70.14.	m. 71.40.54.
27.	m. 86.26.58.	
28.	m. 102.138.1	
29.	m. 152.22.63.	
30.	m. 180.240.	m. 178.115.
31.	m. 229.34.	m. 228
32.	m. 257.32.	m. 233.60.
33.	m. 263.33.	m. 264.23.
34.		p. 285.61.
35.		m. 292.65.

TABLE I.

The first column shows the passage to which the Transmissibility Index belongs. The transmissibility was not examined at every passage, but at frequent intervals — as frequently, in fact, as the requirements of other and more important investigations permitted.

*Direct Transmission:*

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
Orig.	June '24	195	2	1	1	1	0.5	1.0
1.	"	165	1	0.6	1	1	0.6	0.6
2.	"	278	10	3.5	4	8	1.4	1.7
3.	June & Sept. '24	96	2	2	2	2	2	2.0
4.	Aug. & Sept. '24	1,006	17	1.6	6	12	0.6	0.8
5.	Nov. '24	338	1	0.29	1	1	0.29	0.29
6.	"	223	5	2.2	1	3	0.4	0.7
9.	"	180	5	2.7	2	3	1.1	1.8
13.	Apr. '25	201	1	0.49	0	0	0	0
14.	"	290	4	1.3	2	2	0.6	1.3
16.	Sept. & Oct. '25	445	8	1.5	2	4	0.3	0.7
17.	Oct. '25	261	5	1.9	2	2	0.7	1.9
19.	Nov. '25	189	3	1.5	2	3	1.0	1.0
20.	Dec. '25	251	2	0.79	1	1	0.3	0.79
22.	Jan. '26	343	3	0.8	1	3	0.2	0.2
23.	Feb. & March '26	357	5	1.4	2	4	0.5	0.7
24.	March '26	544	36	6.6	10	22	1.8	3.0
25.	March & May '26	456	16	3.5	4	12	0.8	1.1
28.	June & July '26	472	40	8.4	8	29	1.6	2.3
29.	Aug. '26	644	58	9	13	45	2.0	2.6
30.	Oct. '26	550	61	11	7	31	1.2	2.4
32.	Dec. '26	440	2	2	0	2	0	0
33.	Jan. '27	253	1	0.3	0	0	0	0
35.	Feb. '27	253	1	0.3	0	0	0	0
	March '27	171	1	0.5	0	0	0	0

The behaviour of this strain is thus similar to that of the other strains examined in former years. (DUKE, 1924.) Loss of transmissibility took place between the 30th and the 32nd passages. The transition stage, when only gut-infections develop, was not seen; presumably this stage was passed in monkeys Nos. 229 and 228. The experiments carried out at the 32nd passage showed that in both the monkeys of this

passage, Nos. 257 and 233, the strain was non-transmissible. At the 33rd passage a single positive fly was obtained on the seventh day ; but it is doubtful whether this was a case of genuine development, as only a few flagellates were seen. The infected fly at the 35th passage, which died on the eighth day of the experiment, contained only two or three flagellates in all its intestine.

It is interesting to note the increase in both infectivity and transmissibility that took place between the 24th and the 29th passages.

The last cyclical transmission of this strain occurred at the 30th passage. Several clean monkeys were cyclically infected from monkeys Nos. 178 and 180. One of these, No. 225, was fed upon by clean flies to see whether a cyclical passage, interposed after 30 direct passages and just at a time when the power to invade the glands was on the point of disappearing, would restore the transmissibility of the strain. Unfortunately, it was not possible to examine very thoroughly the behaviour of the strain in No. 225. The results obtained were as follows :

Animal.	Date.	Total flies dissected.	Total infected flies.	Percentage of infected flies.	Number of infected flies alive on 25th day.		Percentage of gland infections.	Transmissibility Index.
					With glands infected.	Total.		
m. 225	June '26	123	2	1.5	0	2	0	0

It will be seen from Table I that the percentage of infected flies at the 30th passage was 11 ; at the 32nd passage it had dropped to 2, and the transmissibility to zero. If the processes of cyclical transmission had revived the strain and restored its weakening transmissibility, we should have expected a fairly high percentage of positive flies in the experiments fed on monkey No. 225. The low figure actually obtained suggests then that cyclical passage through the tsetse has not noticeably affected the constitution of the trypanosome, and has not arrested its progress towards complete loss of power to develop in the fly. This inference supports that previously suggested by the experiments on the transmission of arsenic-fast strains given in the Interim Report of the Commission. (DUKE, 1927a.)

5.

THE TRANSMISSIBILITY OF TRYPANOSOMES BY *GLOSSINÆ*:  
SOME REFLECTIONS ON THE SPREAD OF HUMAN  
TRYPANOSOMIASIS.

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In this Paper we will discuss some of the more obvious factors that influence the distribution in nature of the two human trypanosomes known as *T. gambiense* and *T. rhodesiense*.

We will include in our survey certain allied species, the polymorphic trypanosomes of game and domestic stock, as these organisms are easier to study experimentally and their behaviour in the insect intermediary is essentially the same.

The special object of the enquiry is to attempt to explain the way in which human trypanosomiasis spreads. Attention will therefore be directed especially on the relation of these trypanosomes to the insect intermediary.

\* \* \*

The species of trypanosome that infect man and his domestic animals in Africa may roughly be distinguished from other less important mammalian trypanosomes as the "pathogenic group". It is the economic disturbance they produce that has brought them into prominence.

For the purpose of this discussion, we will confine ourselves to these so-called pathogenic species. They include the *vivax*, the *congolense* and the polymorphic groups, on the one hand, and the monomorphic species such as *T. evansi* and *T. equiperdum*, on the other.

If we examine the distribution of these organisms in Africa, it is at once apparent that the first three groups are to all intents and purposes conterminous with the distribution of the *Glossinæ*, whereas the monomorphic species are found mainly outside the tsetse zone. For example, though strains indistinguishable from *T. evansi* may be found in tsetse areas, the main distribution of this species is in Northern Africa, a region where the three first-mentioned groups do not occur.

If we restrict our field still further to the human trypanosomes, we find that these parasites, as far as is known, cannot spread from man to man in the absence of tsetse.

In keeping with these two main divisions of the pathogenic trypanosomes — the tsetse-dependent and the tsetse-independent — we find that the latter, *T. evansi* and *T. equiperdum*, are not transmissible by *Glossinæ*, whereas the former are as a general rule capable of cyclical development in tsetse.

Between these two extremes lies the *congolense* group of cattle and game trypanosomes. These are primarily dependent on cyclical passage through tsetse for their survival in nature, but there are reasons for believing that they may sometimes be propagated directly by biting flies other than the *Glossinæ*. *T. congolense* and its very near relative *T. nanum* are very commonly found established cyclically in wild game-tsetses; but their development in *G. palpalis* is much less certain, and calls for careful investigation. The *vivax* and the polymorphic groups are, on the other

hand, found in nature alike in the game-tsetses and in wild *G. palpalis*. Again, in transmission experiments with *G. palpalis*, the *congolense* group behaves in a less regular manner than the other two. (DUKE, 1912*d* and 1923*b*.)

The three main groups into which fall the tsetse-dependent species are distinguished from one another by pronounced morphological characters, and also by their behaviour in *Glossinæ*. It is now well known that in all these species cyclical development is only complete when the infecting or metacyclic forms appear in the "anterior station" in the fly. This "anterior station" is for the polymorphic group the salivary glands, for the *vivax* and *congolense* groups the proboscis; the last two differ from one another in that *T. congolense* utilises the gut as well as the proboscis, whereas in *T. vivax* the whole cycle takes place in the proboscis.

There is little doubt that the *Glossinæ* are the natural intermediate hosts of these trypanosomes. But, since a tsetse can transmit a trypanosome by two methods, the direct and the cyclical, it behoves us to try and determine in which main capacity the *Glossinæ* act in nature in spreading human trypanosomiasis — as direct or as cyclical transmitters.

The biting flies that attack man in Africa are the mosquitoes and their allies, *Simulium*, and the *Glossinæ*. Man cannot be regarded as a regular host for the large number of species that infest game and stock — the Tabanids, *Hæmatopota*, *Lyperosia*, *Stomoxys* and *Chrysops*; though *Chrysops* is in certain localities a serious pest to man.

All of these flies will bite man on occasion, but they do not depend on him for food. Some of them are known to transmit trypanosomes in nature, and others are under grave suspicion. They function as direct, not cyclical, transmitters: as far as is known, there is no cyclical development of any of the pathogenic African trypanosomes in other flies than the tsetses.

Thus, once a trypanosome has lost the power of infecting the glands of *Glossinæ*, it has only direct transmission between it and extinction on the death of its host.

I have discussed elsewhere at considerable length the possibility of the transmission of human trypanosomiasis by the direct method by tsetse. (DUKE, 1919.) Epidemiological studies have shown that it is exceedingly improbable that mosquitoes play any rôle in the spread of the human disease, and no other species of fly has hitherto fallen under suspicion; we have seen, too, that man is, to all intents and purposes, outside the regular feeding range of the proved direct transmitters of animal trypanosomes.

The investigations made by this Commission have not supplied any evidence of the occurrence of direct transmission of trypanosomes from man to man. That it ever occurs in the endemic form of the disease is very improbable; but it is possible that it does happen occasionally, and this fact must be borne in mind in dealing with the movements of native populations in such areas. In order that the direct method may operate, trypanosomes must be at any rate fairly numerous in the peripheral blood, and there must also be close contact between hosts.

With a virulent strain of parasite in a crowded community, where there are plenty of hungry tsetse and a limited number of reptiles and hippo, it may well be that the trypanosome can pass easily from man to man by the direct method.

My attention was first focussed on the possible importance of this method by the study of the history of the Uganda epidemic.

During the last few years, experiments have been in progress at Entebbe on the effect of repeated direct transmission on various strains. In each case, after a year or so, the strain — easily transmissible on its first isolation — lost its power to develop in tsetse, and changes in morphology and virulence also took place.

These results lent support to the opinion that direct transmission might play an important part in the epidemiology of the disease. It was only when my inclusion in this Commission enabled me to concentrate on the study of the human trypanosomes that I realised the extreme variability of these organisms in relation to their intermediate host.

I now know that the changes which appeared after two years or more of direct transmission, involving some thirty passages, may occur, for reasons at present unknown, at any moment in the life of a strain, with or without the operation of direct transmission. In other words, the transmissibility of a trypanosome is a very variable and unstable character.

As a result of recent work, I am inclined to modify, but not to abandon, my original views on direct transmission.

First of all, it is evident from experiment that this method may function for a long time without producing any effects at all on the strain; and, as BRUMPT has pointed out, it will be rare in nature that conditions exist in which prolonged direct transmission can occur. (BRUMPT, 1924.) Secondly, other causes, at present unknown, may produce the same effects, and may produce them suddenly.

On the other hand, it must still be admitted that direct transmission is one of the factors that may affect the constitution of a trypanosome in its natural surroundings; and the sequence of events that ensued in the Laboratory experiments may, in different circumstances, operate much more rapidly.

In passing, it may be mentioned that there is no evidence that a tsetse cyclically infected by a trypanosome is *infective* until the metacyclic forms have appeared in the "anterior station" — be this labium, hypopharynx or the salivary glands KINGHORN, YORKE and LLOYD obtained positive results by injecting the gut-contents of certain flies infected with *T. rhodesiense* (YORKE, 1912); but, even if this result be not explained by leakage from the glands, the combined experience of those who have worked with the two human parasites goes to show that the trypanosomes developing in a *Glossina* cannot be transmitted by the fly during the act of feeding unless the salivary glands of the insect contain the metacyclic forms.

The weight of evidence at the present time thus points to the metacyclic forms of the human trypanosomes as the main infecting agents. Two biological considerations lend support to this view; human trypanosomiasis never spreads in the absence of *Glossinæ*, and the *Glossinæ* are the only insects in which these trypanosomes are known to develop cyclically.

From a human point of view, therefore, the power of these trypanosomes to develop cyclically in tsetse is of supreme importance, and the transmissibility of a strain is one of its most significant attributes <sup>1</sup>.

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<sup>1</sup>Dr. VAN HOOFF has kindly directed my attention to two cases of Europeans who were, beyond all reasonable doubt, infected by the bite of a single cyclically infected *Glossina*:

Case No. 1. — Male, working in an office, who had never visited any tsetse area and had but recently arrived from Europe for the first time. While at work one day in his office, found a tsetse, which bit him. In the town where he was stationed tsetse were very rarely to be found, being only very occasionally brought in by porters or arrivals by canoe. He suffered from fever several days later, and the medical man, on making an examination before administering quinine, found trypanosomes in his blood. (RODHAIN.)

Case No. 2. (observed by Dr. VAN HOOFF himself). — A trained lady hospital assistant from the Brussels School of Tropical Medicine. After an epidemic of hog-cholera that destroyed large numbers of pigs, tsetse, *G. palpalis* and *G. tachinoides* appeared in the station searching for food. During a siesta, the patient was bitten by a tsetse; the bite was painful, became erythematous, and the local signs persisted for some five weeks. Fever, digestive troubles, rheumatic pains, and Kerandel's sign developed, and numerous trypanosomes were found in a thick film of her blood. The erythematous patch on the leg disappeared immediately after the first injection of trypanamide.



The virulence of a strain, on the other hand, appears to be entirely independent of its transmissibility. Our strains of *T. gambiense* may thus be grouped either according to their virulence in man or according to their transmissibility by *Glossina*. But these groupings do not necessarily correspond or coincide in any way. One might look upon the transmissibility of a trypanosome as its virulence for the tsetse; whereas the virulence, in the ordinary sense of the word, is to be estimated by its action on the mammalian host, *i.e.*, rapidity of invasion, onset of nervous symptoms, resistance to drugs, etc.

We will now consider the phenomenon of loss of transmissibility as exemplified by certain strains of polymorphic trypanosomes, both in the laboratory and in nature.

The manner in which a trypanosome loses the power to develop in tsetse is shown by the behaviour of three strains already studied at the Entebbe Laboratory. (DUKE, 1924a.)

All these three strains were at their first isolation readily transmissible cyclically by laboratory-bred *G. palpalis*. The first originated from wild lake-shore *G. palpalis*; the second from a native infected with *T. rhodesiense* from the Mwanza infected area; and the third is the strain dealt with in Paper No. 4.

The power to invade the glands of the fly disappears first. For a time the strain remains able to develop in the intestine, and may give rise to a relatively high percentage of infected flies. Gradually, however, this power is also lost, the first indication being the appearance of very slight gut-infections in flies of relatively advanced age: for example a ++ or a .+ condition of the intestine in flies dissected from thirty to forty days after their original infecting feed instead of the normal ++++. In a readily transmissible strain, infections of this kind are rarely if ever found. These slight infections must, of course, be distinguished from the early stage of a secondary infection due to the fly having taken up trypanosomes during the incubation period in the hitherto clean animal, before parasites were visible in the peripheral blood.

The final stage consists in the complete loss by the trypanosome of all power to develop, even in the gut.

Turning now to the various strains collected by the Commission, it will be seen that they comprise examples of all these stages.

Strain No. XXIII, the latest acquisition from the island of Damba, and human strain No. XVII, have all but completely lost the power of development in *G. palpalis*; strains Nos. XVIII and XIX are entirely non-transmissible; strains Nos. III, VI and VIII cannot infect the salivary glands of the fly; strain No. IV, though cyclically transmissible on its first recovery, very soon became non-transmissible on being passed cyclically from host to host; whereas strain No. V has remained transmissible for over a year, during which time it has undergone six cyclical passages in ruminants.

Possibly, had they been studied with the same care, other strains would have been found to maintain their transmissibility as well as No. V. This strain has, however, always been distinguished by its readiness to develop in tsetse, and may be regarded as somewhat exceptional in this respect — at any rate, for the infected areas hitherto studied.

The difference between the transmissibility of the different strains is very striking, and must make a strong appeal to the student of the epidemiology of human trypanosomiasis.

It may be objected that the conclusions drawn in these Papers are not warranted by the evidence, and that the number of flies is too small. This criticism is, of course, unanswerable at present. But I have acquired a strong opinion that, with the

technique described above, it is possible, with a comparatively small number of laboratory-bred flies, to obtain a very good idea of the transmissibility of a strain. This point will be considered later on.

\* \* \*

Let us now discuss the various factors that may influence the transmissibility of the trypanosome, and try to explain this strange crisis in its life-history. Four possible explanations present themselves : the determining cause may be : (1) the fly ; (2) climate ; (3) the vertebrate ; (4) the trypanosome itself.

### 1. *The Fly.*

Muriel ROBERTSON, in discussing possible explanations for the variation in the percentage of flies infected by different strains of trypanosomes, or by the same strain at different times, concluded for various excellent reasons that the physiological variations of individual flies played little or no part in determining these differences. She decided that the explanation lay rather in the properties of the trypanosomes taken up by the tsetse. (ROBERTSON, 1913.) KLEINE came to the same conclusion as a result of his transmission experiments in the Tanganyika region.

My own experiments have afforded additional evidence that the tsetse itself is neutral in regard to the subsequent development of the trypanosomes it takes up from the infected animal ; in other words, if the appropriate forms of the parasite are taken up by the fly, they will develop.

The following considerations support this conclusion :

(a) Different strains possess different degrees of transmissibility when first isolated from their natural host. These differences are manifested by the number of positive flies they produce when exposed to laboratory-bred *G. palpalis*. Where these differences are sufficiently striking to be easily demonstrable, it is found that they remain constant, for a time at any rate, whenever these strains are subjected to transmission experiments with laboratory-bred flies.

(b) Non-transmissible strains are consistently non-transmissible, no matter how many tsetse are employed in their investigation. Such strains as No. III readily established themselves in the gut, but invariably failed to reach the glands of the fly. These facts naturally require confirmation, and time alone can give this ; but they are based on considerable practical experience.

(c) The extent to which flies become infected when fed on an animal varies according to the day or days on which they feed. A box fed on an infected animal to-day may yield 10 per cent positive flies ; another, fed on the same animal to-morrow, none — and so on.

(d) The diet of the fly appears to exert no effect on the development of the flagellates, nor does their presence appear to harm the fly in any way.

Whether all species of *Glossinæ* are equally efficient in spreading trypanosomes in nature is another question altogether. The habits and particularly the food propensities of the different species of tsetse may, for all that we know to the contrary, exert an important influence on the trypanosomes they transmit. These are points that demand investigation.

### 2. *Climate.*

ROUBAUD and BAGSHAWE have both put forward the suggestion that climatic influences may affect the transmission of trypanosomes by tsetse. ROUBAUD also suggested that there exist geographical races of tsetse which differ from one another

in their ability to transmit trypanosomes. He invokes this explanation in discussing the strange failure of the disease to spread in certain circumstances. (ROUBAUD, 1920.)

ROUBAUD'S results are, however, explicable on the assumption that he was working with non-transmissible strains, a possibility that was not at the time appreciated. KLEINE'S experiments on the effect of climate are also easily explicable on the same basis; moreover, it is clear from his other publications from this region that non-transmissible strains of *T. gambiense* did exist in his field of work. KINGHORN, YORKE and LLOYD have produced evidence that extreme variations in climate may affect the cyclical transmission of a trypanosome by tsetse. But it is unlikely that the seasonal differences in any given infected area will be sufficiently pronounced to produce a similar effect to those obtained in their experiments. (KINGHORN, 1913.)

### 3. *The Host.*

It can be affirmed with some confidence that, of the mammalian hosts ordinarily available to the trypanosome in nature, no species exerts a definite and immediate inhibitory effect on the transmissibility of the parasite by tsetse. More than this cannot be said, the reactions of the trypanosome are so inextricably involved with those of the host.

We shall see later on that there is reason to believe that certain species of antelope are the hosts best suited to the biological requirements of the polymorphic trypanosomes.

### 4. *The Trypanosome.*

By a process of exclusion, from the experience of workers such as ROBERTSON and KLEINE, and by the results given by my experiments, we are led to the conclusion that the transmissibility of a trypanosome by *Glossina* is a function inherent in the trypanosome itself; but the close relationship existing between the trypanosome and its vertebrate host makes it highly probable that the latter exerts a very potent influence on the conduct of the protozoon.

The first observer to recognise the existence of non-transmissible strains of polymorphic trypanosomes was ROUBAUD, who, working in Dahomey with introduced strains of *T. brucei*, *T. evansi* and *T. gambiense* from the Pasteur Institute in Paris, found that he could not transmit them by *G. palpalis*. (ROUBAUD, 1910.)

Later on, KLEINE, in Tanganyika, noted the disappearance of transmissibility in certain strains of *T. gambiense* with which he was working. In 1912, he came very near to the explanation to which I have been led by my work with this Commission. The following is a translation of what he wrote:

"It may be remarked incidentally that, as is well known, in spite of apparently favourable conditions, the disease very often does not spread. In these cases apparently the trypanosomes have lost the capability of developing in *Glossina*. The causes of this are not clear. As a matter of fact, we find occasional strains of trypanosomes with which it is impossible or very difficult to infect *Glossina*." (KLEINE, 1913.)

A year later, KLEINE again refers to the disappearance of transmissibility in strains of *T. gambiense*. His explanation of the phenomenon was that strains that have recently passed through tsetse are readily transmissible, but that prolonged sojourn in the mammal led to a diminution of transmissibility. (KLEINE, 1914.) KLEINE did not, however, develop these views; he makes no reference to the possible influence of non-transmissible strains in his interpretation of his transmission experiments with ruminants, etc.; nor did he investigate the frequency of these

strains in nature. For reasons which are not quite clear, he seems to have attached comparatively little importance to the phenomenon in considering the epidemiology of human trypanosomiasis.

It was REICHENOW who first reported the existence of non-transmissible strains of *T. gambiense* in man himself. (REICHENOW, 1921.) REICHENOW found that flies fed upon "old cases" of human trypanosomiasis due to *T. gambiense* did not develop infections of the salivary glands, and that even simple gut-infections were rare. Unfortunately, he does not give details as to the clinical condition of these cases, nor do we know how the flies were applied. Nevertheless, it is highly probable that REICHENOW was dealing with genuine non-transmissible strains of *T. gambiense*; moreover — and this is very interesting and important — he was experimenting directly on the patient.

Among the strains I have studied recently at Entebbe, six were in all probability non-transmissible — four in the patients themselves (strains Nos. VIII, XVII, XVIII and XIX), one in the monkey subinoculated from the patient (strain No. V) and one in its second passage from man (strain No. III).

The experiments of Paper No. 1, Part II, suggest that *T. gambiense*, after a few months' sojourn in certain mammals, generally tends to lose its transmissibility. This is noticeable in the case of sheep and goats, and it is perhaps dangerous to infer too much from the behaviour of this somewhat specialised parasite in such hosts. But the same process is apparent in monkeys; and, on the other hand, the discovery of the non-transmissible strain in the Damba antelope (strain No. XXIII) shows that this phenomenon also occurs in game-trypanosomes in nature, at all events in *palpalis* areas.

In sharp contrast to these results stands the behaviour of the antelope infected at Mpumu Laboratory with *T. gambiense*. (DUKE, 1912*b*.) Seven of them were found able to infect the glands of laboratory-bred *G. palpalis* respectively five, six, thirteen, six, nine, eight and ten months after the original infection of the buck. These figures, too, are a minimal estimate. Moreover, in the case of one of the reedbuck and one of the bushbuck, blood inoculations into monkeys showed these animals to be still harbouring trypanosomes fifteen and twenty-two months respectively after infection. The trypanosomes recovered in these monkeys proved to be very readily transmissible. The Transmissibility Index of the reedbuck strain was 5.2 when first recovered in the monkey, and ROBERTSON speaks of both these two strains as giving an unusually high percentage of heavily infected flies. (ROBERTSON, 1913.) Apparently, therefore, these antelope are biologically more perfectly adapted to the requirements of *T. gambiense* than are domestic ruminants. We will revert to this point again later on.

What the experiments given in these Papers have revealed, and what the German workers were not in a position to realise, is that a strain may lose its transmissibility very rapidly on transference to a new host, even though the passage may be effected by cyclically infected fly. This is illustrated by the behaviour of strains Nos. IV and VII, in subpassage into goats.

REICHENOW's observations are of great interest. The details of his experiments are not given, and it is impossible to see how he applied the flies used in testing his "old cases", or to ascertain the exact stage of the disease. Assuming, however, that the flies fed for several consecutive days — in other words, that every opportunity was given to them of picking up the infecting forms — then these experiments form a valuable confirmation of my own. It is a pity that details are not given of the

condition of the cerebro-spinal fluid in these cases. REICHENOW's view on the significance of the changes shown by the cerebro-spinal fluid in human trypanosomiasis are, however, at variance with those held by this Commission.

My own experience with flies fed upon infected man has been somewhat different. The cases examined at Entebbe included several, such as Nos. II, IX, X, XII and XIX, that were far advanced in the disease, but no typical chronic cases of several years' standing have yet been studied.

In all our advanced cases, the trypanosomes were transmissible; in fact, strain No. IX gave the second highest Transmissibility Index of any polymorphic strain hitherto examined. REICHENOW obtained a certain number of intestinal infections in his flies, which shows that at all events some of his strains had not completely lost touch with the tsetse: indeed, in the absence of details, it is difficult truly to appraise his results, in view of the established fact that on certain days the most transmissible of strains may fail to infect fly.

We are now ready to summarise the results of our survey of the behaviour of the human trypanosomes in relation to tsetse.

The trypanosomes in the majority of the human cases we have examined have proved to be transmissible by laboratory-bred *G. palpalis* at the Entebbe Laboratory. There is no valid reason for doubting that they are also transmissible by the same tsetse in nature — at all events, under approximately similar climatic conditions. In a minority of the cases the strain was non-transmissible.

The cases examined include early and advanced cases, judged by the standard of the changes in the cerebro-spinal fluid. No chronic cases of long standing have yet been studied at Entebbe.

REICHENOW's experience with cases of this kind goes to show that, in long-standing infections in man, the trypanosome loses its transmissibility. The same thing may happen when *T. gambiense* is conveyed by cyclically infected *G. palpalis* to clean domestic ruminants or to clean monkeys. In the early stages of the infection, the trypanosomes in these animals are, as a rule, transmissible; later on, the transmissibility diminishes, in many cases to vanishing point. Even after a few months' sojourn in these animals, the human trypanosomes may lose the power of invading the glands of the fly; later still, all power of development is lost. Different strains vary greatly in their transmissibility and in the tenacity with which they retain this property.

There is reason to suppose that the same series of events occurs in other species of polymorphic trypanosomes, such as *T. brucei* and the Damba trypanosome, when monkeys or sheep and goats are employed as hosts. It would seem, indeed, that, for the trypanosomes of this group at all events, loss of transmissibility is a normal sequela to prolonged sojourn in these mammals. In certain circumstances, the disappearance of transmissibility takes place quite suddenly. A strain may be cyclically transmitted from one animal to another, and from the moment of its establishment in the second animal be found to be non-transmissible.

Owing to the impossibility of investigating by direct experiment man's resistance to trypanosomes, we cannot determine with certainty whether man, as a host of the polymorphic trypanosomes, should be classed with these "experimental animals" or with the Mpumu antelope. But we have already seen that non-transmissible human strains are by no means uncommon, and epidemiological studies have shown that the spread of human trypanosomiasis is very often unaccountably slow and uncertain. It seems, therefore, a reasonable assumption that man is not by any means an ideal host for these parasites, and we may fairly class him with monkeys and domestic ruminants for the purposes of our argument.

All this evidence suggests a reasonable and satisfactory explanation of what occurs in native communities — an explanation, moreover, which accounts for many of the apparent anomalies in the spread of human trypanosomiasis. Loss of transmissibility is a danger constantly threatening the polymorphic trypanosomes in nature. The longer the parasite remains in its host, the less transmissible it becomes. Although individual strains vary greatly in stability, it is evident that it is of supreme importance to the trypanosome that it be frequently exposed to tsetse during the early stages of its invasion of the mammal, while it is still transmissible. This need will be fulfilled when susceptible hosts are numerous and the contact between them and the fly is close ; then the trypanosome will be found widely distributed, *i.e.*, a high percentage of the principal mammalian hosts infected, and the Transmissibility Index of the strain will be high. In nature, we find this state of affairs obtaining generally in game-tsetse areas ; in human communities, only where the disease is in epidemic form.

For several reasons, life is more difficult for the trypanosomes that depend on man. It appears probable that he is less susceptible, generally speaking, to trypanosomes than are the ruminants ; and this handicaps the transmission of the parasites, especially in game-tsetse areas. Then again, where *G. palpalis* is the principal or sole insect vector, the partiality of this fly for reptiles tends to reduce the contact between it and man.

And so it is that the human trypanosomes comparatively seldom enjoy the conditions that are necessary to epidemic spread, and, when they do, the mortality they produce in man soon puts an end to their career. We may not be certain that the *T. brucei* type of trypanosome never destroys its wild-game hosts ; but, at all events, we do know that it never produces a mortality in the game comparable to the havoc wrought by epidemic human trypanosomiasis.

But this frequent passage from host to host, in the early stages of its sojourn in the mammal, aids in another way the propagation of the trypanosome. *There will be frequent opportunity for reinfection or superimposed infection of the same host by fresh strains of trypanosomes.*

Experiments performed with the antelope at Mpumu showed that reinfection can occur, and that it is followed by a recrudescence of infectivity. (DUKE, 1913a.) In other words, an animal whose trypanosomes have from long sojourn become non-transmissible can, on reinfection with another strain, once again readily infect flies. Experiments on these lines are at present being carried out at Entebbe, and the results will be of interest and bear on our discussion.

These two factors acting together will ensure a plentiful supply of transmissible strains for the fly to take up and propagate.

The ideal adjustment between the trypanosome and its mammalian host would seem to be a minimum of pathogenicity combined with a maximum of transmissibility. How far this ideal is ever realised in nature we do not know ; probably the adjustment between host and parasite is nearest to perfection in the relation between the big game of Africa and their trypanosomes.

\* \* \*

In applying our explanation to *T. rhodesiense* we are met with more difficulty, because at present little is known about this trypanosome and its affinities.

Man's relative insusceptibility to trypanosomes undoubtedly plays an important part in determining his relation to this type of human trypanosomiasis. In *G. palpalis* areas — that is, where *T. gambiense* is the human trypanosome — man is, as a rule, the principal and often the only mammal available for the trypanosome. He is, *as*

far as is known at present, invariably susceptible to the metacyclic forms of this trypanosome inoculated by the bite of a *Glossina*. In game-tsetse areas, he is generally only an alternative food-animal for the tsetse, and of secondary importance to the trypanosome ; and, as long as game is available, his susceptibility does not seriously affect the survival of the parasite.

\* \* \*

Comparing the two human trypanosomes in respect of transmissibility, it appears that what we now call *T. rhodesiense* is better able to avail itself of the tsetse intermediary.

Of the nineteen *T. gambiense* strains studied by the Commission, six were non-transmissible when first examined, *i.e.*, 31.5 per cent ; 10.5 per cent of these strains showed a Transmissibility Index of 5 or over.

Of the five untreated *T. rhodesiense* strains we have been considering, all were transmissible when first examined, and 60 per cent showed a Transmissibility Index of 5 or over. But it must be remembered that many strains of *T. rhodesiense* run a rapid course in man, and the trypanosome has thus less chance of losing its transmissibility.

Our experience of freshly isolated *T. brucei* strains from game-tsetse areas is unfortunately too limited to justify any comparison with this organism.

So far as they go, these figures are in keeping with the explanation put forward above. In *T. rhodesiense* areas, the human strains represent strains in general circulation among the game of the area. They can be transmitted from man back to game, and their use of man as a host is largely incidental to the ultimate survival of their species.

*T. gambiense*, on the other hand, is essentially a trypanosome of which man is the chief mammalian host. It has had and still has a formidable task before it ; and, judged by biological standards, the success of the parasite is not very striking.

\* \* \*

Is it, then, mere prejudice to see in all these observations support for the contention put forward elsewhere that *T. gambiense* has diverged from a *brucei* stock ? and does not its comparatively imperfect adaptation to man suggest that this divergence took place, biologically speaking, not such a very long time ago ?

A practical question arising from this theory is : To what extent has natural selection fixed the difference between the two types ? In other words : Can a typical *T. gambiense* revert to the *brucei* type ? The importance of this point is obvious.

When I began work with this Commission, I hoped to be able to test this view by maintaining *T. gambiense*, by cyclical passage through tsetse, for a number of generations in domestic ruminants. The results obtained with these animals have shown that they are not good hosts for this trypanosome, and they are therefore unsuitable for experiments of this kind. Evidently, the only way to test this view is by the use of antelope such as those employed at Mpumu, in which the trypanosome seems thoroughly at home ; and I hope in the near future to be able to pursue this line of investigation.

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6.

NOTES ON THE  
BIONOMICS OF THE POLYMORPHIC TRYPANOSOMES  
ON DAMBA ISLAND, VICTORIA NYANZA.

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Damba Island is situated in Lake Victoria some four miles south of the coastline of the Chiagwe province of Buganda Kingdom, on the north-eastern edge of the Sesse group of islands.

The island is some six miles long by three broad at its broadest point. Near the middle it is almost cut in two, a narrow isthmus of a few hundred yards in width separating the heads of two great swamp-filled bays. During the Uganda epidemic of sleeping-sickness, the population of the island suffered severely, and was literally decimated by the disease.

Damba is forested from end to end ; entirely covered, in fact, save for a few small open spaces where rocky outcrops deny the trees a hold. The population was removed by Sir Hesketh Bell's orders when the islands were depopulated in 1909. In August and September of that year, the people left Damba and the neighbouring island of Kome for fly-free settlements on the mainland.

After the departure of the natives, the *sititunga*, of which there was always a considerable number in the great papyrus swamps that cap the deep bays of the coastline, began to multiply rapidly. There were no large carnivora, and the antelope, freed from their great enemy, man, had nothing to fear save the crocodile and disease. As time went on they increased exceedingly, and soon their habits changed and they spent more and more of their time on dry land, in the forest. By 1912, they had multiplied to an astonishing extent. The intensive grazing of all these animals changed the very nature of the forest, and the branches all along the shore-line were nibbled off to a height of from four to five feet in a sharp well-defined line.

Tsetse occurred all along the coastline, and also followed the animals far into the forest. At one point on the shore is the well-known breeding ground that for many years past has supplied pupæ to the Uganda Laboratories.

From the beginning of 1910, the infectivity of these flies has been studied from time to time, first from Mpumu and later from Entebbe. The species of trypanosomes found in the wild fly were a member of the polymorphic group, considered by BRUCE to be *T. gambiense*; *T. vivax*; and *T. uniforme*. The *congolense* group of trypanosomes has never been found in wild fly on the Sesse Islands.

In 1911, I isolated from four *sititunga* shot on Damba two strains of a polymorphic trypanosome whose reactions, carefully examined, appeared to resemble *T. gambiense* rather than *T. brucei*. (DUKE, 1912 *a* and *c*.) In 1924 and again in 1926, polymorphic trypanosomes were recovered from antelope on this island ; on both these occasions the strains showed many of the attributes of *T. brucei*. Between 1912 and 1926, a good deal of indirect evidence has been accumulated to show that these Damba trypanosomes are not pathogenic to man, and I conceived the idea that a change had occurred in the constitution of the trypanosome as a result of the long sojourn in antelope. (DUKE, 1921.)



From 1912 to 1926, I visited the island from time to time and examined the infectivity of the wild tsetse there, both by feeding the flies on susceptible animals and by dissection of freshly caught tsetse.

So much for the history of the island from the laboratory worker's point of view. We will now examine the experiments carried out since the depopulation of the island in 1909.

From January to June 1910, the Royal Society's Commission fed 6,456 wild Damba flies on seven monkeys. Two of these became infected with the polymorphic trypanosome which the Commission called *T. gambiense*. Supposing that only one fly in each positive experiment was infected, this gives a percentage of 0.03 infected flies at this date. (BRUCE, 1911.)

In May 1911, CARPENTER obtained an infection in a single experiment in which he fed 885 Damba flies on a monkey. (CARPENTER, 1912.) His object was merely to see whether the flies were still infective, and, having obtained this information, he made no further investigation. The percentage of 0.11 given by this single experiment cannot of course be taken as a definite indication of the infectivity of the fly in 1911.

Between September and December 1920, I fed 3,816 wild Damba flies on three monkeys. (DUKE, 1920.) All three monkeys developed the polymorphic trypanosome, *i.e.*, at least 0.07 per cent of the flies were infected. During this time, 625 flies were dissected and the gut and proboscis of each fly examined. The percentage of proboscis infections I called the "Game Index". The trypanosomes developing in this organ are essentially game parasites; and in a region such as Damba, where there are no cattle or domestic animals, the percentage of this type of infection gives a rough idea of the degree of contact existing between the fly and the antelope. Both *T. vivax* and *T. uniforme* develop very readily in *G. palpalis*, and are common in the situtunga of the Sesse group of islands wherever the animals are in contact with tsetse. On the occasion we have just referred to the Game Index was 3.8.

In 1922, when 1,755 flies failed to infect a monkey, 1,000 were examined for proboscis-infections and the Game Index was found to be 1.1. (DUKE, 1922.) These 1922 dissections were all performed at the one of the two regular camps on the island, where the Game Index is always small. The other Game Index figures represent the average for the two camps.

In February 1924, 3,455 flies were fed on monkeys without any infection resulting, and 1,163 flies were examined for proboscis-infections; the Game Index was 4.3. (DUKE, 1924*b*.) In September 1924, the Game Index was found to be 1.3. From 4,390 flies one infection was produced, being a percentage of 0.02 infected with the polymorphic organism; in December, 3,000 flies failed to infect two dogs. The total percentage of infections with the polymorphic trypanosome in the 10,845 flies fed on clean monkeys during 1924 was thus 0.009.

In October 1925, 5,139 flies were fed without infecting the monkeys.

Inoculations into clean monkeys of the blood of situtunga shot on the island were made during these visits. Thus in 1920, September and December, the blood of thirteen situtunga was injected into two monkeys, one of which became infected. (DUKE, 1920.)

In October 1922, the blood of five antelopes was inoculated without any infection occurring; three of these animals contained *T. uniforme*. (DUKE, 1922.)

In February 1924, nine animals were tested by subinoculation into one monkey, which became infected.

In September 1924, the blood of three animals was inoculated without infecting the monkey. (DUKE, 1924*b*.)

In September 1926, eight antelope were shot and their blood divided among two monkeys, one of which became infected with the strain No. XXIII referred to in Paper No. 3.

These investigations have been carried out as occasion allowed me to leave the Entebbe Laboratory. They are very incomplete, and it may well be objected that such random fragments of information do not constitute evidence. But there are certain facts that are significant in thinking over these notes.

Damba is separated from Kome Island by a narrow strait three or four hundred yards wide. At the time of writing, Damba is still uninhabited; but since 1921 there have been natives living on Kome, and their number has been slowly increasing. Damba has long been famous in the lake-area for the number of its *sititunga*, and poaching by natives has always occurred to some extent. Since the return of the inhabitants of Kome, poaching has been rife on Damba; a number of animals has been killed, and the survivors, though still numerous, have become much more timid and wary, and are reverting more to their old habits and are not so often seen along the lake-edge by daylight. All these considerations apply especially to the side of Damba adjoining Kome, and this is the part of the island where most of the experiments were carried out.

For these reasons, one must conclude that the contact between the antelope and the fly is less close than in 1912 or in 1920. The fly is not and never has been entirely dependent on the antelope, though it undoubtedly utilises them freely (*cf.* Game Index); there have always been plenty of crocodiles, *Varanus* and hippo.

The figures we have considered confirm this opinion, for the Game Index seems to be diminishing and so does the percentage of flies infected with the polymorphic organism.

The species of trypanosomes responsible for the Game Index on Damba, *T. vivax* and *T. uniforme*, are very much more readily transmitted by *G. palpalis* than is the polymorphic organism; the percentage of positive flies is considerably higher, both in nature and in the laboratory.

Evidently, in nature these two species will survive a reduction in contact between fly and antelope that would be fatal, or at all events very unfavourable, to the polymorphic species. When the interruption is complete, the Game Index will be zero, and the polymorphic organism will not be found in the fly. I have shown that, on islands where there are no antelope but only hippos and reptiles, the polymorphic organism is not found in the *G. palpalis*; and, though this point was not entirely settled, it is almost certain that, in such circumstances, *T. vivax* and *T. uniforme* will also be absent. (DUKE, 1913 *c.*)

The recovery in September 1926 for the first time of a completely non-transmissible strain of the polymorphic organism from the blood of a *sititunga* on Damba is therefore of peculiar interest.

Examined in the light of the explanation put forward in Paper No. 5, what do we find? The return of the natives to Kome Island has led to an increase in the amount of hunting on the adjoining island of Damba. The antelope, in the last few years, have practically ceased to show themselves along the shoreline during the daytime, whereas formerly they were frequently to be found there, at any rate up till 9.30 a.m. and from 2.30 or 3 p.m. onwards. To a varying extent the same applies all along the Damba shore. The antelope are more wary and more prone to keep under shelter. That they are still fed on by tsetse is obvious, and is shown by the presence of proboscis-infections and by occasional evidence of the presence of the polymorphic organism.

In other words, the contact between fly and antelope is less than it was. The effect of this on the two groups of trypanosomes is shown by the diminishing Game Index in the case of *T. vivax* and *T. uniforme*, and by the increasing rarity of the polymorphic organism in the wild fly of the island. This means for the polymorphic organism that the transference of this trypanosome from host to host among the antelope takes place less and less frequently. Fewer animals will thus be infected, and those that have been infected will not be reinfected so often. In this way, according to our theory, we should expect that, sooner or later, strains would lose their transmissibility owing to prolonged sojourn, without re- or superinfection, in the same mammalian host.

All this reasoning may be specious and plausible. It is frankly admitted that the evidence on which it is based is fragmentary. In the circumstances, it was impossible to devote more attention to Damba and its secrets, and I have merely stated the available facts and examined them in the light of a new theory.

At all events, the interpretation it affords seems reasonable and satisfactory.

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## Appendix A.

### AN INVESTIGATION OF THE ACTION OF "BAYER 205" ON *TRYPANOSOMA GAMBIENSE* DEVELOPING IN *G. PALPALIS*.

In the Interim Report of the Commission some experiments were described on the prophylactic effect of "Bayer 205" on *T. gambiense* developing in *G. palpalis* (DUKE, 1927 b). On that occasion, the flies were fed on the Bayer-containing blood twenty-four hours *before* they took up the trypanosomes from the infected animal. Various periods elapsed between the administration of the drug and the feeding of the flies, and the conclusion was reached that "Bayer 205" administered in this manner had no effect whatever on the subsequent development of flagellates in the tsetse.

In the present series of experiments, the procedure was varied, and the flies were given their feed on Bayer-containing blood *after* having fed on the infected animal. Evidently this test is the more severe.

The actual technique is in general that followed in all transmission work at Entebbe. The drug was injected into the monkey intramuscularly, 0.013 gr. per kilogram body-weight.

The two *T. gambiense* strains used in these experiments were Nos. V and IX, both of which were known to be readily transmissible. The flies were fed for two consecutive days on the infected animal, then starved for twenty-four hours, and finally given one feed on the Bayer-monkey at different intervals after the administration of the drug.

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			It gut only.	In gut and salivary glands.		
292	222	6	41	25	20	45	32	120	2	0	,6 ,27	2 & 3. XII. 26.
293	"	6	35	22	25	47	32	Control	2	1	,6 ,8 ,32	2 & 3. XII. 26.
294	217	5	26	19	24	43	30	Control	0	0		4 & 5. XII. 26.
295	"	12	27	21	16	37	30	168	1	1	,23 ,30	4 & 5. XII. 26.
296	338	4	36	35	27	62	31	192	2	0	,31 ,31	6 & 7. XII. 26.
297	"	7	16	10	14	24	31	Control	1	0	,7	6 & 7. XII. 26.

These experiments, limited though they are, show that the development of *T. gambiense* in *G. palpalis* is not affected by the presence of "Bayer 205" in the blood taken up by the fly forty-eight hours after the infecting feed. We may presume, therefore, that the drug will have no effect in fully established infections of the fly.

"Bayer 205" therefore appears to exert no sterilising effect on tsetse infected with *T. gambiense*.

### Appendix B.

## DOES A PRELIMINARY FEED ON CLEAN BLOOD AFFECT THE DEVELOPMENT OF TRYPANOSOMES TAKEN UP TWENTY-FOUR HOURS LATER BY A NEWLY HATCHED *G. PALPALIS* ?

Two pairs of experiments were performed. In the first pair, one box of the newly hatched flies were fed on a clean monkey, and then twenty-four hours later put on an infected animal carrying a transmissible strain of *T. rhodesiense*; the other box was starved while its companion was on the clean animal, and then twenty-four hours later it too was placed on the same infected animal.

In the second pair of experiments the same procedure was followed, save that, after the first infecting feed, both boxes were starved for forty-eight hours and then given another feed on the infecting animal.

The results are shown in the following table.

Experiment No.	Infected animal.	Day of experiment on which dissection began.	Number of flies alive on 25th day.	Number of flies dissected during the experiment.			Duration of experiment in days.	Number of generations re-moved from man.	Number of flies containing flagellates.		Ages of the infected flies dissected during experiment.	Dates of infecting feeds.
				Males.	Females.	Total.			It gut only.	In gut and salivary glands.		
164	clean monkey & goat 143.	9	41	24	26	50	42	0	0			12. VIII. 26. & 13. VIII. 26.
165	starved goat 143.	17	42	18	27	45	42	2	1	,17 ,42 ,37		
166	clean baboon & goat 144.	7	50	28	30	58	42	2	5	,42 ,42 ,36 ;38 ;40 ;42 ;42		13, 14 & 16. VIII. 26.
167	starved goat 144.	7	31	24	27	51	42	1	0	,34		

These experiments indicate that it makes no difference to a young laboratory-bred fly whether it feeds before its first infecting feed or not.

## Appendix C.

### THE NOMENCLATURE OF HUMAN TRYPANOSOMIASIS IN NATIVE AFRICAN LANGUAGES AND ITS POSSIBLE BEARING ON THE ANTIQUITY OF THE DISEASE.

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The Baganda are a highly intelligent Bantu race, with a history behind them of a long line of hereditary kings, and a wonderfully complex system of administration peculiar to themselves. Their history, customs and folk-lore have been exhaustively studied by ROSCOE. His work, *The Baganda and their Customs*, shows conclusively that the Baganda occupied a unique position among surrounding Central African races, and their superiority was demonstrated by frequent raids upon their neighbours far and near. It is therefore without surprise that we find the Baganda possessed of a fairly wide acquaintance with disease, both of man and domestic animals. Naturally, judged by modern European standards, this knowledge is rudimentary. Nevertheless, they recognised and named quite a number of human ailments.

In the Luganda language some diseases possess names descriptive of a cardinal symptom; many are, however, designated by a word that has no other meaning than the disease itself. In the first class, we find one of the native names for diarrhœa and dysentery, which, though disagreeable and sometimes no doubt fatal complaints, do not often assume serious epidemic proportions in undisturbed native communities in Buganda. Thus diarrhœa is known as *ekidukano* (from the verb *okudukana*), an easily recognised modification of *okuduka*, to run; dysentery is *ekidukano kyo musai* or *kya masera* or *kya ma'zi*, according as blood or mucus or fluid predominates. There is also another word for diarrhœa, the verb *okuwaluka*, which has no other meaning, and is also commonly used in conjunction with the supplementary word, *omusai*, *amasera* or *ama'zi*. *Okuwaluka* is undoubtedly a much older word etymologically than *okudukana*. In the second category we find a number of easily recognisable pathological conditions, such as epilepsy, gonorrhœa, etc., and also the infectious and epidemic diseases, including chicken-pox, measles, smallpox and plague. Severe chicken-pox is not uncommon in Uganda to-day, and often closely resembles smallpox; measles is a serious inconvenience owing to its symptoms, sequelæ and rapid spread: smallpox and plague have always been the dreaded scourges of African native races, causing, in the one case, loss of life, disfigurement and blindness and, in the other, a terrible mortality.

The Luganda word for measles is *lukusense*, derived from a verb *okukusensa*, meaning to suffer from measles. Chicken-pox is *namusuna*; smallpox, *kawali*; and plague, *kawumpuli*. *Kawumpuli* is the name of the god to whom the disease was ascribed by native superstition. None of these words have any alternative meaning. And so on, for a number of other diseases of old standing in the country.

Turning now to sleeping-sickness, the word in use in Luganda is *mongota*, derived from *okubongota*, to nod with sleep; so that this disease falls into the first category, *i.e.*, those named from a prominent symptom. I believe that, actually, this name was applied by one of the White Fathers when the disease first appeared in Uganda. There is no other Luganda word for human trypanosomiasis, and the evidence all points

to the conclusion that the disease is of comparatively recent introduction. The mortality caused by sleeping-sickness is too serious to escape the notice of the native in regions where it is endemic.

Now, the contention here advanced is that, where a disease as grave as human trypanosomiasis has been for centuries endemic, the language of the inhabitants will contain a word for the disease, and a word, moreover, that will have no alternative meaning, and will bear no reference to the symptomatology. Where populations have migrated, the word in use may be a relic of an earlier race. On the other hand, where sleeping-sickness is of comparatively recent introduction, the disease will either be denoted by an imported word or by one which, like *mongota*, describes a striking symptom.

Among the Azande we found an imported word in general use all over the country, *pongi*, which VAN HOOFF tells me is used wherever the *lingua franca* is Lingala. Close enquiry from two old witch doctors, interviewed by VAN HOOFF at Dungu, revealed the existence of another word, *pupe*, which, said these authorities, would only be known nowadays to a few of the old people. This latter word indicates the general behaviour of an insane person, and is obviously a symptomatic name.

According to the view here advanced, this would indicate an invasion by sleeping-sickness many years ago, followed by a practical disappearance of the disease, followed again, since the arrival of European civilisation, by yet another invasion.

In the Kavirondo country, Dr. VAN HOOFF has found that there are several words for sleeping-sickness. On the Nzoia and Yala rivers, the word is *nandolo*, meaning sleep; in the Yalua language, *tshonindo*, also meaning sleep; here one also finds the Bantu word *kawongera*, also meaning to sleep. The most ancient word seems to be *kilulume* or *ndulume*, meaning tremors, also employed for *petit mal* and slight attacks of genuine epilepsy. Yet another word applied to sleeping-sickness is *akunuga*, meaning stupor or coma, and applied to advanced cases of human trypanosomiasis. Here, again, all the words are descriptive of striking symptoms of the disease, and are not in any way specific, indicating, according to this view, a relatively recent introduction of human trypanosomiasis into Kavirondo.

This notion may be baseless; it may even have been already put forward and disposed of. If so, an apology is necessary for an unavoidable oversight. It cannot be tested in Uganda, because here the disease is of comparatively recent introduction. The West Coast and parts of the French and Belgian Congo, where for centuries sleeping-sickness has been known to the natives, are the districts where one must look for words denoting this disease and it alone. In such countries, as with plague in Uganda, the name may indicate a divine origin according to native ideas on ætiology.

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## Appendix D.

### THE RESISTANCE OF *T. GAMBIENSE* TO ARSENIC.

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Little or no progress has been made with the experiments on the transmission of arsenic-fast strains of *T. gambiense*, which were promised in the Interim Report of the Commission.

A number of monkeys infected with this trypanosome has been under treatment, but the response is exceedingly slow and the animals remain "negative" for months at a time.

VAN HOOFF's experience in the Semliki Valley shows that there are, in that region at all events, a number of infected natives carrying relapse-strains after treatment with considerable quantities of atoxyl. It was hoped to bring back some of these strains to the Laboratory, and there to examine their transmissibility. But not a single monkey or guinea-pig inoculated during that safari developed trypanosomes. So nothing could be done.

This very important question therefore remains still in abeyance, pending the recovery of a suitable strain.

One interesting fact that has emerged from these attempts to produce an arsenic-fast strain in monkeys is that two strains of *T. gambiense*, from untreated patients, resisted, in monkeys, a dose of 0.01 gr. atoxyl per kilogram body-weight of the monkey.

This shows that *T. gambiense* possesses a greater natural resistance to atoxyl than has hitherto been suspected.

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## Appendix E.

### ON THE POSSIBLE CHEMOTACTIC INFLUENCE OF THE SALIVARY GLANDS OF *GLOSSINÆ* IN THE DEVELOPMENT OF THE POLYMORPHIC TRYPANOSOMES IN TSETSES.

By Drs. H. Lyndhurst DUKE, G. LAVIER and M. M. PRATES.

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During the dissections of laboratory-bred *G. palpalis* carried out in the course of numerous transmission experiments at the Entebbe Laboratory, it was often noticed, in examining positive flies, that flagellates developing in the intestine congregated in numbers about the salivary glands of the fly, especially when the gland-contents were protruding.

In view of the importance of the glands of the fly to the polymorphic group of trypanosomes, it was decided to investigate this phenomenon. The technique adopted was to dissect out the glands of clean tsetses bred at the Laboratory, break up the glands, tease up the fragments on a slide, and then introduce, under the same coverslip, a drop of the gut-contents of a positive fly. Normal saline was employed throughout these manipulations. The preparations were ringed with vaseline, put away in the dark, and examined at intervals.

Other experiments were also performed in which small Wassermann tubes were employed instead of slides, and samples removed at intervals. In each of these latter experiments, one tube was kept under anærobic conditions.

#### *First Series.*

*T. gambiense* taken from a positive fly that showed a gut-only infection. Salivary glands of clean flies partially crushed, to produce a saline extract of salivary glands, in which were suspended morsels of gland.

After five hours in the dark nearly all free-swimming forms were dead. Large numbers of flagellates were seen attached by their posterior ends to the gland fragments, especially where a hernia of the contents was protruding (*cf.* Figure 1, by G. LAVIER, and 1a by M. M. PRATES).

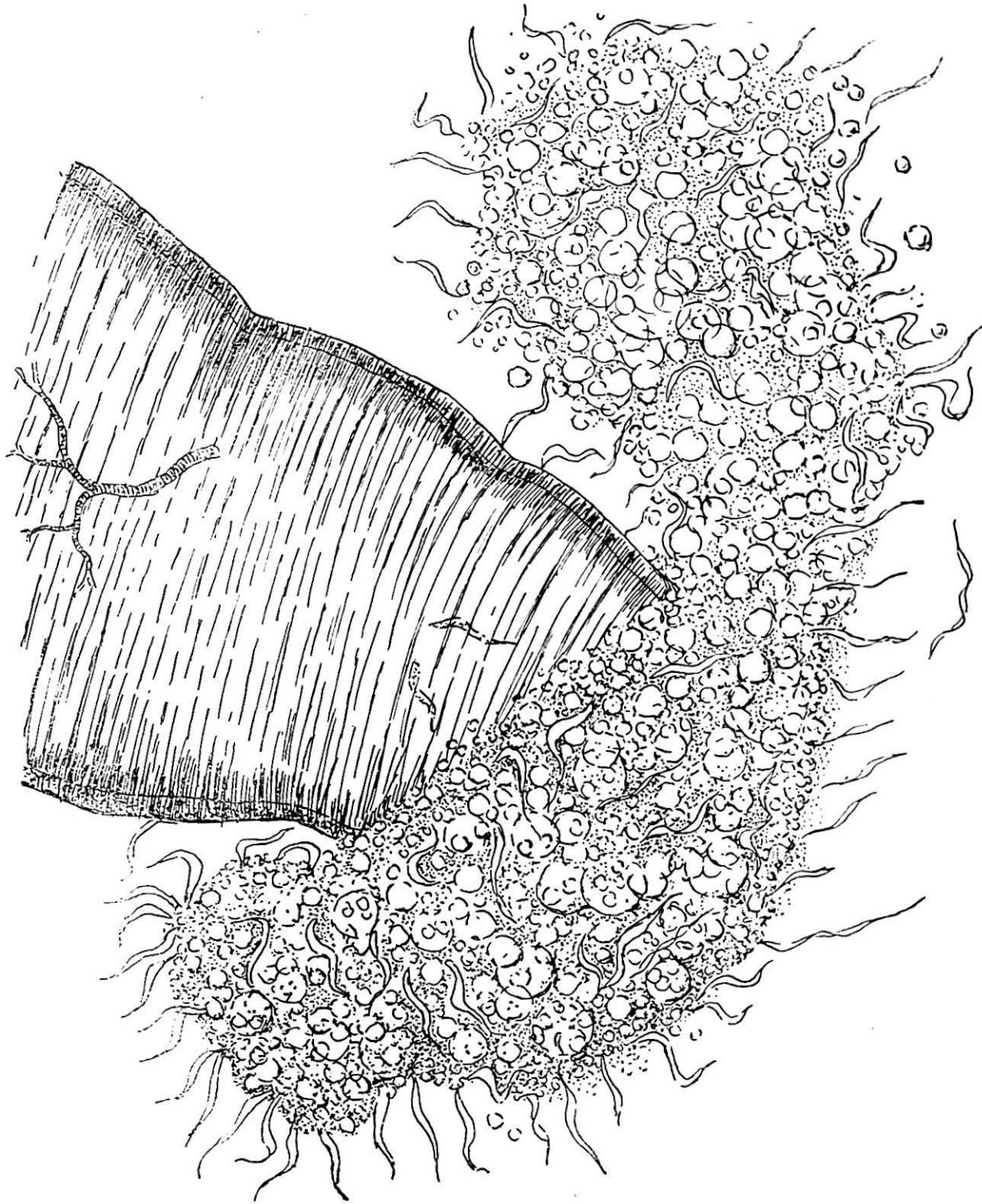
#### *Second Series.*

The same experiment was repeated with a fly infected with *T. gambiense* (again a gut-only infection), and using the glands of *Stomoxys* and *Musca domestica*. The flagellates did not attach themselves to the glands.

These experiments with the glands of *Glossina palpalis* and the intestinal flagellates of developing infections with *T. gambiense* were repeated on several occasions. Attachment of flagellates to the glands was not a constant occurrence, but nearly always occurred where there was a definite hernia of gland substance to which the trypanosomes could obtain access.

Occasionally attachment was observed to other viscera, *i.e.*, the fat-bodies, Malpighian tubes, etc., but never with any regularity and never to the same extent as to the glands. The intestinal flagellates of flies whose glands were already infected did not behave differently from those derived from flies showing mature gut-only infections.

Fig. 1



0 50 μ

Whether the glands of different individual flies vary in their attractiveness for the flagellates we do not know. More work is necessary, and will be done as occasion allows.

*Experiments with T. grayi.*

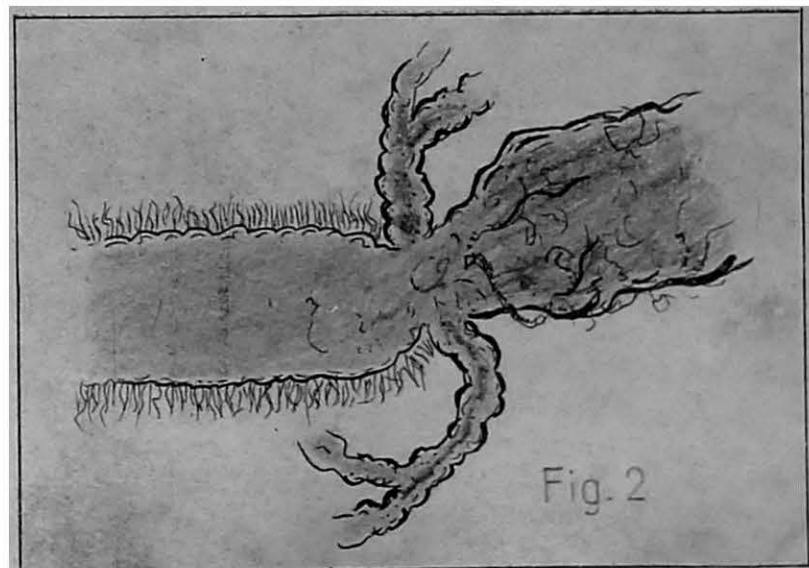
*T. grayi* from eight wild *G. palpalis* were exposed to the chopped-up intestines of ten clean *Glossina*. Two definite agglutinations were observed — one at the terminal opening of a piece of gut, and the other along the outer surface of another piece (*cf.* drawings 2 and 3 by M. M. PRATES).

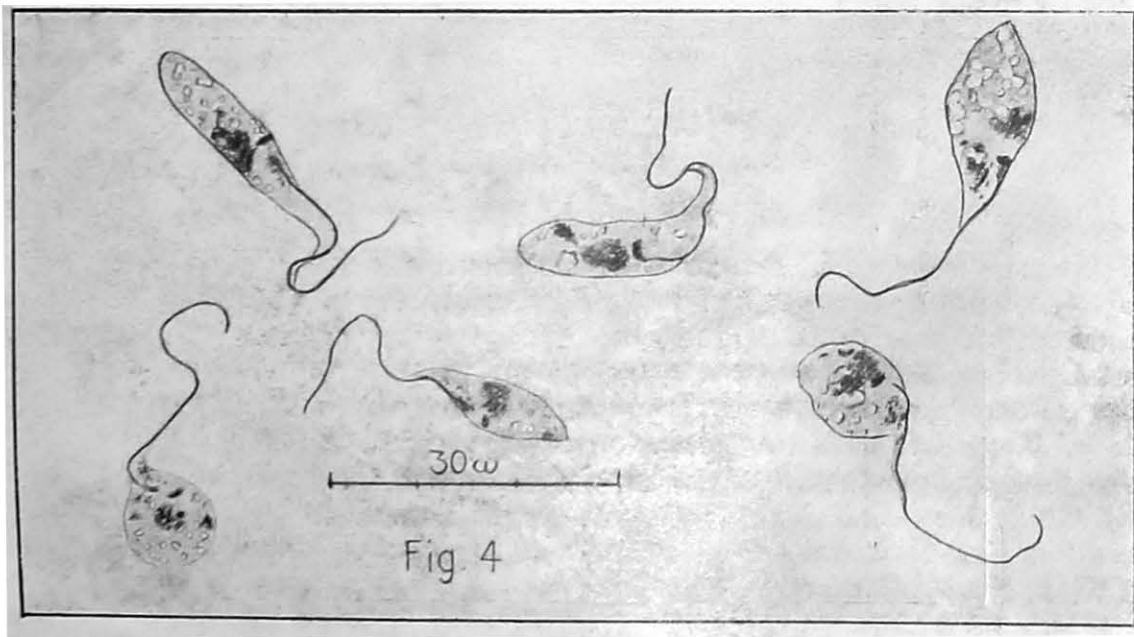
*Experiments with T. gallinarum (cf. drawing 4, by M. M. PRATES).*

A very pronounced agglutination of flagellates took place around the end of a piece of salivary gland of *G. palpalis*.

*T. gallinarum*, as far as we know, does not undergo complete cyclical development in *G. palpalis*; it is therefore curious that the flagellates should be attracted by the glands.

These experiments may have no significance whatever as an indication of the manner in which the salivary glands become invaded. The chemotaxis we have described may be manifested only *in vitro*, and not play any part in the developmental processes of the trypanosomes. On the other hand, these observations suggest the advisability of repeating and extending this line of investigation, and this will be done when an opportunity recurs.





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**III.**

**REPORTS BY Dr. G. LAVIER**

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1.

ATTEMPTED INFESTATION OF BABOONS  
BY *TRYPANOSOMA RHODESIENSE*

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It is known that baboons are resistant to infection by trypanosomes of the group *brucei*. THOMAS and BREINL, however, have reported some experiments made in England by which they have succeeded several times in producing in some baboons, by simple intra-peritoneal inoculation, a fatal infection by *T. gambiense* or *T. brucei*. Their experiments, which have never been reproduced successfully by any other of the experimenters who have repeated them, allowed one to suppose that the natural immunity of the baboon to trypanosomes could, in circumstances undetermined, diminish or even totally disappear. This furnished an argument to those authors who maintained the thesis of the identity of *T. rhodesiense* with *T. brucei*. Reasoning by analogy, they saw the possibility of a lowering in the natural immunity of man to *T. brucei*, a lowering by the favour of which this parasite of big game might become a human parasite.

It was for this reason that the study of the conditions which determine the diminution or disappearance of the natural immunity of the baboon to trypanosomes was included in the programme of the International Sleeping-Sickness Commission. On my arrival in Uganda, I was put in charge of that part of the work. I shall say at once that, like all my predecessors, except THOMAS and BREINL, I obtained no result. The natural immunity of the baboon, in my experiments, remained absolute<sup>1</sup>; I shall therefore only relate them briefly, as a memorandum.

I endeavoured to obtain the lowering of this immunity by helminthiases, by recurrent fever from *Treponema duttoni*, and by tuberculosis. I also tried to produce hæmopoietic disturbances by benzene and lead salts, but the injection of these substances rapidly produced in the animals severe localised necrosis, complicated by infection, and I had to stop the experiment before it had yielded any appreciable effect.

HELMINTHIASES.

These and, in particular, ankylostomiasis have been considered by some writers capable of diminishing the resistance of man against *Trypanosoma brucei-rhodesiense*. I therefore attempted, first of all, to infect a baboon with ankylostomata.

Baboon No. 1.—May 14th, 1926.

Blood : 3 million red corpuscles, 4% eosinophilia.

Fæces : amœbic cysts, *coli* type ; iodophilic cysts ; eggs of strongyloid ; scanty eggs of œsophagostoma.

On May 15th, cultures rich in strongyloid larvæ of *Necator americanus*, obtained from various natives, were applied subcutaneously in the belly, thighs and axillæ. Examination of fæces, up to June 6th, showed no appearance of eggs of *Necator* (there were none, moreover, afterwards).

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<sup>1</sup> The baboons of the Entebbe Laboratory belonged probably to the species *Papio tessellatus*.

It seemed, therefore, as though this worm could not evolve in the baboon. I then decided to reinfect this animal with its own parasites (oesophagostomata) put in culture.

The extremely rich cultures were applied subcutaneously on June 7th and August 5th.

Eosinophilia : June 9th, 3%; June 21st, 6%; June 28th, 6%; September 8th, 9%.

Fæces : Eggs of oesophagostomata were present in all the examinations. Although there were variations in the apparent quantity, they were generally numerous.

On September 8th, subcutaneous inoculation of 1 c.c. of the blood of ape No. 172; trypanosomes (*T. rhodesiense*) extremely numerous.

Result absolutely negative; not even transitory infection.

#### RECURRENT FEVER.

This experiment was attempted by reason of the distribution of tick fever, which disease exists in the regions where sleeping-sickness from *T. rhodesiense* rages.

Baboon No. 7 received subcutaneously on July 8th, 1926, 10 c.c. of the blood of a man with tick fever (second recurrence), the presence of spirochætes being determined in the blood.

A large drop of the animal's blood was then examined daily. Spirochætes appeared July 18th; very numerous on the 19th and 20th; on the 20th,  $\frac{1}{2}$  c.c. of blood from rat 9, swarming with *T. rhodesiense*, was inoculated. Spirochætes were present on the 21st and 22nd, although diminishing in number; the following days they were absent and reappeared, very scantily, on July 29th; the number increased on the 30th, and on the 31st they were very numerous; on the last date, a second inoculation was made of  $\frac{1}{2}$  c.c. of blood from the same rat, still richer in trypanosomes. Spirochætes were scanty on August 1st, and afterwards definitely disappeared. The presence of trypanosomes in the blood of the baboon was therefore never determined and this animal was alive and well in June 1927.

#### TUBERCULOSIS.

Tuberculosis exists in the regions where human trypanosomiasis abounds, but is still a rarity; it is therefore not known to play a predisposing part. I have nevertheless tried its effect on the baboon.

Baboon No. 4 received on August 17th, 1926, a subcutaneous injection of expectoration rich in Koch's bacillus. Four or five days afterwards, an ulceration appeared at the site of inoculation; at the same time a bilateral inguinal adenitis was noticed, with the presence of a very large and indurated element on each side. There was also costal and bilateral axillary micropolyadenitis. The temperature showed fairly extensive oscillation, but it is to be noted that, in a control baboon, oscillations of nearly the same order occurred.

On August 31st, subcutaneous inoculation of  $\frac{1}{2}$  c.c. of the blood of ape No. 172, very rich in *T. rhodesiense*. During October, all trace of adenitis disappeared; the thermic oscillations had a feebler amplitude. The ulceration of inoculation, which closed after a fortnight, leaving a large and exuberant scar, appeared afresh, but this time with characters of cutaneous tuberculosis.

Towards November 12th the animal showed, at the level of his collar, an abundantly suppurating ulcer. The following days the whole of the neck region was indurated; the temperature increased; the animal remained suffering from a manifest torticollis. The abdominal wound showed an abundant suppuration due to streptococcic super-infection.

On November 20th, the animal, being in great torment, was killed. At the autopsy, no lesion attributable to trypanosomiasis was revealed; there were never, moreover, any trypanosomes in the peripheral nor in the visceral blood. Curiously enough, there was no manifestation of visceral tuberculosis, at least macroscopically; no mesenteric adenopathy, no lesion of the spleen, lungs, or meninges. Microscopical examination of the skin revealed, at the point of inoculation, typical cutaneous tuberculosis, with numerous giant cells, thus confirming the macroscopical aspect of the lesions.

This baboon, then, apparently died of streptococcic septicæmia, the microbe having gained entry by the wound in the neck. It is interesting to note that in this animal, which could very certainly be considered as new to tuberculosis, infection by Koch's bacillus was not generalised, but, on the contrary, evolved locally as in animals in a state of allergy.

Finally, despairing of the cause, and thinking that subcutaneous inoculation perhaps exposed the trypanosomes too much to the action of the secretions of the tissues of the baboon, I decided on the intra-spinal route. Taking advantage of the fact that my colleague, Dr. VAN HOOFF, had just made a suboccipital puncture in baboon No. 1, I injected, on October 9th, 1926, in the spinal canal of that animal  $\frac{1}{2}$  c.c. of the blood of ape No. 172, extremely rich in *T. rhodesiense*.

The result was absolutely negative. At the end of June 1927, the baboon was alive and well, without ever having shown trypanosomes in the blood.

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2.

A MORPHOLOGICAL STUDY OF ISOLATED STRAINS  
AT ENTEBBE LABORATORY  
BY THE INTERNATIONAL SLEEPING-SICKNESS COMMISSION

The object that had been assigned to the studies of the Commission was principally to investigate the possible affinities between the animal trypanosomiases from *Trypanosoma brucei* and the human trypanosomiases. This necessitated investigations of a very different order, among which the compared morphological study of parasites could not be neglected. At the request of the President of the Commission, I took charge of this part of the programme. These are the results of the researches made on viruses of different sources, isolated and preserved at the laboratory of Entebbe.

As, in the general opinion, there are only minimum morphological differences which are practically nil between the "species" *T. brucei*, *T. gambiense* and *T. rhodesiense*, it was necessary for me to pursue this study as far as possible and, in a word, to study the detailed cytology of these protozoaires. I shall be excused, therefore, for having devoted a part of the time that I spent in Africa to applying methods that seem more appropriate for use in Europe than in this territory; but I have been able also to study some viruses quite recently isolated and this explains why, in several ways, I deviate from classic descriptions based on strains isolated for a long time in the laboratory. I have not, however, been able to pursue this study as far as I should have wished; in this kind of research the limit of resources that a colonial laboratory can offer is very quickly reached; I hope to complete certain of these studies elsewhere, in Europe, under more favourable conditions.

I could not, moreover, enter here into details of the cytological observations that I have made; I will content myself, therefore, with indicating the principal results—at least, so far as concerns the possible differentiation of viruses.

I. STRAINS STUDIED.

One can divide them quite naturally into four groups according to their origin:

- Isolated strains in man in the region of the *G. palpalis*;
- Isolated strains in animals in the region of the *G. palpalis*;
- Isolated strains in man in the region of the *G. swynnertoni*;
- Isolated strains in animals in the region of the *G. swynnertoni* or *morsitans*.

Finally, we shall see a strain of human origin which is very interesting, but where the locality of the infection has not been able to be determined with certitude.

I. Human strains arising from regions of the *G. palpalis*. They amount to seven<sup>1</sup>:

- Strain I (*V. H.* 8);
- Strain II (*V. H.* 34);
- Strain III (*V. H.* 9);
- Strain IV (*Blasio*);
- Strain V (*Kl.* I);
- Strain VI (*Kl.* II);
- Strain X (*V. H.* 141).

<sup>1</sup> For the details concerning these strains, consult the *Provisional Report*.

2. Animal strains arising from regions of the *G. palpalis*. There are two of these :

Strain *Damba I*. — Isolated in September 1925, by Dr. H. L. DUKE, from a Situtunga antelope killed by him in the Isle of Damba (Sessé group of islands).

Strain *Damba II*. — Same origin, but the isolation of the strain from the Situtunga war made in November 1926.

It is to be noted that in the Isle of Damba there certainly does not exist any other species of glossina than the *G. palpalis*.

3. Human strains originating from regions of the *G. swynnertoni*. There are two of these :

Strain *Tang. I*. — Isolated in the region of Mwanza (Tanganyika) by Dr. CORSON, from the patient Marenya, May 10th, 1926.

Strain *Tang. II*. — Isolated in the region of Mwanza by Professor KLEINE, from the patient Boga, in December 1926.

4. Animal strains originating from the regions of the *G. morsitans* or *G. swynnertoni* :

Strain *brucei Kl*. — Isolated by Professor KLEINE at Ikoma (Tanganyika) in a *G. swynnertoni* zone, from an infected cow.

*Mbarara Dog* Strain. — Isolated at the laboratory of Entebbe from a dog naturally infected in the neighbourhood of Mbarara (Uganda). In this region, there only exists *G. morsitans* ; the dog, very thin and affected by a double keratitis, was taken, in great torment, to the laboratory of Entebbe, April 10th, 1927, date of the isolation of the strain.

5. *Bambu* Strain.

I place on one side this strain, to which I shall return later on in detail, because of the interest that it presents.

It was isolated April 27th, 1927, by Dr. DUKE and Dr. VAN HOOFF from the patient Risiki, dying at Bambu (eastern province of the Belgian Congo). There are no glossinæ in this region, and there is a doubt as to the region where the patient had been infected.

## II. TECHNIQUE.

These strains have been studied in the guinea-pig, in the monkey (*Cercopithecus* sp.) and, exceptionally, in the rat, owing to the small number of these animals at disposal. The susceptibility of this species appears variable ; no durable infection has been obtained in the white rat with human strains originating from *G. palpalis* regions. Certain of these animals have never shown trypanosomes ; others have presented rare examples in the blood circulating during twenty-four or forty-eight hours, on which a crisis occurred, followed, as far as one can judge, by a definitive cure.

The guinea-pig has been shown difficult to infect even by human or animal strains coming from *G. morsitans-swynnertoni* regions. Nevertheless, this animal is the one in which the morphological study gives the most interesting results, for in it the trypanosome manifests a polymorphism much more marked than in the cercopithecus. The infection is also more abundant, and, thanks to the easier manipulation of this animal, one always obtains better smears.

It is therefore in the guinea-pig that I have followed the various strains, and it is to the infection of this animal that the morphological characteristics belong of which I shall speak later on.

Except in the cases of special cytological researches, I have studied the morphology of trypanosomes on smears quickly dried and immediately fixed by the May-Grünwald solution, then stained by Giemsa solution diluted in distilled water of pH = 7.5, slightly alkaline.

Apart from the virus obtained by experimental infection, I have had at my disposal some smears made at Tanganyika by Professor KLEINE from the blood of infected men. I have studied, moreover, smears that I have obtained with the blood of patients of the Semliki region. None of these viruses, however, could be followed in animals, the inoculations effected not having been followed by infection. In short, I have been able to study twice the blood of the naturally infected dog in the region of Mbarara ; this strain it was possible to follow.

### III. PRINCIPAL MORPHOLOGICAL AND CYTOLOGICAL CHARACTERS OF THE *Brucei* GROUP.

I will say at once that the morphology and the cytology of the several strains that I have studied are extremely alike. For the convenience of the statement, I will not then separate at first the different viruses, whatever may be their origin ; I will return to the differences of detail that I have been able to establish.

#### *Polymorphism.*

The morphological character that is most typical of the *brucei* group is its polymorphism. This phenomenon, which is very easy to determine, is much more difficult to define. One may take for the two extremes the "short" form, without a free flagellum, and the "long" form, very slender and elongated, with a long, free flagellum. But between these two forms there are all the transitions possible ; the term of intermediate form does not give more precision, for it is itself indefinable.

In any case, the terms "short" form and "long" form, which would gain by being altered to thickset form and slender form, should in no way be presumed from the absolute dimensions of the individuals, for there are some short forms of remarkably large dimensions, larger than some slender forms, even taking into account the free flagellum of the latter. This alone suffices to make it understood how deceptive is the procedure which exists for manifesting the dimorphism in constructing a curve of the length of the individuals ; and this all the more that one deals with a heterogeneous value for flagellated individuals while adding to the length of the axis of the body the length of the free flagellum. In reality, the best manner of taking count of the polymorphism is simply to examine the smears of the blood.

The polymorphism is, moreover, very visible when fresh ; it is to be noted that the movements of the thickset forms and the slender forms can be a little different : while the thickset form moves on one spot, the slender form is capable, at times, of suddenly effecting a rather rapid and extensive movement, even to the point of going out of the microscopic field. The polymorphism is manifest quite as well in the natural hosts as in the inoculated animals.

#### *Natural Infections.*

I have not had at my disposal more than one naturally infected animal, the dog of Mbarara ; the examination of its blood at two different times showed a polymorphous trypanosome. As regards man, it has been often said that the trypanosome was monomorphous in him, and I have therefore had to study the morphology of the

trypanosome in man in thin smears of blood. This technique, which has the great inconvenience of requiring very long examinations under the microscope, has the advantage over that of the thick drop employed for the same end by REICHENOW in showing well-fixed and well-stained individuals and also of permitting interesting cytological observations.

The smears of human blood examined had two origins :

(a) Patients infected in the region of the *G. swynnertoni* of Tanganyika :

1. Dr. C. — A smear of blood showed numerous parasites ; the thickset forms dominated, but the intermediate forms were not rare. This slide was unfortunately broken by accident before a complete study had been made.

2. Patient Simiyu. — A smear of blood taken December 20th, 1926, by Professor KLEINE showed two long forms.

3. Patient Mukili. — A smear made October 23rd, 1926, by Professor KLEINE showed eight individuals : six short and 2 long.

(b) Patients infected in the *G. palpalis* region :

1. Patient Mangasi (see preliminary report). — The blood, on direct examination and after centrifugation, and the cerebro-spinal fluid showed only long forms similar to *T. vivax*.

2. Patient Okongo (Van Hoof 160). — Infected in the territory of Kadimo (Kenya). The examination was made as an exception on a thick drop ; numerous thickset and intermediate forms.

3. Patient Kambaya. — Infected in the region of Beni (valley of the Semliki). Four slides showed three individuals ; two long forms, one intermediate ; the inoculation of the guinea-pig was not followed by any infection.

4. Patient Kabuhu. — Infected in the valley of the Semliki ; five slides showed two trypanosomes, both of which were of the intermediate form ; the inoculation of the guinea-pig was not followed by any infection.

5. Patient Matali. — A very interesting case. This child was infected near Bambomé, in the valley of the Semliki, at the foot of the Ruwenzori. The *G. palpalis* are abundant all along the streams of this neighbourhood, but there are also *G. fusca*. The trypanosomes in this patient were numerous at the examination (fresh). Ten stained slides showed sixty individuals. The plate (Fig. 1, pages 126 and 127) shows the extent of the polymorphism. The inoculation of the guinea-pig, in spite of the abundance of parasites, was not followed by any infection.

It is to be noted that all these examinations in man have been made only once in each case, and under these conditions it is possible, even in the little animals of the laboratory, that they took place at a moment when the polymorphism was only revealed a very little or even not at all. Nevertheless, we see that where the trypanosomes are rare in man, and this is more frequent, they can all be of the same type, but this type varies according to the cases. In cases where one can observe a sufficient number of individuals, the trypanosome in man is plainly polymorphous (Mukili

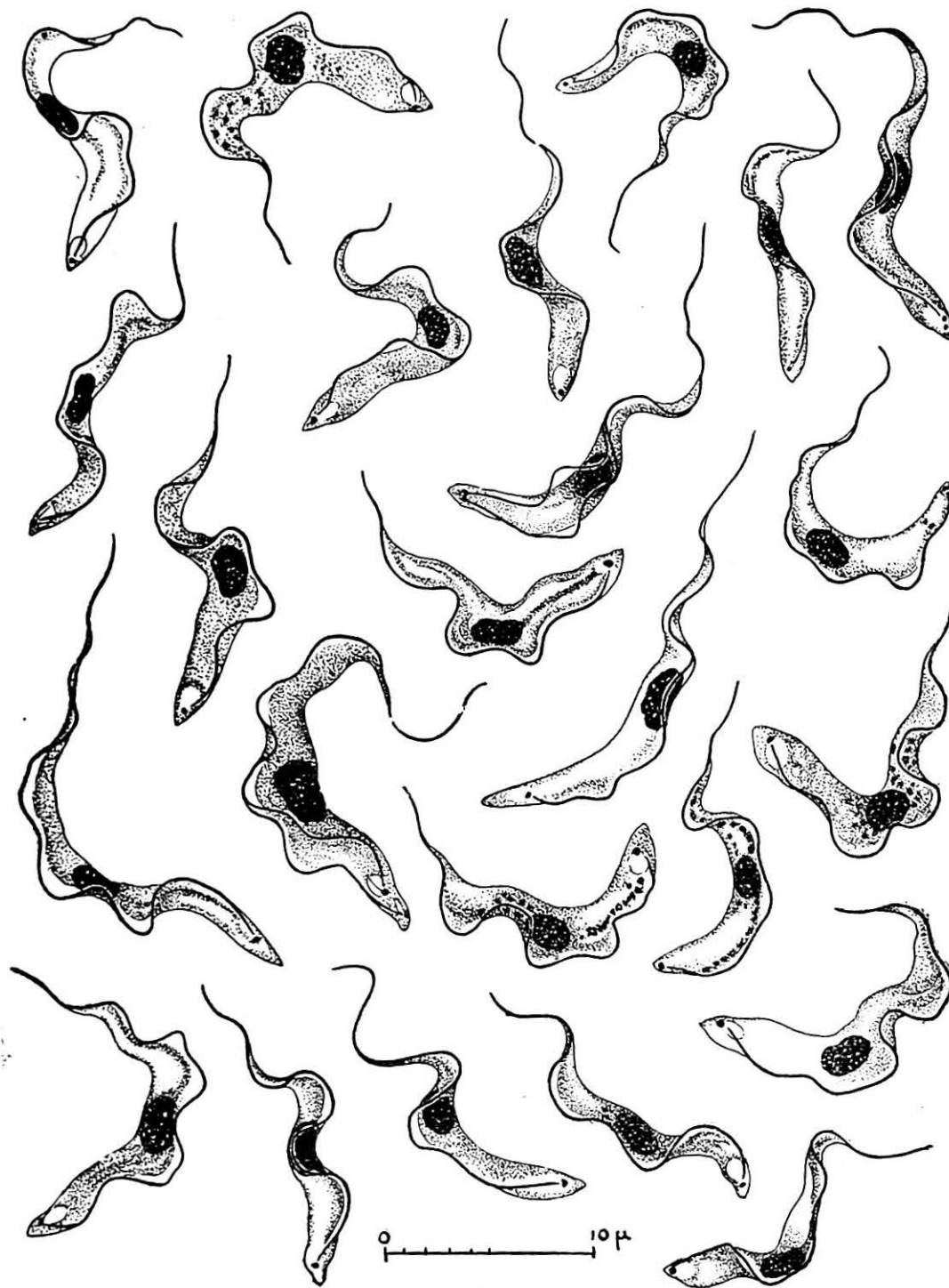
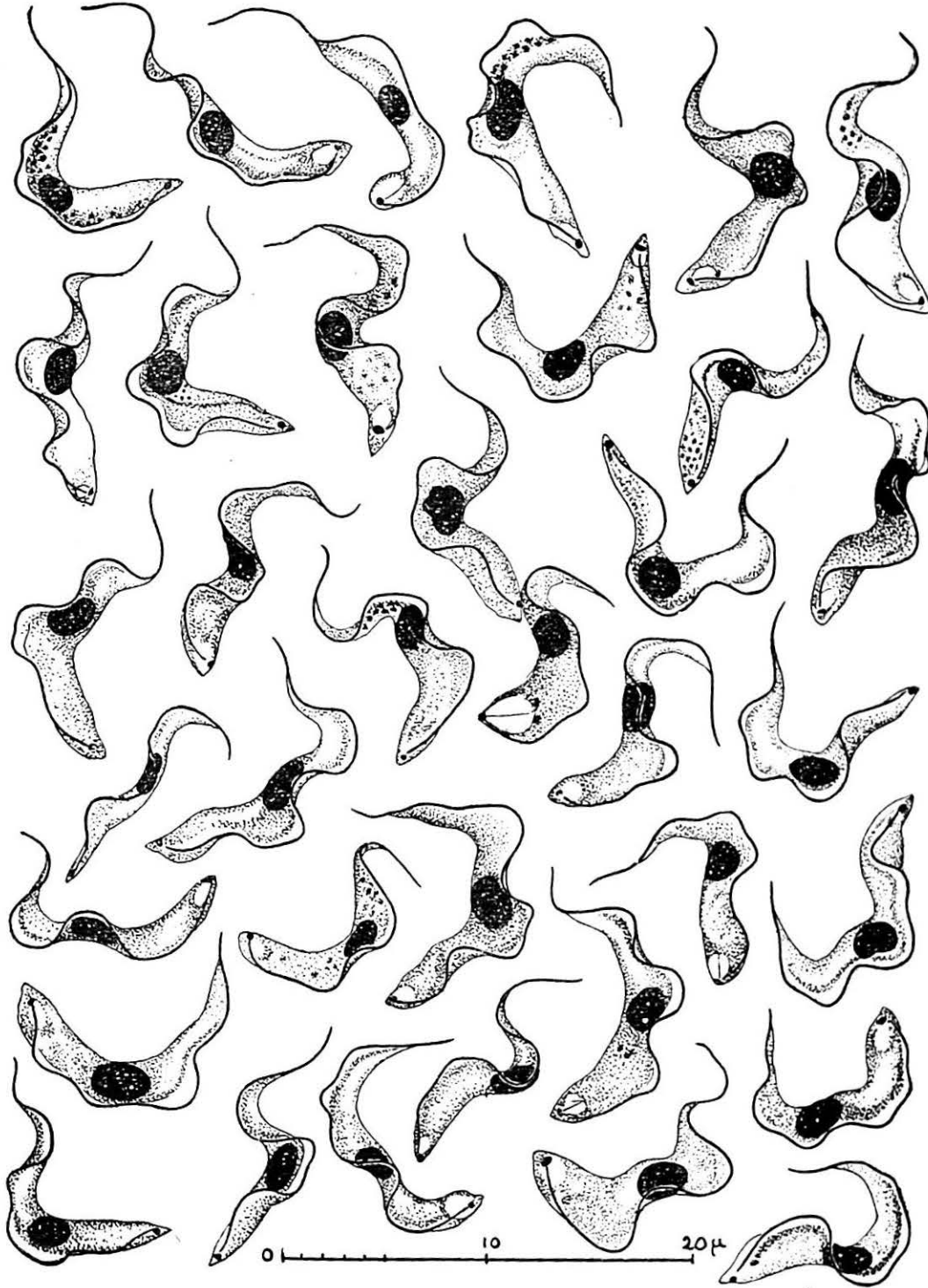


FIGURE 1. — *Trypanosoma gambiense* in human blood





(as shown in 10 blood smears from patient Matali).

case and especially the Matali case), as in the animal infections ; the Mangasi case, where the individuals were numerous and of the same type, seemed to constitute an exception.

### *Experimental Infections.*

The experimental infections, by allowing the virus to be followed daily, have furnished me with much more data than the natural infections.

In the inoculated animals, the strains that I have had to study all showed marked polymorphism. The degree of polymorphism has varied greatly according to the kind of animal infected. In each of the inoculated animals, the dominating aspect was susceptibility to variation according to the weather, without, however, any regularity, and nothing was seen which favoured an " endogenous cycle".

It is, as I have said, in the guinea-pig that I have especially studied the isolated strains. The polymorphism one can thus observe exceeds all that the study of strains preserved in the European laboratories leads us to expect. The recently isolated trypanosome has, as much in its morphology as in its cytology, a live aspect quite different from that of the strains kept for years ; I have always regretted not having been able to devote days to the examination of certain slides, as they reveal such unforeseen aspects. I hope, however, to complete this study on the rich material that I have collected.

One can say that the short and thickset form best characterises the polymorphism of the *brucei* group. This does not exist either in *T. evansi* or in *T. equiperdum*, which have undoubted affinities with *T. brucei*. We note, nevertheless, that, of *T. evansi*, BRUCE writes : " A small percentage are without free flagellum, but this, in my experience, is rare ". In *T. maroccanum*, a Moroccan race of *T. evansi*, VELU shows that nearly 4 per cent of the individuals in the horse, the dog and the rat are devoid of flagellum. " The dimorphism, however ", he says, " is very little accentuated, because, apart from the presence or the absence of the free flagellum, there does not exist any distinctive characteristic between the two types."

In the *brucei* group, on the contrary, if one reserves the name of short forms for those that are devoid of flagellum, the individuals thus defined generally differ plainly from the long forms with free flagellum, but are capable themselves of a great variety of aspects. They can present notable differences of length according to the inoculated hosts ; from this point of view, the monkey and the rat are capable of furnishing " short " individuals remarkably long, whereas in the guinea-pig, on the contrary, this form is generally of very small size.

But, moreover, in the same infected animal there can be considerable variation of size of this form. It is here also that one may find veritable dwarfish forms. Certain of these forms are indistinguishable in aspect and dimensions from forms typical of the *T. congolense*; they can even often present, as is generally the case in this last species, the parabasal body situated marginally. In general, however, these small forms are a little larger than those of the *T. congolense* type and more resemble, accordingly, the *T. simiae*.

Certain of the short forms are relatively very long and have a very thickset aspect as well. This type is shown in all the strains, but is shown with particular frequency in the virus isolated from the antelopes of Damba.

The intermediate and long forms are much less characteristic of the group. It is to be noted that certain intermediate forms are found with the posterior extremity obtuse instead of being sharp or tapering ; if, in this case, the parabasal body is

voluminous, these forms are identical with the common forms of *T. vivax*. Small numbers of such individuals can be found in all the strains ; in the case of the patient Mangasi, they constituted the rule.

#### *Position of the Core or Nucleus.*

The deviation of the nucleus either towards the front or towards the back has been observed in the guinea-pig in certain of the short forms of all the strains studied. The existence of "postero-nuclear" elements would not then serve as distinctive characters between the various trypanosomes of the *brucei* group.

The appearance of these forms is, one knows, capricious, varying according to the kind inoculated and according to the moment of infection. The abundance itself of these forms would not serve as a basis of differentiation. If they have always been rare in the human strains of *G. palpalis* and frequent in the human strains of *G. swynnertoni* that I have studied, they showed themselves very rarely indeed in an animal strain of *G. morsitans*; this last fact is even more remarkable as, having observed in the naturally infected dog (an animal, however, very unfavourable to the appearance of the postero-nuclear forms) a notable nuclear aberration in the short forms, I expected to find in the subinoculated guinea-pig a big percentage of postero-nuclear forms.

The antero-nuclear forms are found equally in all the strains. One can say that, generally speaking, they are, in comparison with the postero-nuclear forms, equally abundant in the human strains of *G. palpalis*, less abundant in the human and animal strains of *G. morsitans-swynnertoni*, and, as a curious fact, plainly more abundant in the strains of the Damba.

Finally, a very interesting thing is to be noted : a certain number of individuals are completely devoid of nuclei. Their attitude in the smears, however, well shows that they were perfectly alive at the moment when the fixer attacked them. Such forms have already been noted among the Herpetomonidæ and generally attributed to degeneration of the nucleus. In reality, the study of the phenomena of division shows clearly that these individuals are born without nuclei ; they can no doubt persist some time and die when they have exhausted the cytoplasmic reserves.

These individuals without nucleus are shown at certain moments with relative frequency in the human strains of *G. swynnertoni* and animal strains of *G. morsitans-swynnertoni* and *G. palpalis*. I have not found them in the human strains of *G. palpalis*, but I have not been able to devote sufficient time to their study ; I consider it very probable that they exist there also, but, infection being with these strains always more discreet, it demands a much longer investigation.

#### *Parabasal Body (Blepharoplaste, Kinetonucleus).*

This is, in all strains, of extremely variable size. In certain strains, it is very voluminous in all the individuals as in *T. vivax*. In the Matali case, it was always more voluminous.

In the other strains studied, certain individuals had a parabasal body as much developed as it was very restrained in others. In extreme cases, certain individuals present a considerable hypertrophy of this body, while in the others, on the contrary, although much more numerous, it is hardly distinguishable, giving the impression of a veritable atrophy.

Finally, and this is not the least interesting, each of the strains enclosed a certain number of individuals which were totally devoid of this body, and were therefore "ablepharoplastic", to use a bad term, but one sanctioned by use.

From a special study of these "ablepharoplastic" forms, to which I devoted myself, it results that :

1. They can exist in the naturally infected host. This, at any rate, was the case of the Mbarara dog ; in man, I have not been able to observe a sufficient number of individuals to have a chance of finding them.

2. They exist in experimental infections, whatever may be the infected species ; I have observed them in the guinea-pig, the white rat and the monkey.

3. In experimental infections, they appeared a very short time after the first trypanosomes ; their proportion then grows rapidly for a few days, then attains an approximative equilibrium, which continues indefinitely until the death of the infected animal.

4. Subinoculation does not seem to augment the proportion of these forms. It is probable, on the contrary, that a certain number of subinoculations brings about their progressive disappearance ; they have not been noted in the strains preserved in the European laboratories.

5. These forms seem, in the near absence of the parabasal body, to behave exactly as the others. They multiply, giving "ablepharoplastic" individuals which perpetuate the race. They do not seem to be influenced by the trypanolytic crisis any more unfavourably than the normal individuals.

6. Their existence is explained in a very simple manner by following the process of division. Perhaps a small quantity accrue from the extreme degree of atrophy of the parabasal body which I have noted above.

7. The point of equilibrium of the percentage of these forms is variable according to the strains. Here are the figures that I have observed :

- (a) Animal strain of *G. swynnertoni* :  
Strain *brucei* Kl. : 5.8 to 9.4% about.  
Animal strain of *G. morsitans* :  
*Mbarara Dog* strain : 6 to 10% about.
- (b) Human strains of *G. swynnertoni* :  
Strain *Tang. I* : 1 to 2% ;  
Strain *Tang. II* : 1 to 2%.
- (c) Animal strains of *G. palpalis* :  
Strain *Damba II* : 1 to 2%.
- (d) Human strains of *G. palpalis* :  
Strain *V. H. 8* : 0.5% ;  
Strain *V. H. 34* : 1.7 to 2% ;  
Strain *V. H. 9* : 1.7% ;  
Strain *Blasio* : 0.5% ;  
Strain *Kl. I* : 0.5% ;  
Strain *Kl. II* : 1% ;  
Strain *V. H. 141* : 1%.

Being, for the total of these strains, 0.5 to 2%.

#### Division.

The cytological study of the trypanosome enables, apart from the cytoplasmic elements (mitochondries, metachromatic granules and others), the following structures to be distinguished: the nucleus, the basal grain or true blepharoplast from where the flagellum springs and which is joined to the parabasal body ; the basal grain and the parabasal body are applied to the posterior pole of a vacuole.

Of these structures, three only are divided: the nucleus by promitosis, the basal grain and the parabasal body by simple scissiparity. The new vesicle and the new flagellum are formed afresh

But the interesting part in the division is not so much the modality of these partial divisions as their chronology. For the two new trypanosomes to be identical, it is necessary that all these partial and independent divisions be concluded and joined before the scission of the cytoplasm. But this relative simultaneity is far from being the rule: it is specially towards the second and third weeks of the infection of the guinea-pig that one observes strong parasitic pressure accompanied by intense and rapid multiplications; it is then that the anomalies are more frequent, tending to incomplete and partial divisions.

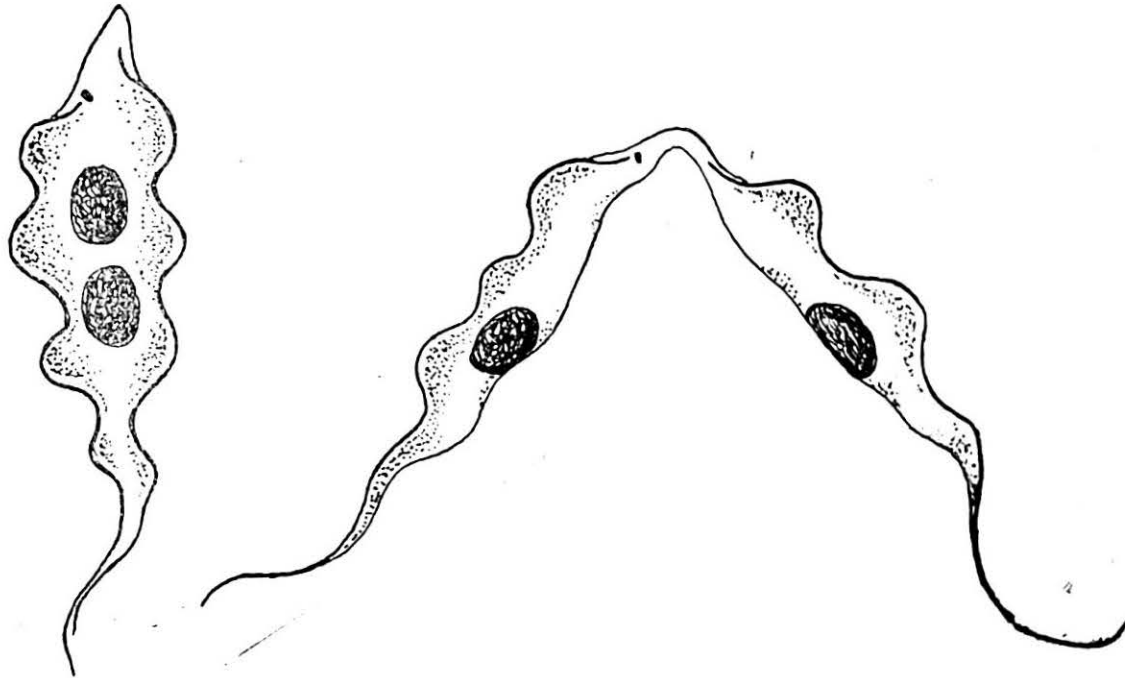


FIGURE 2. — Formation of "ablepharoplastic" individuals (shown diagrammatically).

It is difficult to say which is the organule of which the division is accelerated or behind, but the relative result remains the same:

The nucleus, for example, can commence its division first either by acceleration on its part, or by lateness on the part of the other. In this case occurs the process that I have mentioned in the preliminary report and which ends in the deviation of the nucleus in one of the new individuals either towards the front or the rear.

Another case is that there, after the division of the nucleus and the basal grain, the parabasal body divides too late or not at all; one of the two individuals is then devoid of it and is therefore "ablepharoplastic" (Fig. 2).

If the division of the nucleus is very late, or has not taken place, the basal grain and the parabasal body divide; a second flagellum develops; the cytoplasm divides at last and one obtains on one side a normal individual and on the other an individual devoid of a nucleus (Fig. 3).

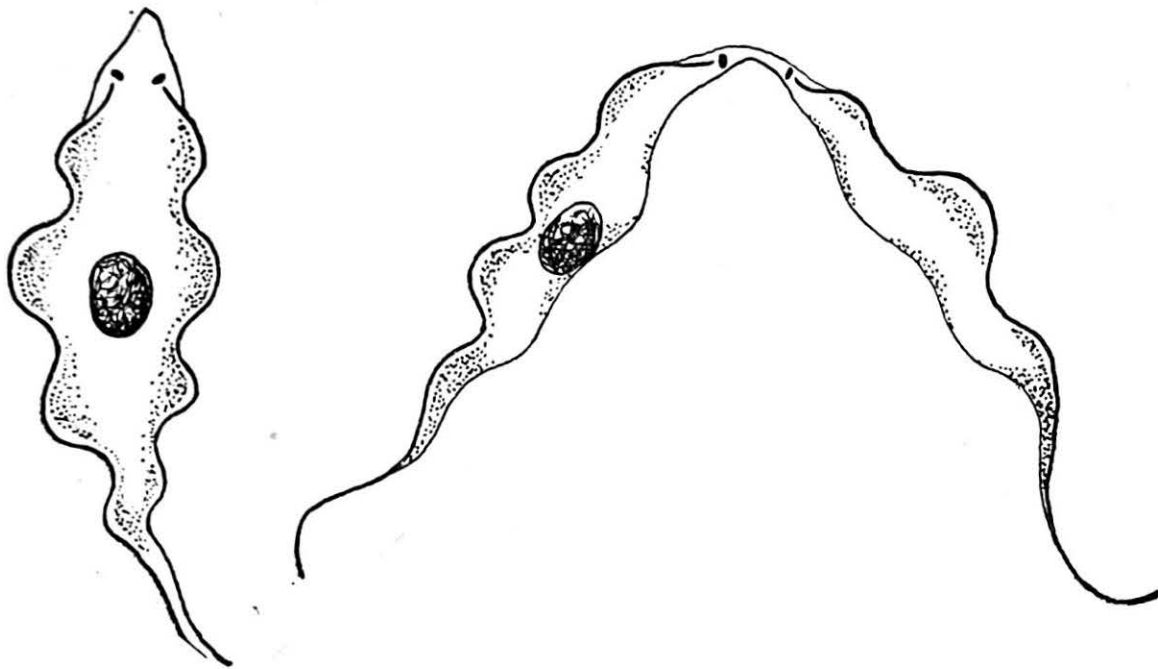


FIGURE 3. — Formation of anucleate individuals (shown diagrammatically).

Finally, the division of the cytoplasm itself may be late and one then obtains a single mass with several nuclei and as many flagella and parabasal bodies. Of such forms one is often noted and drawn.

As I have already said, it is difficult to give an account of this process of the organelle where the division is accelerated or where it is late, but if one takes as a pivot the basal grain, the only formation that never defaults, one can summarise in the following table the mechanisms ending in the appearance of the abnormal individuals :

Speed of division of			Result
Nucleuse	Parabasal body	Cytoplasm	
Augmented	Shortened or nil	Shortened	Deviation of the nucleus (antero-nuclear and postero-nuclear forms)
Shortened or nil			One of the individuals is "ablepharoplastic".
			One of the individuals is devoid of nucleus.
			Form with four nuclei or more and as many flagella.

All these modalities can, moreover, combine to end in forms even more complex.

It is to be remarked that the adherence of the parabasal body to the basal grain, which seems to be, in a state of repose of the trypanosome, quite strong, becomes very feeble at the moment of the division, and that these two forms can then easily separate one from the other, often in a definite manner. The independence of division is such, moreover, between these two organules, that one can find individuals where the parabasal body is plainly divided, leaving no indication whatever of division either in the basal grain or in the nucleus.

One has there, in short, an excellent example of that "independent game of the cellular and nuclear constituents" on which CHATTON has insisted. It is interesting to note that this author, developing his thoughts, even cited as an example, "hypothetic moreover": "If, in an organism like a trypanosome, one could be able to realise the multiplication of the flagellar centrosome or mastigosome and of the flagellum without concomitant nuclear division . . .". The hypothesis of CHATTON is found realised in the strains freshly isolated from the group *brucei*. The plasmodieresis, moreover, is not affected, and ends in this paradoxical result of the individual devoid of nucleus and yet, however, adapted to at least a limited life. Does the succession of the phenomena of division just described assume the rôle of centrosome attributed to the basal grain? If, after all, there are no individuals without basal grain, then no general division without that of this organule, this division of the true blepharoplast, is far from preceding, in all the individuals, that of the nucleus and the parabasal. But it is very possible that at these moments of rapid division the succession of phenomena overlaps several generations; I have at least found an example of them: two individuals joined by their posterior extremities, one of the two being devoid of nucleus; this signified, then, that the primary nucleus, found in the other individual, was too late in dividing; but, on the preparation, it was seen that this nucleus was just commencing to divide. If the individuals had been detached, that is to say, if the smears had been made a few seconds or a few minutes later, one would have admitted that, in the isolated nucleated individual, the nucleus was divided before the basal grain; however, the form without nucleus still being present showed that this division of the nucleus did not belong to the generation of the new individual, but to the preceding generation; then, in spite of the appearance, the basal grain was divided before the nucleus. This example would suffice to show how difficult it is to interpret in an exact manner these phenomena, which are, on the contrary, so easy to determine in their relativity.

#### IV. DIFFERENCES ESTABLISHED BETWEEN THE STRAINS ACCORDING TO THEIR ORIGIN.

If I have not revealed essential differences in the morphology and the cytology of the different strains that I have studied, there are, certainly, between them some differences, of a secondary order.

The human strains of *G. palpalis* have always given in the guinea-pig discreet infections; the parasites have never been seen extremely abundant. Besides, by utilising the Giemsa staining solution in distilled water of p H 7.5, the short forms of these strains have generally shown a clear and homogeneous cytoplasm and always of a lilac tint. The other strains, on the contrary, have presented the short forms, which, in the majority of cases, in the same conditions, were stained distinctly blue, often intense (these reactions of staining refer, I repeat, to the trypanosome infecting the guinea-pig).]



In addition, the abundance of morphological anomalies has shown to be variable according to the regime of the virus :

1. The two animal strains of *G. morsitans* and *G. swynnertoni* are shown, from this point of view, absolutely identical ; the forms devoid of nucleus here were relatively abundant, and the proportion of "ablepharoplastics" was very high in comparison with the others ; it is to be noted that this proportion was the same in the two strains, notwithstanding their difference of geographical and epidemiological origin.

2. The two human strains of *G. swynnertoni* have shown quite abundant forms with posterior nucleus ; the forms without nucleus were found there without great difficulty. The "ablepharoplastic" forms, much rarer than in the animal strains, attained the same proportion as in the two following groups.

3. The two animal strains of *G. palpalis* (Damba antelope) were most easily characterised to the eye by the abundance of the very long "short" forms, the rarity of forms with nucleus displaced and, in this case, the very distinct frequency of the nuclear deviation in front. This aspect in the smears enabled the animal strains of *G. morsitans-swynnertoni* to be easily distinguished.

4. The last group, that of the human strains of *G. palpalis*, contrasts with the others by its calm aspect. Atypic divisions are there much more rare ; forms without nucleus probably exist, but certainly in a very slight proportion ; the "ablepharoplastic" forms, however, attained a proportion hardly inferior to that of the second and third groups. The forms with deviated nucleus either towards the front or towards the back demand careful research.

These differences of stained reaction of the cytoplasm or of morphological anomalies are, in short, connected with the cellular activity of the trypanosome, and, consequently, to its virulence in relation to the host. It is therefore not impossible to find between these four aspects all the transitions. Morphological study, usually too much neglected, might show this in the strains isolated daily in the various regions of Africa. In any case, I can say that, concerning those which I have followed, these characters were sufficiently distinct to enable me, in favourable moments of the infection of the guinea-pig, to recognise, from only one examination of the smears, the group with which I had to deal.

The very interesting case of the Bambu strain may serve as an example of the subject. On May 14th, 1927, at the request of Dr. DUKE, I made and examined smears from guinea-pig 350 ; this animal had been inoculated by him and Dr. VAN HOOFF, at Bambu (Ituri, Belgian Congo), with the blood, very rich in trypanosomes, of the patient Risiki, then on the point of death. The success of this direct inoculation was the more remarkable as the incubation had been particularly short ; when the guinea-pig 350 was sent to me, two days after the appearance of the first trypanosome, the parasites were already swarming, on direct examination. The smears showed very abundant and very polymorphous trypanosomes ; a large number of forms with posterior nucleus, with an intensity of the nuclear deviation going as far as the situation of the nucleus at the level of the parabasal body or even its passage farther behind. The forms without nucleus were quite easily found ; the "ablepharoplastic" forms amounted to two in five hundred (or 0.4 per cent) ; the next day, the aspect was the same ; on June 16th, the animal was found dead. (Dissection showed a strong bilateral pulmonary congestion and a spleen of normal size ; death could not, then, be attributed to infection by trypanosome.)



Two monkeys, 353 and 354, were subinoculated on May 12th ; they both presented trypanosomes on May 17th ; monkey 354 died on June 9th and monkey 353 the following day. In these two animals the parasites were always abundant and the anomalies of division particularly frequent, reaching the appearance of very rare postero-nuclear forms, an exceptional thing in the monkey. "Ablepharoplastic" forms were seen on May 31st in monkey 353 in the proportion of 1.6 per cent (calculated on five hundred individuals).

To sum up, the morphology of this trypanosome places it in the group of the human strains of Tanganyika (Rhodesian group) ; and it even presented the characters of this group to a degree still more marked than the strains of Tanganyika that I had had at my disposal. The clinical information, which will be found elsewhere, agrees, moreover, very well with this determination, which is the first made for the Belgian Congo.

As concerns the region where the patient was infected, the information gathered suggests that it was in the valley of the lower Semliki, a place where *G. palpalis* only exists. Must it then be admitted that this glossina is capable of transmitting under natural conditions a virus of the Rhodesian type ? I will point out that this patient, in order to return to Bambu, had to traverse the zone of the western escarpment, where *G. pallidipes* are known to exist in great numbers ; this fly, which has great biological similitudes with *G. morsitans*, propagates in Zululand, in Italian Somaliland and Northern Kenya, a polymorphous animal trypanosome which seems to present characters morphologically identical with those transmitted by *G. morsitans* and *G. swynnertoni* (first group) ; it is not impossible, then, that one can inoculate in man a trypanosome having the same characters as that of Tanganyika. But it is as well to note that the dipterous fauna of this region is far from being completely studied ; but zones of *G. morsitans* are known with certainty in districts not far away, particularly in the symmetrical region of the eastern escarpment in English territory ; the presence of *G. pallidipes* shows that *G. morsitans* can live there, and it is very possible that new research will reveal this species in the regions where may be placed the probable point of infection of the patient Risiki.

It would be interesting to make this enquiry and to extend it even to the countries bordering on the Upper Nile, on the Uelle and on the Ubanghi-Chari. It is quite possible also that the unforeseen determination of Bambu would repeat itself in other points.

I would also desire to direct attention to the third group, that of the animal strains of *G. palpalis*. The two strains studied came from the Isle of Damba, where the game cannot emigrate ; they can at the most pass on to the neighbouring island, which does not modify the epidemiological characters ; in fact, the only glossina that can be found there is *G. palpalis*. But these strains present quite special morphological characters that are already found in a strain of the same origin studied by DUKE in 1921. It may be suggested that this special aspect is due to the insular life of the antelope, the isolation having evolved in a certain sense the trypanosome of the Sessé Islands. One would not, of course, be able to reply definitely to this objection without fresh studies in other regions. It can, however, now be remarked that one of the strains studied by DUKE in 1921, having these same characters, came from wild flies of terra firma. The older information is difficult to utilise. The "Jinja cattle trypanosome" (Sleeping-Sickness Commission, Royal Society, 1903) has been later assimilated by BRUCE and his collaborators with the *T. brucei*, Uganda, 1909 ; but as far as the mediocre Fig. 1, pl. I, of Vol. V., *Sleeping-Sickness Reports*, allows one to judge, the "Jinja cattle trypanosome" seems to have been a polymorphous trypanosome associated with *T. congolense*; the origin of the infection is difficult to determine,

the sick animals having traversed the *G. pallidipes* zones before living in the *G. palpalis* zone. On the origin of the *T. brucei*, Uganda, 1909, one has even less information, and one may even be allowed to ask why BRUCE and his collaborators, who had up to then attributed to *T. gambiense* all polymorphous trypanosomes, either human or animal, isolated in Uganda, have considered this as being *T. brucei*; it is certain, in any case, that part of the trypanosomes described by the Commission as *T. gambiense* were not of human origin; in particular, one of the two strains studied by Muriel ROBERTSON for the cycle of the trypanosome in the vertebrata came from wild flies of Kibanga, a region evacuated by the population for several years, and had every chance of being in reality a trypanosome of the antelope.

However that may be, to consider only the strains that I have had through my hands, it seems to me extremely difficult, if not impossible, to take for identical the human trypanosome and that of the antelope, and in consequence seeing in the Situtunga a reservoir of virus for man. It is true the hypothesis has been put forward that the human trypanosomes could, after prolonged sojourn in the antelope, have acquired new characters. I will simply remark that, in reality, the trypanosome was well studied in the Situtunga after the great epidemic of the Sessé Islands, and it seems to me more simple to admit that this parasite existed for a long time in the antelope with the same characters as now, and long before the appearance of the human virus. For the rest, the numerous epidemiological data and, in particular, the recent repopulation of the Sessé Islands show well that there is a complete independence between the parasite of man and that of the antelope.

One question is still to be considered: are the differences determined between the four groups of a permanent or temporary order? In other words, will a human trypanosome of the *G. palpalis* region preserve its characters, or will it evolve towards the animal trypanosomes *G. morsitans*? It is a long time since authors have maintained that *T. rhodesiense* was a *T. gambiense* modified by a new invertebrate host.

It is not impossible, in fact, that the changing of vectorial insects involves a modification of the parasite. The virus passes a part of its existence in the invertebrate host and the other in the glossina. In the first state, it is evidently in a constant condition; we know, however, what defensive regulators the interiors of mammals have and, consequently, what opposition they can furnish to the variations of characters on the part of the trypanosomes. One can follow, moreover, this phenomenon in the course of the experimental infection of the guinea-pig. We have here the beginning of a period, lasting a fortnight or a month, in which the forms are more various and more atypical; the "ablepharoplastic" forms, which are easy to follow concretely because of their striking character, are at this moment in a regularly increasing number, for not only do the individuals already devoid of parabasal multiply, but in addition they constantly re-form themselves; their number then ceases to increase and varies but slightly after; this means that the period of atypical division is ended and one is confronted with a true unification of the strain; the majority of the second generations which had arisen from it disappear by a selection due no doubt to the reaction of the host, and the trypanosome then takes a much more uniform aspect. We already know that, if the passage through mammals is sufficiently prolonged, this uniformity continues until it results in a complete monomorphism. The vertebrate host seems then to play also a regular rôle.

In the second state, on the contrary, the trypanosome lives in an insect, being practically unsheltered from the variations of its environment, temperature, dryness or humidity in particular, and one knows how much these conditions influence the cyclic evolution of trypanosomes. It seems logical to admit that the exterior actions would then have more influence on the protozoa and the better chance of determining

modifications in them. We observe, by the way, as regards the trypanosomiases that they transmit, that the glossinæ can be classed in two large groups : the group of humid regions, *G. palpalis* and *G. tachinoides*, transmitting *T. gambiense* and the Damba type; and, on the other hand, the group of the dry regions, *G. morsitans*, *G. swynnertoni*, *G. pallidipes*, transmitting *T. rhodesiense* and *T. brucei*.

We are able, then, to envisage the possibility of the passage of one type to another by changing the vectorial host; this would be, besides, not a change of zoological species, but the modification of the conditions of existence of the intermediary host.

This hypothesis could not be verified except by an epidemiological study or by staying in a region where one could follow the deposit of a new virus, and especially by the experimental study of a strain having a change in its customary glossina. In this case, three results would be possible : either the trypanosome would not be able to adapt itself to its new conditions and would rapidly become intransmissible, or else it would adapt itself but would keep its characters; or, again, it would adapt itself and would take on new characters due to the new glossina. The future alone will show which of the three possibilities really happens.

#### V. DOES THE *Brucei* GROUP CONSIST OF ONE OR FOUR SPECIES ?

The morphological differences that I have just exposed are very small and very difficult to establish. If their constancy were proved, they would help to establish demarcations in so homogeneous a group. If, on the contrary, they are only fortuitous, is it to be admitted that this group only forms one species ?

That depends on the conception one makes of the species. In this respect there are two tendencies : for it is certain that the notion of species can only be based on exclusively morphological characters; trypanosomes morphologically established as identical should then belong to the same species, all other characters being considered devoid of value. For others, in defect of morphological characters, biological characters should be taken into account. Such is the conflict of the "biological species", which each one resolves according to his own idea, and often changes the next moment.

We first observe that the morphological and biological characters can be no better defined than the species. If we hold a physico-chemical conception of life, it is difficult to disentangle the vital phenomena from the substratum which produces them. In the particular case of the protozoa, and especially of the trypanosomes, in which the number of organules is particularly restrained and yet forms the only morphological basis, these organules, invisible on the living, are brought out only by fixing and staining, which are nothing more than a succession of physico-chemical reactions; the information thus obtained is as much of a biological as of a morphological order, as a very simple example will show. We have previously seen that all the strains presented individuals devoid of parabasal. This character may seem morphological at the first instant, so much so that it has served as a basis for establishing the *T. equinum* species. But as the study of these forms shows how they are derived from normal forms, we are obliged to abandon this morphological character, striking as it may be.

However that may be, after having determined that the trench between the two fields is less deep than it seemed, if one adopts the first view, that the morphology is the basis of the classification, one is confronted with only one species, *T. brucei*, embracing even *T. gambiense*. But this last trypanosome has never been considered,

even by the most ardent unicists, as identical with *T. brucei*. The only morphological argument (and was it perhaps right ?) invoked for a long time—that of the presence or not of postero-nuclear forms—has not, as we have seen, any value whatever. But this trypanosome—the best known, moreover, of all the group—is essentially human; all the epidemiological facts prove that, even if animals can be infected by it, man is the only reservoir of this virus in nature. It will anyhow be necessary to distinguish it from the others; if we do not wish to make a species of it, we must make it a subspecies, a variety, a type or a race; no one, in fact, disputes the necessity of the distinction, but only what name to give it; it is, then, simply a wrangle in words.

But the important side of the problem, and the most discussed, is the identity or not of *T. brucei* with *T. rhodesiense*.

Admitted that they are morphologically identical, the problem may be faced in three ways.

1. Treating of two different species, one *T. rhodesiense*, infecting man, the other, *T. brucei*, incapable of doing so; this, then, is the only eminently biological character which separates them.

2. There is only one species, *T. brucei*, capable of infecting man when the natural immunity of the latter for some reason or other disappears. This opinion is evidently inspired by the experiments of THOMAS and BREINL on baboons, experiments the results of which unfortunately have not been obtained by any other author. The lowering of the human immunity has not yet been experimentally established. This thesis does not agree, moreover, with what we know concerning the epidemiology of *T. rhodesiense*, in particular with the fact that the time of distribution of this last trypanosome only slightly covers that of the *T. brucei*.

3. From a third point of view, *T. brucei* would be capable, after having been able to infect a man whose natural resistance had disappeared, of adapting itself to this new habitat and of infecting other men just as easily. This opinion is, one sees, intermediate between the two preceding; instead of there being only one species, *T. rhodesiense*, there would be a certain number of races of *T. brucei* adapted to the human host.

The pivot of the discussion is, then, definitely the human immunity towards *T. brucei*, and the possibility of its disappearance. This immunity is admitted by the dualists as absolute, following a certain number of inoculations made in man with the blood of infected animals without result. This number, the unicists object, is too slight. It is exact, but not considerable, and a single infection obtained in these conditions would ruin the arguments of the dualists. But it is no less true that this first success has still to be obtained.

In short, we have not enough material at our disposal to yield any hope of settling the dispute. There is urgent need of other data. Must we again attempt inoculation in man? We could not really find there proof against all criticism; even if the inoculation of the blood of antelope were followed by infection, it could always be objected that the virus of the animal had perhaps been of human origin.

The real argument capable of settling this long dispute is the epidemiology of their origin. Enquiries made with care and without preconceived idea, in the countries where *T. rhodesiense* abounds, for elucidating the mode of appearance and propagation of each centre, if prolonged for a certain number of years, would bring definitely into

the light the rôle played by big game on the one side, and the reservoir of human virus on the other <sup>1</sup>.

But as these data are not available, all discussion on the subject becomes fruitless.

There are many other cases, besides, where two parasites, one being strictly human and the other strictly animal, are nevertheless morphologically identical; the separation of them into distinct species is based on the information furnished by natural reciprocal immunity and by epidemiological research. By this means have been separated, among the Protozoa, the amœba of the tadpoles, *Entamœba ranarum*, and the human dysenteric amœba; the *Giardia* of the rat and that of man; among the Cestoda, the *Hymenolepis* of the rat and that of man, and among the Nematoda, the ascaris and the ankylostoma of the pig and those of man. But the *rhodesiense-brucei* discussion is approached so nearly by that on *Brucella melitensis* and *B. abortus* as to resemble it in its smallest details; the same arguments exactly have been invoked on the one part and on the other and comprise, for the unicists, the passage by ruminants, and for the dualists, the conclusion: the absolute impossibility of distinguishing the two viruses not only by the morphological but also by the cultural and immunological characters; but the one is pathogenic for man and the other is not, the ingestion by man of *B. abortus* not being followed by any infection.

Finally, it must not be forgotten that, if we ever acquired enough evidence to consider *T. rhodesiense* as a purely human parasite, the question would then plainly become whether it were not simply a race of *T. gambiense*, the two trypanosomes merely representing two modalities of the same virus. This opinion, maintained by several authors and in particular by KLEINE, might well end in becoming the most conformable to facts.

## VI. ATTEMPT AT PHYLOGENY OF THE *Brucei* GROUP.

It is generally admitted that there is a certain affinity between the *brucei* group on the one side and the *T. evansi-T. equiperdum* group on the other. The trypanosomes of this last group, in fact, morphologically correspond to the long and intermediate forms of the trypanosomes of the first.

But we know that, if sustained for a long time in the laboratory on small animals, the trypanosomes of the *brucei* group tend progressively, with variable speed, to lose the short form and consequently to become monomorphous. They are then indistinguishable morphologically from the *evansi-equiperdum* group. Certain authors therefore admit that this last group is derived from the *brucei* group by loss of the cyclic transmission and adaptation, of *T. evansi* to direct transmission by insect puncture, and of *T. equiperdum* to direct transmission by contact.

The arguments in favour of this thesis are that the glossinæ at certain geological epochs have had a larger distribution than now, and especially that the *brucei* group,

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<sup>1</sup> In the abundant literature on *T. rhodesiense*, there is only one work from this aspect, namely, the recent paper of MACLEAN, *Annals of Trop. Med. and Parasitol.*, XX, 1926, page 329. This author, having followed the advance of an epidemic in the district of Ufipa (Tanganyika), has been able to establish progression by solely human agency. The reading of this work, made with great precision and method, shows the value of the data that could be collected if this method of enquiry were enlarged and generalised.

Since this note was written, another paper upholding the same views has been published by W. H. DYE in *Trans. of the Royal Soc. of Trop. Med. and Hygiene*, Vol. XXI, No. 3, November 1927, page 187. The author, after having studied another focus in Tanganyika, believes also in the human origin of the disease; he states that the epidemic disappears after suppression of the human virus reservoir, although neither the amount of game nor its contact with man has been modified.

by cyclic evolution in an intermediate host, seem more fixed—more “ancestral”, one might say.

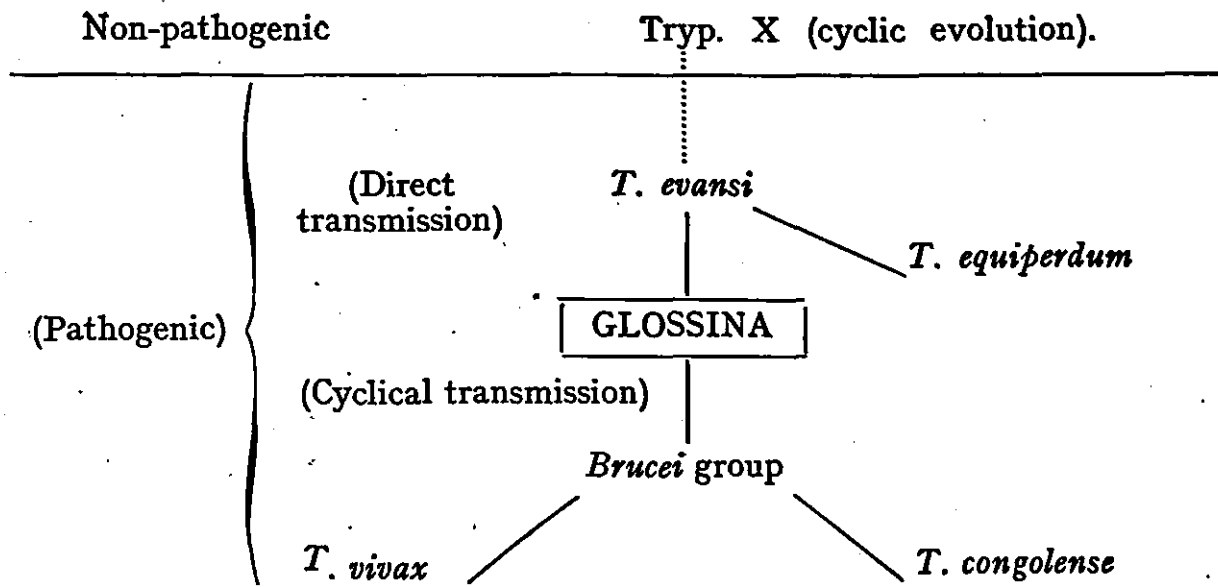
I believe it is possible, however, and perhaps with more reason, to maintain the opposite thesis. First of all, is the *brucei* group well adapted to glossina? There is always considerable difficulty in experimentally infecting this insect, the proportion of individuals in which complete cyclic evolution takes place being always very small; in short, the aptitude of the trypanosome to evolve cyclicly is very variable, and quite frequently, even in recently isolated strains, it is absolutely nil (see on this subject the experiments of H. L. DUKE), although the trypanosome is as polymorphous as anything capable of evolution; in fact, if a transmissible strain is followed for some time, it will be seen that it generally loses this character quite rapidly. This power of evolving cyclicly seems then so labile and, one must say, so mysterious that it does not seem possible to give it a morphological basis. Muriel ROBERTSON has expressed, after an insufficient number of experiments, the hypothetical view that only the short form is capable of evolving, yet the intransmissible strains at the Entebbe Laboratory showed as many short forms as the strains capable of evolving entirely.

From such facts the transmission of the *brucei* group is put aside; with the other trypanosomes the evolution of which has been specially followed (*T. inopinatum*, *T. granulosum*, *T. lewisi*, *T. cruzi*, for example), although there is a tolerable facility in obtaining cyclic evolution in the invertebrate host, comparable facts are not observed.

We may, then, imagine that, on the contrary, the *brucei* group is derived from *T. evansi*. This last trypanosome, as far as is known, is transmitted directly by the puncture of insects, but epidemiological facts show that the nature of the insect is not indifferent, and this induces one to think that this transmission is not strictly mechanical, but that a reciprocal adaptation between the trypanosome and the invertebrate has already commenced (it is, however, possible that in the course of time a true evolution will be discovered). One can imagine that this commencement of adaptation determined in certain species of gadflies is produced more deeply with diptera of the glossina kind. This adaptation, however incomplete, would explain the difficulty and the rarity of this transmission. The new conditions, where the trypanosome is then found, may have caused this morphological modification by exaggeration of the polymorphism, which is found already in *T. evansi* during the latent state. The return to the ancestral conditions of the direct transmission would thus explain the return to the ancestral monomorphism.

One may be allowed also to believe that the other trypanosomes evolving in some glossinæ, that is to say, *T. vivax* and *T. congolense*, have an analogous origin. We have seen that, in the strains of the *brucei* group, forms are met with identical with those of these trypanosomes. One can imagine that these characters have been fixed in certain races of the *brucei* group, and these are more closely adapted to glossina by adopting a different evolution.

If, finally, the views of C. A. HOARE are admitted, who sees the origin of the pathogenic trypanosomes of mammals in the non-pathogenic, transmitted by the digestive tract, after a cycle in very adapted insects, as in the case of *T. lewisi* and others, the following phylogenetic table could then be drawn up :



I do not hesitate to say that this is all largely hypothetical.

IV.

**REPORTS BY DR. M. MAXIMO PRATES**

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LOURENÇO MARQUES, MOZAMBIQUE.



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## PREFACE

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In my work on the precipitin test applied to the blood in the alimentary tract of glossinæ, I followed the general technique usually employed in forensic medicine for the analysis of blood-stains. I was fortunate enough to find at the Entebbe Laboratory the valuable work by NUTTALL, GRAHAM-SMITH and STRANGWAYS, dealing not only with medico-legal analyses but more particularly with the blood relationship between animals. I obtained from this study the most lucid, practical and accurate information, without which I should have found it difficult to pursue my task.

The material difficulties which I was to encounter in my work would also have been insurmountable but for the loyal and devoted help of Dr. DUKE, who did everything possible to remove them : he offered me the use of the Entebbe Laboratory, and I was given the benefit of his knowledge and vast experience in regard to the tsetse-fly. I take this opportunity of conveying to all my colleagues my admiration and grateful thanks.

I should be wanting in gratitude were I to omit all mention of my indebtedness to Dr. VAN HOOFF, who sent me from various places all the blood specimens he was able to obtain ; to the Director of the Miguel Bombarda Hospital, with which the Lourenço Marques Laboratory is connected ; to Dr. Manuel PORTUGAL, one of the most zealous promoters of Portuguese public interests, who was most generous in supplying me with material for my investigations ; and, lastly and most of all, to Professor Ayres KOPKE ; who, by his lucid and practical teaching, aroused in me an interest in tropical medicine and, in order to maintain and stimulate that interest, spontaneously suggested my unknown name for work of great responsibility—that undertaken by the International Commission on Sleeping-Sickness—and who, lastly, gave me the continual benefit of his learned advice in order the better to enable me to accomplish that task.

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1.

## BRIEF NOTES ON THE MICROSCOPIC DIAGNOSIS OF TRYPANOSOMIASES.

Having been asked by the President of the Commission to collaborate in the study of ruminants as virus reservoirs, I spent some months examining the blood of inoculated animals. I was also asked to treat and examine the blood and cerebro-spinal fluid of persons suffering from sleeping-sickness sent to the Laboratory for the purposes of the Commission's investigations. I took a number of notes suggested by the difficulties that I sometimes encountered in diagnosing trypanosomiasis. These notes supplied me with the material for the present article.

### I. PERSONS SUFFERING FROM SLEEPING-SICKNESS.

#### 1. *Blood.*

In all the cases of sleeping-sickness sent to the Laboratory, I obtained positive results by centrifuging the blood; on certain days, however, I obtained none by this method, although the results of the thick-film method and of gland puncture had been positive. The inverse also occurred.

#### 2. *Cerebro-spinal Fluid.*

I had occasion to practise repeated lumbar punctures with examination of the cerebro-spinal fluid before, during and after treatment in the case of almost all the sleeping-sickness patients sent to the Entebbe Laboratory. I took a number of notes, which I am publishing not as definitive results but simply as personal impressions, until I can confirm them by the study of a larger number of cases.

(a) I found, in persons suffering from sleeping-sickness, trypanosomes which could easily be discerned by microscopic examination of the peripheral blood or the juice of glands, the cerebro-spinal fluid being normal or nearly so.

(b) After treatment with 24 to 30 grammes of tryparsamide, the cerebro-spinal fluid became normal in practically all the patients treated, or contained at most 5 to 12 cells per cubic millimeter, even in serious cases, in which 600 or 1,000 cells per cubic millimeter had been found, and which had presented symptoms of somnolence.

(c) When the cerebro-spinal fluid contained a large number of cellular elements, the albumin content was rather higher in non-centrifuged fluid than after the centrifugal elimination of leucocytes and other cells.

(d) The Nageotte chamber, with its 100 cmm. capacity, offers this valuable advantage, that it makes it possible to obtain rapidly a truer average number of cells per cubic millimeter than if the smaller Thoma, Fuchs-Rosenthal chambers or others of small capacity be used.

(e) The Nageotte chamber, however, has one drawback as compared with smaller, shallower chambers, namely, that it does not permit of differential diagnosis of the various cellular elements. Only with No. 6 or No. 7 objectives is it easy to identify muriform cells, so important from the point of view of the nervous complications of

human trypanosomiasis. If the big cover-slips belonging to the Nageotte chamber are used, it is impossible to adjust the microscope so as to see the cellular elements deposited at the bottom of the chamber ; nor is this possible, even with a No. 3 Leitz objective. But if, instead of the cover-slip belonging to the apparatus, an ordinary fine cover-slip be used, it is quite easy, with a No. 3 objective, to count the cells deposited at the bottom of the Nageotte chamber ; a morphological study can be carried out more satisfactorily, however, with higher-power objectives in the little chambers already referred to, or simply by examination of the centrifuged cerebro-spinal fluid between the slide and the cover-slip, if only occasional cells have been found.

(f) Provided that a few small technical details are borne in mind, it is quite easy, with the Nageotte chamber, to examine the cerebro-spinal fluid for trypanosomes. Red corpuscles and leucocytes are known to settle more rapidly than trypanosomes, a phenomenon which is turned to account in examinations for trypanosomes by blood centrifugation. If the Nageotte chamber is filled with cerebro-spinal fluid and left for about ten minutes or a quarter of an hour, all other cells fall to the bottom ; the trypanosomes alone remain for some time on the surface near the cover-slip or float on the top of the liquid, but quite separate from the cellular elements. The latter drop to the bottom of the Nageotte chamber, out of focus of the objective, and leave the supernatant fluid clear and transparent under microscopic examination ; all objects such as trypanosomes, stand out separately darkly silhouetted in the bright microscope field like the "negative" of a dark-ground preparation. With a low-power objective—and this is the only kind that can be used with the Nageotte chamber—it is quite easy to examine 50 or 100 c.mm. of cerebro-spinal fluid and to detect any trypanosomes that may be present.

The following is the practical method I employed in looking for trypanosomes in the cerebro-spinal fluid :

If I found a large number of cellular elements in the cerebro-spinal fluid in making a cell-count in the Nageotte chamber, I then proceeded to look for trypanosomes. If the cell-count was low, I first centrifuged the fluid and filled the Nageotte chamber with the lower layer, covering it not with the thick cover-slip supplied by the makers of the apparatus but with an ordinary fine cover-slip, so as to permit of an examination of the bottom of the chamber with a No. 3 objective. I then let the preparation stand for 10 minutes or a quarter of an hour, long enough for the cellular elements to settle, but not the trypanosomes, which continue to float for some time. Using a No. 20 objective and a No. 6 Zeiss compensating eyepiece, after 10 or 15 minutes' sedimentation, I used to find the trypanosomes, chiefly by focussing between the under surface of the cover-slip and the level at which the lines on the bottom of the Nageotte chamber were beginning to be faintly visible, though not well defined.

The difficult part of the examination consists in identifying the trypanosomes with low-power objectives. In the search for flagellates each microscopic field should be examined at all depths from the cover-slip right down to the bottom of the chamber where the cells are deposited. The trypanosomes moving in the fluid sometimes move out of focus.

The microscopic examination must be carried out most carefully ; the minute silhouettes of the trypanosomes may escape the notice of the observer if the movements of the coarse adjustment be too rapid. If the fine adjustment be used, it would do away with the advantage of this particular method, which permits of rapid examination of a large volume of cerebro-spinal fluid. An observer who is not forewarned might

miss the trypanosomes, not recognising them with the low-power objectives, but, once he has caught sight of their little forms darkly silhouetted against a clear background, he will realise, when carrying out subsequent examinations, the real advantage offered by a deep chamber such as that of Nageotte in detecting the presence of trypanosomes in the cerebro-spinal fluid.

## II. ANIMALS.

I was placed in charge, for several months, of the examination of the ruminants at the Entebbe Laboratory experimentally infected with *T. gambiense*, I took a number of notes, and have extracted what appeared to me to be the most interesting for the purpose of this article.

For nearly a month I made daily examinations of the blood (fresh preparations between slide and cover-slip) of from 12 to 20 inoculated animals. Out of 423 animals, 6 only gave positive results, *i.e.*, 1.4 per cent. Another person engaged in the examination of thick films from the same animals obtained only two positive results.

I then carried out 83 centrifugalizations of the blood of these and other animals and found trypanosomes in 13 examinations, *i.e.*, approximately 15 per cent positive results; but allowing for the fact that I examined from three to five preparations of each centrifuged blood, the percentage of positive findings in relation to the number of slides examined is very much lower.

While I was engaged in these centrifugalizations, an examination was also being made of fresh blood preparations between slide and cover-slip and of thick films, dried and stained. Of the samples which had given thirteen positive results by centrifugalization, trypanosomes were found only in three in fresh blood films and only in two by the thick-films method, one of these last two positive findings being from an animal (No. 342) in whose case examination after centrifugalization had always given negative results, despite repeated and protracted attempts.

Dr. DUKE was successful in infecting glossinæ bred in captivity on inoculated animals of whose blood microscopic examination had been negative.

When trypanosomes are few in number, as in the case of *T. gambiense* in domestic ruminants and even in certain persons suffering from sleeping-sickness, their microscopic diagnosis is very uncertain. Whatever the means actually employed to discover the trypanosomes in the peripheral blood, negative results do not preclude the possibility of trypanosomiasis. When there are clinical grounds for suspecting trypanosomiasis, every possible means of diagnosis should be employed and repeated before eliminating this hypothesis. The following are the methods generally employed at the Entebbe Laboratory: examination of fresh blood, directly or after centrifugalization; examination of the blood by the thick-film method or stained films; inoculation of susceptible animals; examination of gland juice and of the cerebro-spinal fluid; recovery of the organism from animals infected from the patient and the application of the formol-gel test. The results of this last-named method of diagnosis are dealt with in a special article.

For the results of transmission experiments, the reader is referred to the interesting results to be found in Dr. DUKE's report. This process has given the highest percentage of positive results. The only drawback is that it requires a special organisation, like that of the Entebbe Laboratory for the breeding of glossinæ, and that it is not applicable to certain human and animal strains of trypanosomes, which—as Drs. DUKE, KLEINE, ROUBAUD and REICHNOW have shown—are not originally transmissible by the tsetse-fly.

Cases have occurred of ruminants inoculated a number of times by means of the syringe and bitten by infective glossinæ, in which it was impossible to find trypanosomes by microscopic examination of the blood, although their presence was sometimes revealed by transmission experiments or on post-mortem examination.

I did not find, as I say, that centrifugalisation of the blood of ruminants offers any great advantage as compared with the direct examination of fresh preparations or stained thick-films. Their blood has one peculiarity which may, perhaps, explain the negative results obtained by centrifugalisation. I began by centrifugalising the blood of two animals at once with a hand centrifuge with two tubes; later, when the electric centrifuge with its four tubes, was set up at the Entebbe Laboratory, I was able to treat simultaneously the blood of four different species: human beings, heifers, sheep and goats.

Employing the same quantity of blood and sodium citrate (9 c.c. of blood, and 1 c.c. of citrate solution), I observed that sedimentation of the corpuscles occurred more rapidly in the case of the human blood than in that of the ruminants, and that as regards the latter it was slower in sheep's blood than in heifer's and slower still in goat's blood.

Is the explanation to be found simply in the viscosity of the plasma? It should be noted, however, that the rate of sedimentation of the different bloods which I centrifugalised was in direct ratio to the size of the respective red cells, a fact which may be more conveniently seen from the following table:

	Human Beings	Heifers	Sheep	Goats
Diameter of red corpuscles in microns . . . . .	7 $\mu$	6 $\mu$	5 $\mu$	4 $\mu$
Time, in minutes, required for centrifugalisation of the red corpuscles . . . . .	15'	20'	25'	30'

In the centrifugalisation of human blood the separation of the trypanosomes from the red blood corpuscles is simple, for the latter are deposited after 10 minutes' or a quarter of an hour's centrifugalisation and the trypanosomes take from 30 to 40 minutes. On the other hand, the smaller corpuscles of ruminants take practically the same time to centrifugalise as polymorphic trypanosomes. This may, perhaps, be the reason why less satisfactory results are obtained with the blood of ruminants than with human blood, after centrifugalisation.

In studying *T. gambiense* infection in ruminants, therefore, there is risk of our assuming sterilisation to have occurred in an animal which is really infected if we rely simply on the microscopic examination of the peripheral blood.

I now propose to submit a few observations which I made when endeavouring to introduce certain modifications into the technique for the microscopic diagnosis of trypanosomiasis, owing to the uncertainty of the results obtained in domestic ruminants by the usual methods of diagnosis.

My first experiments were on ruminants, but as trypanosomes were rarely found in them (the parasites having disappeared definitively or temporarily during trypanolytic crises), and as I wished to find out for certain whether the negative results were due to the inadequacy of the methods of diagnosis employed or to the absence of trypanosomes in the blood examined, I next proceeded to carry out experiments on guinea-pigs and monkeys infected with *T. gambiense*, which, on examination of thick films or of fresh preparations between slide and cover-slip, revealed the presence of occasional parasites in the peripheral blood (monkeys 222 to 273 and 274 and guinea-pig 248, infected with the trypanosome from Damba antelopes).

I. "*Hæmoclastic*" Shock.

I decided to try adrenalin injections, a procedure recommended by certain experimenters in the diagnosis of malaria. I gave to monkeys (weighing about 3 kg.) two or three drops of a normal solution of adrenalin, and to guinea-pigs I gave injections of 0.5 to 1 c.c. of a solution composed of three drops of adrenalin and 10 c.c. of distilled water.

I also inoculated fairly big doses of old cultures of bacteria isolated from the intestines of tsetse-flies.

I examined 500 microscopic fields of fresh preparations and took an average of the trypanosomes found in each field, before the injection and half an hour, 1 hour, 2 hours, 3 hours, 5 hours and sometimes 24 hours after the injection producing the "shock".

The results varied very considerably: in some cases there was a big increase in the number of trypanosomes per area, while in others the changes were inconsiderable. The highest percentages were obtained 3 or 4 hours after the injection.

Blood centrifugalisation before and after the "shock" also gave good results on occasion, but these were not sufficiently constant for it to be possible, without control-experiment to recommend this process as a real improvement.

2. *Centrifugalisation of Much-diluted Blood.*

It will be noted that when the blood contains a fairly large number of trypanosomes, easily discernible on direct examination of fresh preparations between the slide and cover-slip, the parasites did appear in point of fact to be more densely concentrated in the sediment obtained from the third centrifugalisation, but some were still found attached to the blood corpuscles in the residues of the first and second centrifugalisations, carried out in accordance with the inventors' original technique. We may conclude, therefore, that in these partial centrifugalisations the extraction of the trypanosomes is not complete, only a certain proportion, sometimes quite small, being separated from the blood corpuscles.

Since, further, the red corpuscles in the centrifugated tubes take longer to settle, the greater the volume of blood centrifugated and the more highly concentrated it is, I resolved to carry out centrifuge experiments with small quantities of blood highly diluted with a citrate solution. Instead of the classic solution of 9 c.c. of blood + 1 c.c. of 6 to 10% sodium citrate solution, I employed practically inverse proportions: 1 c.c. of blood + 5 c.c. of 1% sodium citrate solution.

The results which I got by this process were practically the same as those obtained with the classic triple centrifugalisation, that the centrifugalisation of diluted blood possesses the advantage that it does not take so long and requires only one-tenth of the blood usually employed in the Martin-Leboeuf process. I completed all the diluted blood centrifugalisations in the space of 7 to 10 minutes; after 2 minutes, the red cells settled, forming a thin layer. When the supernatant liquid was decanted and recentrifugated at the proper speed, after 5 to 7 minutes the leucocytes, the red corpuscles and the trypanosomes were deposited. The whole of this small deposit could be examined in one or two fresh preparations between the slide and cover-slip. Classic centrifugalisation takes from 30 to 45 minutes and the results obtained do not compensate for the expenditure of time or the larger quantity of blood required, which is not easily obtainable from all animals and veins.

### 3. *Centrifugalisation of Hæmolysed Blood.*

As already mentioned, we sometimes found trypanosomes in a thick film, after hæmolysis and staining, when repeated centrifugalisation of the blood of the same animal had not revealed them, that is to say, we discovered trypanosomes *in a single thick film* but found none in 9 c.c. of centrifugalsed blood, equal approximately to 200 drops; accordingly, I am becoming more and more convinced that centrifugalisation separates only a small proportion of the parasites contained in the peripheral blood so treated.

I endeavoured to combine the advantages of centrifugalisation and of the hæmolysed thick-film method by diluting the blood with a liquid which at the same time fixes the trypanosomes and hæmolyses the red blood corpuscles. I tried several media, always with small quantities of blood (five to ten drops, or 0.25 to 0.5 c.c.), simply varying the hæmolytic agent.

(a) *Noguchi's fluid*: Affects the morphology of the trypanosomes very little, but does not hæmolysed sufficiently, and it is difficult, after the centrifugalsed deposit has been dried and stained, to discover the trypanosomes in the mass of red corpuscles remaining.

(b) *Giemsa Solution*: I examined the hæmolysing and staining properties of this solution. Hæmolysis of the blood corpuscles was effected, but Giemsa solution, in the presence of blood, produced a precipitate, so that it was difficult to recognise the trypanosomes in the mass of the precipitate, even when present in large numbers. The results obtained with hæmatoxilin, anilin and Leishman solutions were no more encouraging.

(c) *Ross-Ruge's Reagent* (solution of formal acetic acid): Hæmolysis is complete, and the trypanosomes can be readily diagnosed, although somewhat deformed and imperfectly stained by the Romanowsky process (or the Leishman, Giemsa, processes, etc.)

(d) *1 in 3 Solution of Absolute Alcohol*: The trypanosomes are less deformed and can be better fixed and stained than when the blood is hæmolysed with Ross-Ruge's reagent, but hæmolysis is incomplete and it is difficult to distinguish the trypanosomes among the remaining red blood corpuscles accumulated in the preparation.

(e) *1 in 5 Solution of Methyl Alcohol*: Hæmolysis is perfect, provided that not much blood is added (one drop per cubic centimeter, as otherwise precipitates also appear and render microscopic examination difficult. When trypanosomes are treated with this solution, they can be almost as characteristically stained by the Romanowsky method as in the case of dry films.

This process, like the thick-film method, is to be recommended not for the purposes of a morphological study of the trypanosomes but simply to determine their presence in the blood.

The following was the method that I adopted :

I put into a centrifuge tube 4 to 5 c.c. of distilled water + 1 c.c. of methyl alcohol. I pricked the ear or paw of the animal as is done in making blood smears or fresh preparations to be examined under a cover-slip. By means of a capillary pipette with a rubber cap, I then drew up the drop of blood and squirted it into a solution of methyl alcohol, stirring the latter so as to wash out the pipette and facilitate hæmolysis.

After collecting and hæmolysing from six to ten drops of blood in this way, I centrifugalsed immediately, for 7 to 10 minutes, as rapidly as seemed desirable; I



then decanted the liquid and with a capillary pipette drew out the small centrifuged deposit, which was placed on a slide, dried and stained by the Giemsa or Leishman method.

The microscopic field appeared entirely covered with leucocytes, hæmatoblasts and trypanosomes, as in a thick film, but naturally in a denser mass, since in any given field there were the leucocytes and trypanosomes obtained from ten times the quantity of blood. This fact of concentration, together with more perfect hæmolysis, constitutes the advantage of this particular method : it collects in a small area of the preparation the contents of not one drop of hæmolysed blood (as in the thick-film method) but from six to ten drops, better hæmolysed than by the classic thick-film method. The centrifugalisation of the hæmolysed blood in the alcohol solution is effected by one single operation in from 7 to 10 minutes.

If more blood is to be employed, a larger quantity of methyl alcohol solution must also be used, so that hæmolysis may be complete and the remnants of the red cells may not interfere with the examination.

The trypanosomes are slightly swollen, but when stained by the Romanowsky method the flagellus, nucleus and kinetoplast can be sufficiently differentiated and it is easy to distinguish them from the leucocytes and hæmatoblasts in the hæmolysed blood.

If the addition of a large quantity of blood to the alcoholic solution resulted in incomplete hæmolysis, the addition of a single drop of 10% acetic acid solution was sufficient to ensure immediate hæmolysis.

I have not used methyl alcohol to which acetic acid had already been added, as I thought that such a reagent would be likely to distort still further the trypanosomes and make them staining with Giemsa more difficult, even if the solutions were subsequently neutralised. Accordingly, I simply mixed the blood with the methyl alcohol solution, without acetic acid, and only added the latter if this was necessary, to complete hæmolysis. With hæmolysis obtained by this double operation, the distortion of the trypanosomes was less marked, perhaps because the acetic acid came in contact with flagellates already more or less fixed by the methyl alcohol.

\* \* \*

I have made a number of trials of these methods but only when the Commission's work was drawing to its close and I was busy preparing the reports. I thus have not had time to elaborate large statistical data confirming the encouraging results which I had obtained with monkeys infected with *T. gambiense*.

If a solution can be prepared that will produce complete hæmolysis of the blood corpuscles and at the same time fix the trypanosomes without interfering with their staining properties, in the microscopic diagnosis of trypanosomiasis in the early stages of infection will be greatly facilitated. It is in the early stages that diagnosis is so important, since it is only then that there is any likelihood of a *Therapia Sterilians magna*.

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2.

POSTERIOR-NUCLEAR FORMS OF POLYMORPHIC TRYPANOSOMES  
(BRUCEI, GAMBIENSE AND RHODESIENSE).

A great deal has been written concerning the identity or non-identity of the three polymorphic trypanosomes (*brucei*, *gambiense* and *rhodesiense*) since STEPHENS and FANTHAM discovered that the last of these is characterised by the presence of forms in which the nucleus is displaced towards the posterior extremity.

Dr. DUKE (1915, 1921, 1923 and 1926) is one of the investigators who, during the last few years, has made the most determined effort to prove that these trypanosomes are simply varieties of a single zoological species.

Professor BRUMPT (1924), on the contrary, in a critical review of the facts and hypotheses concerning the specificity of *T. rhodesiense* in which he refers specially to the arguments put forward by Dr. DUKE, seeks to prove that the three trypanosomes, *brucei*, *gambiense* and *rhodesiense*, are all distinct and valid species.

Epidemiological and geographical facts, the virulence and crossed immunity reactions of the three trypanosomes, and their vertebrate and invertebrate hosts, etc., can be made to serve the supporters of both theories.

There is, however, one rather more remarkable feature, namely, the morphological character of the position of the nucleus in the posterior-nuclear forms, forms which are unknown in *T. gambiense* but are easily demonstrated in *T. brucei* and *T. rhodesiense* and have already been used to distinguish the latter from *gambiense*.

Dr. DUKE wrote in 1923 that he was convinced that these posterior-nuclear forms could also be found in virulent strains of *T. gambiense*, suitably inoculated into certain laboratory animals.

Dr. DUKE, a supporter of the thesis of the identity of species of polymorphic trypanosomes in African mammals, was appointed President of our Commission, whose programme included a study of the relations between *T. gambiense* and *T. rhodesiense*. One of his first steps accordingly was to obtain recent strains of *T. gambiense* and to inoculate them into guinea-pigs and rats and examine for posterior-nuclear forms, the fundamental argument on which defenders of plurality of species based their theory.

When I arrived at the Entebbe Laboratory, at the end of April 1926, I found guinea-pigs inoculated with *T. gambiense* recently isolated by Dr. VAN HOOF from sleeping-sickness patients.

One of the first tasks which the President of the Commission gave me was to look for the much-discussed posterior-nuclear forms in the blood of these infected guinea-pigs.

It had already been shown that posterior-nuclear forms of *T. rhodesiense* could only be found in the case of heavily infected rats and guinea-pigs, when flagellates were present in large numbers in the peripheral blood.

Day after day I examined the guinea-pigs to determine their state of infection and devoted much time to looking for the shorter forms in the animals most heavily infected with *T. gambiense*, in case any of them might show a displacement of the

nucleus towards the posterior extremity, as in *T. rhodesiense*. I must admit that I undertook the work simply from a sense of duty, finding it irksome, and proceeded without enthusiasm. *Post tantosque labores*, I discovered a shorter form, with the

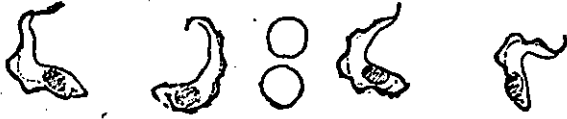


Fig. 1

First posterior-nuclear forms found in *T. gambiense* by members of the Commission.

nucleus somewhat out of position. This was more encouraging and gave me patience to pursue my researches with greater faith. After this, to discover typical posterior-nuclear forms was simply a matter of persevering; I found some practically every day (except during trypanolytic crises) in guinea-pig 54, which was the most heavily infected of all, and had been inoculated about two months previously (Fig. 1).

This is how Dr. DUKE's prevision came to be corroborated.

\* \* \*

Shortly afterwards, Dr. LAVIER, of the Laboratory of Parasitology of the Paris Faculty of Medicine, who had been specially appointed to study the morphology of trypanosomes, found these forms not only in this same guinea-pig 54, but also in many others which had been inoculated with *T. gambiense* recently isolated by members of the Commission from various patients suffering from sleeping-sickness.

Later, Professor KLEINE announced that he had also found posterior-nuclear forms in the blood of a guinea-pig inoculated with *T. gambiense* obtained from one of his patients, and sent the drawings made with a camera lucida.

Forms with a posterior nucleus are undoubtedly more difficult to find in *T. gambiense* than in *T. rhodesiense*. I never succeeded in detecting the extreme forms in which the nucleus, passing behind the kinetoplast, gives the trypanosome the "Crithidia" form.

As I have said, Dr. DUKE had anticipated and prepared everything. It was just by chance that I happened to be carrying out a careful microscopic examination, and was fortunate enough to make this observation and hand it over to Dr. DUKE, its rightful owner. This was the whole extent of my modest collaboration.

The question was subsequently dealt with in full by Dr. LAVIER; the latter has prepared a most interesting study, admirably illustrated by himself, which forms part of the Commission's report.

#### SUMMARY AND CONCLUSIONS.

The posterior-nuclear forms of *brucei* and *rhodesiense* trypanosomes, which were formerly considered to distinguish them from *T. gambiense* (supposed not to possess them), do exist also in this last-named species.

It was my good fortune to be the first to find these forms in guinea-pigs which Dr. DUKE had inoculated with *T. gambiense* recently isolated from sleeping-sickness cases, with a view to searching for the presence of these forms. Later, it was possible to observe them fairly regularly, especially towards the end of severe cases of infection.

The percentage of posterior-nuclear forms in recently isolated *T. gambiense* is manifestly lower than in recently isolated *T. rhodesiense* infections. They may, however, be more numerous than in laboratory strains of *rhodesiense* after many

passages. This trypanosome, as THOMSON AND ROBINSON (1926) pointed out, in the end exhibits neither posterior-nuclear forms nor real polymorphism, adopting a temporary or definitive monomorphism.

From this point of view, *T. gambiense* behaves as if it had been separated longer than *T. rhodesiense* from its primary natural vertebrate host, probably big game, the ordinary natural reservoir of *T. brucei* and *T. rhodesiense*.

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3.

### GATÉ-PAPACOSTAS REACTION (FORMOL-GEL TEST) IN THE DIAGNOSIS OF TRYPANOSOMIASES.

The formol-gel test, discovered by GATÉ AND PAPACOSTAS, originally consisted in adding two drops of commercial formalin to 1 c.c. of quite clear blood-serum and leaving this mixture for 24 to 30 hours at laboratory temperature.

Sera which coagulated and formed a jelly were regarded as giving a positive, those which remained liquid as giving a negative reaction (Fig. 1).

According to the workers, syphilitic sera gave positive reactions corresponding more or less with the results of the Wassermann reaction.

The test appears to have been discovered accidentally.

Those who work in laboratories know how difficult it is to keep blood-serum sterile, forming as it does an excellent culture medium for many micro-organisms.

Physical and chemical sterilising agents are sometimes injurious to certain of its properties. Careful asepsis and cold are the most harmless means if it is important to preserve all the properties of the serum intact.

At the same time, according to the purpose in view, there are cases in which the serum may be heated from to 50° to 60° C., or antiseptics (phenol, chloroform, tricrisol, quinosol, etc.) in small quantity may be added in order to prevent the rapid growth of any bacteria that may have been introduced despite careful asepsis.

In clinical laboratories, which do not have to carry out a very large number of analyses, Wassermann tests are not made every day as the variety of reagents required and their complex preparation entail almost as much work for one reaction as for several dozens. This necessitates the keeping, until the special day set aside for such work, of sera which arrive daily at the laboratory.

It was apparently when adding small quantities of formalin to such sera, in order to preserve them, that GATÉ AND PAPACOSTAS observed that some jellified and others did not; those which did being those which gave positive Wassermann reactions.

Statistics published at the time by GATÉ AND PAPACOSTAS (1920) show that normal sera generally remain liquid, while syphilitic sera give the formol-gel reaction, the results tallying with those of the Wassermann reaction in 85 per cent of cases.

The technique of this reaction is very simple; it is not surprising, therefore, that it should have been immediately tried in a number of laboratories, where it gave very

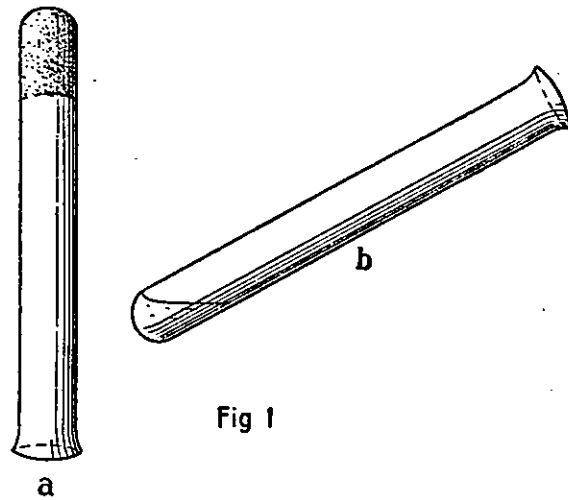


Fig 1

#### TEST-TUBE REACTION.

a. *Positive reaction*: The serum becomes solid and the test-tube can be turned upside down without spilling it.

b. *Negative reaction*: The serum remains liquid.

divergent results, perhaps because some over-estimated its advantages and others its defects.

The reaction has recently been tested by a number of investigators in both human and animal trypanosomiasis. The results obtained were contradictory; accordingly, as the Commission had sufficient material at its disposal to test the reaction, and as Dr. DUKE and myself were trying to find a more efficient method than that hitherto employed in the difficult task of diagnosing trypanosomiasis in ruminants experimentally infected with *T. gambiense*, I decided, at the suggestion of the President of the Commission, to make my contribution to the subject under discussion by carrying out a few experiments.

\* \* \*

The divergent results obtained by different investigators may, in my opinion, be explained by variations in the technique adopted or by different criteria of interpretation. All employ 1 c.c. of clear blood serum, but there are marked differences as regards the quantity of formalin added to the serum, the size of the tube used for the reaction, the number of hours during which the reagents are left in contact before the results are read, and, lastly, the criterion applied in assessing the latter, namely, simple jellification (total or partial) or opacity of the jelly.

*Quantity of Formalin.* — Some add to 1 c.c. of serum a single drop of 40% or 20% formalin; some add two, three or four drops; others indicate the size and angle of inclination of the drop-pipette, and a very few give the volume of the drops employed in fractions of a unit of capacity. If, instead of a big dropper, a narrow capillary pipette is used, it is obvious that the number of drops may vary, for example, from twenty to seventy or even more per cubic centimeter. We shall demonstrate later that the quantity of formalin employed is not without effect on the rate of jellification.

*Calibre of Tube.* — As a rule, jellification is tested by inverting the tube containing the formalised serum. One cubic centimeter of serum, even in the soft jelly state, does not remain suspended at the bottom of an inverted tube if the tube is too large; on the other hand, if the inverted tube is too narrow, capillarity may prevent the serum, from pouring, even though it is liquid.

*Time required for the Action of the Reagents.* — In my opinion, the number of hours during which the serum is left in contact with the formalin before the results are read accounts more than anything for the differences in the results reported by various investigators. Some consider one hour long enough, while others consider from 24 to 72 and even 96 hours to be necessary. Hence, a serum which becomes jellified, for example, in 30 hours gives what is regarded by some as a positive and by others as a negative reaction.

*Simple Jellification or Jellification accompanied by Opacity.* — In India, where this reaction has been regarded as characteristic of kala-azar, it has been made a rule, since jellification of sera occurs also in other indigenous diseases, that opacity of the solidified serum shall be the criterion of a positive reaction in the diagnosis of kala-azar. Accordingly, the solidification of tuberculous, leprosy, malarial, syphilitic and other sera is not regarded as a positive result unless this opaque appearance is found.

\* \* \*

We shall now proceed to consider the special results obtained by certain investigators, and shall compare them with those which we have ourselves obtained.

## I. FORMOL-GEL TEST IN THE DIAGNOSIS OF SYPHILIS.

GATÉ AND PAPACOSTAS (1920), in their first report, recommended the addition of two drops of formalin to 1 c.c. of clear serum, the mixture to be left from 24 to 30 hours at laboratory temperature. Jellification occurred in syphilitic sera, confirming the Wassermann reaction in 85 per cent of cases.

ECKER (1921), on the contrary, concluded from his observations that the reaction does not possess the value which GATÉ AND PAPACOSTAS attributed to it in the diagnosis of syphilis: he had obtained negative reactions in sera that were certainly syphilitic and, again, positive reactions in non-syphilitic sera.

SUFFERN (1921) obtained similar contradictory results, although, in syphilis, the results were very frequently positive (90 per cent).

A. BESSEMANS (1922) did not find the agreement noted by GATÉ AND PAPACOSTAS between the results of this reaction and those of the Wassermann reaction. He obtained jellification of the serum of many persons who were undoubtedly not syphilitic. He also noted that the sera of sick persons jellified more readily than that of normal persons.

N. BETTENCOURT (1922), M. LÉGER (1922), and M. ARMANGUÉ AND P. GONZALÈS (1922) also carried out a comparative study of the two reactions; according to their observations the results were quite different, and they came to the conclusion that the formal reaction was of no value in the diagnosis of syphilis.

GATÉ AND PAPACOSTAS (1922), in a fresh study on the reaction to which they have given their name, followed the original technique with one difference: they read the results after 72 instead of 24 or 30 hours, as they had originally recommended. They observed that the reaction was frequently positive, not only in cases of syphilis but also in various other diseases (tuberculosis of long standing, pulmonary, osteo-articular or ganglionic; blennorrhagia with resultant complications, etc.). *The investigators declare that, in their view, the formalin-gel test is evidence of unknown humoral modifications, perhaps determined by certain generalised chronic infectious processes, which profoundly affect the organism.*

They also declare that, after efficient anti-syphilitic therapeutic treatment, reactions become negative which before were positive.

## II. FORMOL-GEL TEST IN THE DIAGNOSIS OF INDIAN KALA-AZAR.

NAPIER (1921-22) employed this test in India in the diagnosis of kala-azar, using a technique very similar to that originally described by GATÉ AND PAPACOSTAS. He added to 1 c.c. of serum one drop of 20% formalin in a 1/2-inch tube (approximately 12.5 m.m.). After shaking the solution, he kept it for 24 hours at laboratory temperature. He judged the results of the test, however, not by the jellification of the serum but by the opacity and tint assumed by the jelly, whence the name of "aldehyde test", applied to this reaction to distinguish it from the Gaté-Papacostas "formol-gel test". Napier regards as positive only those reactions in which the serum becomes solidified and *completely opaque* in the space of 24 hours. He regards as negative all sera which do not become opaque within the first 24 hours, whether jellification occurs or not.

Adopting this criterion, he carried out the reaction with the serum of 150 persons, 91 of whom exhibited leishmania on microscopic examination. Of these 91 sera, 89 (98 per cent) yielded an opaque jelly within the first 24 hours. This did not occur in the sera of the 59 remaining persons in whom no leishmania had been found.

Apart from kala-azar, in a number of cases of leprosy and advanced tuberculosis the test produced opaque jellification. NAPIER also observed cases of malaria in which jellification was rapid and accompanied by opacity, the opaque jelly assuming a slightly greenish tint, easily distinguishable from the dull-white characteristic of kala-azar opacity, which is like the white of a boiled egg.

NAPIER observed that positive reactions of untreated kala-azar became negative after effective treatment with tanter emetic.

NAPIER carried out a number of experiments to determine the chemical characteristics of the reaction. He declared that the reaction occurred between the aldehydes and the part of the serum which contains the euglobulins. He also observed that it was only necessary slightly to increase the "Ph" of a negative serum to obtain a positive reaction, and that, if the "Ph" of positive sera was modified more or less slightly, negative reactions were obtained.

FOX AND MACKIE (1921) agree that the reaction in question possesses the real value attributed to it by NAPIER in the diagnosis of kala-azar. They suggest a modified technique yielding prompt results. They place one drop of clear serum on to a slide, which is placed upside-down on a watch-glass containing a few drops of formalin. They regard as positive reactions in which the drop becomes solidified and opaque within an hour. To test solidification, the serum need only be cut with a needle, which will divide it into two if it is actually solidified, and will not affect its shape if still liquid (*cf.* Fig. 2).

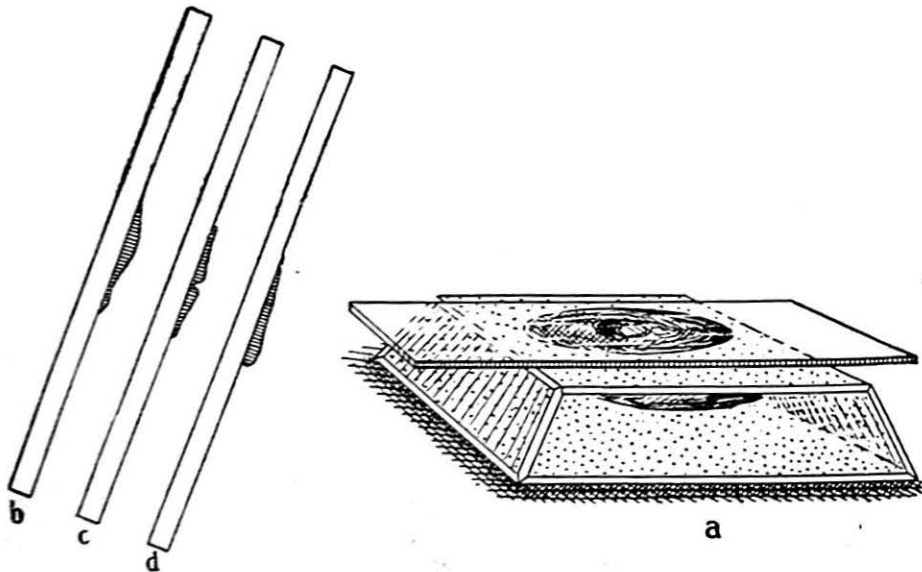


Fig. 2

REACTION BY THE FOX-MACKIE TECHNIQUE.

a. A slide with one to three drops of serum to be examined, over a hollow glass receptacle containing a few drops of commercial formalin ;

b. *Positive reaction* : The drop of solidified serum does not alter in shape if the slide is tilted ; and

c. It can be cut with a needle which will make a furrow in it ;

d. *Negative reaction* : The drop of serum, if still liquid, falls when the tube is tilted and does not alter in shape when cut with a needle.

reaction is due to an increase in the euglobulins of the blood, which is associated with the development of anti-bodies which appear to be intimately connected with these substances.

NAPIER AND MILLIS (1923) carried out fresh experiments, working respectively on microscopic examinations to detect leishmania and on formalin reactions. The results obtained were concordant, except in one case : in this, though the serum jellified and rapidly became opaque, it was not possible to confirm the diagnosis of kala-azar by microscopic and clinical examination.

GANGULI (1925) also experimented with the reaction in the diagnosis of kala-azar and obtained encouraging results. These confirm NAPIER'S contention that the formol-gel



FROILANO DE MELLO AND F. BARRETO (1926) experimented with the reaction, using the sera of persons suffering from various diseases. They employed the original Gaté-Papacostas technique and various modifications. They came to the conclusion that the opacity of the sera on which NAPIER based his diagnosis of kala-azar does not bear a fixed relationship to jellification, but varies in proportion to the quantity of formol added to the serum under analysis.

They do not admit the value of the Gaté-Papacostas reaction in diagnosis, as they have obtained positive results not only in cases of syphilis, tuberculosis, leprosy, etc., but even in normal individuals. As regards the opacity of the jelly, to which NAPIER attributes special importance in the diagnosis of kala-azar, this has also been observed by these investigators in cases of malaria, syphilis, leprosy and other diseases.

### III. FORMOL-GEL TEST IN THE DIAGNOSIS OF ANIMAL TRYPANOSOMIASSES.

PLANTUREUX (1923) applied the test, employing the technique recommended in the latest GATÉ-PAPACOSTAS report, to camels infected experimentally with *T. berberum*, the causative organism of "debats", the trypanosomiasis of North African camels.

The experiments of PLANTUREUX are of special interest in that they were carried out on camels which were healthy, were subsequently infected experimentally, and were finally treated. The formol-gel reaction was repeated before and after infection and before and after treatment. The results obtained were regularly controlled by the methods employed at the Algiers Pasteur Institute by Edm. AND Et. SERGENT AND DONATIEN, in their study on the experimental infection of dromedaries with *T. berberum*.

All the animals examined before inoculation gave a negative formol-gel reaction even after five days. Out of 6 camels infected for less than three weeks, 2 only gave a positive reaction within 24 hours, 1 within 48 hours, 1 within 72 hours and 1 within 96 hours; one other gave negative results, even after five days; in the case of 16 camels infected for over five weeks, the sera of 11 coagulated within 24 hours, of 3 within 48 hours, and of the remaining 2 within 72 hours. In the case of 12 infected camels after treatment, the sera of 2 only became jellified after two days, the sera of the other 10 remaining liquid after five days. Like GATÉ AND PAPACOSTAS, PLANTUREUX adopted an arbitrary three-day period to judge the result of the reaction, and this is why he regarded all the reactions of camels infected for more than five weeks as positive, although 5 out of 16 produced coagulation only at the end of two or three days.

He declares in his conclusions that during the early stages of infection the sera give fewer positive results and that the administration of various drugs, *even if they have no effect from the curative point of view*, prevents or at all events retards the reaction.

SPACKMAN (1923) tried this reaction on the sera of Indian camels infected with *T. evansi*, and found it of great assistance on the diagnosis of "surra."

HORNBY (1923) experimented with the same reaction in Central Africa, employing like PLANTUREUX the Gaté-Papacostas technique. He further defines the calibre of the drop-pipette (1 mm. diameter) and its angle of inclination at the time of use (45 degrees).

Out of 28 healthy oxen :

9	produced	jellification	within	6	hours.
5	”	”	”	12	”
6	”	”	”	24	”
5	”	”	”	48	”
3	”	”	”	72	”

He concluded that the bovines in the region where he had carried out his experiments reacted differently from the North African camels observed by PLANTUREUX. HORNBY even wondered whether the results described by PLANTUREUX were not completely contradicted. However, he examined the serum of an ox infected with *T. vivax*, which also produced rapid jellification (in less than 6 hours). He did not regard the reaction as of great value, at all events as regards the diagnosis of bovine trypanosomiasis in Central Africa.

KNOWLES (1924) applied the reaction to Sudan camels suffering from “tahaga” trypanosomiasis caused by *T. sudanense* (*T. evansi*).

He employed two methods simultaneously : the test-tube method and that of the slide and watch-glass with formalin. He used a tube 3 inches by one-third of an inch (about 7.5 cm. by 0.8 cm.), into which he poured 1 c.c. of clear blood serum and four drops of commercial formalin. He examined the tubes at the following intervals :  $\frac{1}{2}$ , 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours. For the slide method, he followed the Fox-Mackie technique, the only difference being that he poured three drops of serum on to the slide instead of one, as FOX AND MACKIE did in the diagnosis of kala-azar ; as he explains, the reaction being slower in the case of Sudan-camel “tahaga” than in that of Indian kala-azar, a single drop placed on a slide over formalin might harden in drying and lead one to imagine that jellification had occurred.

He tried the reaction on 7 camels infected with *T. sudanense*, and all produced rapid jellification (in the tube, in less than 24 hours, and on the slide, within 1 hour). On the other hand, jellification never occurred at all in the case of 12 healthy camels.

The serum of 2 oxen did not solidify, but in the case of 2 healthy horses “partial” jellification occurred in 24 hours in one, and “total” jellification in 48 hours in both.

In view of the results obtained, KNOWLES regards the formol-gel reaction as very useful in the diagnosis of Sudan-camel disease. The slide reaction, although less accurate, can be used when purchasing camels, as it is more rapid.

KNOWLES observed what PLANTUREUX had already demonstrated, namely, that in the case of recently infected camels the reaction is not always positive.

He also observed that the intensity of the reaction does not depend on the presence or absence of trypanosomes in the peripheral blood.

#### IV. THE FORMOL-GEL TEST IN THE DIAGNOSIS OF HUMAN TRYPANOSOMIASIS.

BRENNAN (1924) applied the formol-gel reaction to 10 sleeping-sickness patients and to 7 healthy persons. The first gave a positive and the others a negative reaction.

MORRISON (1924) applied the same test to 20 persons : 10 suffering from trypanosomiasis and 10 from other diseases. Eight of the 10 trypanosomiasis patients, all treated with Bayer, produced complete jellification in 6 hours ; in the case of the ninth, coagulation occurred in 24 hours, and in that of the tenth, within 48 hours.

*Three of these cases gave a positive reaction, even after the patients were apparently cured.*

Out of the 10 subjects not suffering from trypanosomiasis, 6 gave a negative and 4 a positive reaction (one case of malaria, which produced jellification in 6 hours, one case of smallpox, and a second of malaria in 24 hours, and a third case of malaria in 48 hours).

MORRISON considers the reaction of no great value as an aid to diagnosis, having found a high percentage (20 per cent) of positive reactions in diseases other than trypanosomiasis, unless indeed, these were old cases of trypanosomiasis spontaneously cured, which do not appear to be uncommon in Nigeria; if this be so the reaction would appear to be of great retrospective value.

According to MORRISON, cases apparently cured as the result of recent treatment continue to give a positive reaction. If, in cases treated some considerable time past which are regarded as definitively cured, the reaction should prove negative, it would be highly significant.

MORRISON considers that a reaction of this kind, which, although empirical, gives such uniformly positive results in sleeping-sickness, is deserving of greater attention, although he admits that the results obtained in a limited series of cases cannot be regarded as absolutely conclusive.

As the previous investigator had found 4 positive reactions out of 10 subjects who were ill though not suffering from trypanosomiasis, JOHNSON (1925) decided to try formol and Sachs-Georgi reactions, simultaneously with the serum of patients from Nigeria, where syphilis and other diseases caused by protozoa are fairly frequent.

He employed 1 minim<sup>1</sup> of 40% commercial formalin, to which he added 1 c.c. of clear serum in a small test-tube; he regarded as positive serum reactions which showed complete jellification within 24 hours, as feebly positive those in which partial jellification occurred, and as negative those in which the sera remained liquid after 24 hours.

Out of the 168 sera examined, 99 gave a positive Sachs-Georgi reaction, 28 a feebly positive reaction, and 41 negative reaction. The results of the formol-gel reaction were respectively: 84 positive, 24 feebly positive and 60 negative.

In view of the high percentage of positive results obtained in the case of syphilis (a disease found frequently in hot countries) and other tropical diseases, he concluded that this reaction is only of slight value in the diagnosis of cases of sleeping-sickness in process of evolution or already cured.

DYE (1926) applied the formol-gel reaction to persons infected with *T. rhodesiense* and to animals supposed to be infected with *T. brucei*.

Technique: to 1 c.c. of serum is added 1 minim of formalin. The tube is examined at the end of half an hour, 1 hour, 5 hours and 24 hours. For convenience, the results are arbitrarily classified as follows:

Liquid at the end of 24 hours . . . . .	Negative
One-quarter solid at the end of 24 hours . . . . .	P. 1.
Half-solid at the end of 24 hours . . . . .	P. 2.
Half-solid at the end of 5 hours . . . . .	P. 3.
Three-quarters solid at the end of 5 hours . . . . .	P. 4.
Completely solid at the end of 5 hours . . . . .	P. 5.
Completely solid at the end of 1 hour . . . . .	P. 6.

<sup>1</sup> 1 minim equals one-seventeenth cubic centimeter.

DYE confirmed the results obtained by previous investigators in the case of syphilis, tuberculosis, leprosy, malaria, kala-azar and human and animal trypanosomiases, namely :

(a) Cases infected with *T. rhodesiense* produce complete jellification within from 24 to 48 hours ;

(b) The intensity of the reaction does not correspond to that of the infection, as shown by the number of trypanosomes present in the peripheral blood ;

(c) The formol-gel reaction is also modified by the successful therapeutic treatment of trypanosomiasis *rhodesiense* and from this point of view, the action of "Bayer 205", according to DYE, is stronger than that of tryparsamide.

To sum up his conclusions :

1. In the region where he carried on his work he found 70 per cent of the children suffering from hypertrophied glands, a percentage which he believes does not occur in non-tsetse zones. According to DYE, these high percentages are found in the places where trypanosomiases are most frequent.<sup>1</sup> As the formol-gel test applied to these children gave a high percentage of positive results and these became totally or partially negative under the action of "Bayer 205", he is of opinion that these were cases of trypanosomiasis already cured or in process of being cured, although in the majority of cases he was not successful in proving the presence of *T. rhodesiense*.

2. DYE recognises that game must serve as a reservoir and constitute a constant source of infection. But as game is rare, *T. rhodesiense* is seldom found except in passage from man to man, this being given by DYE as the reason for a reduction in virulence of certain strains of trypanosomes. Proceeding on this assumption, he believes that in the region in question there is a long standing mild endemic of trypanosomiasis *rhodesiense*, characterised by a tendency towards spontaneous cure with a fairly large number of healthy carriers.

3. He likewise considers a high percentage of game to be infected with *T. brucei*, since the game (taken as a whole) gives approximately 35 per cent positive formol-gel reactions, and in the case of certain animals (water-buck), approximately 83 per cent, although out of thirty animals he found one only with trypanosomes, on a single occasion, the formol-gel reaction is modified by "Bayer 205"; this constitutes yet another analogy between *T. brucei* and *T. rhodesiense* noted by the investigator.

4. He recognises also that the effect of tryparsamide on *T. rhodesiense* is less marked than that of Bayer, as evidenced by its therapeutic action and the modification in the intensity of the formol-gel reaction. This observation appears to him to constitute a serious argument in support of the duality of the species *rhodesiense* and *gambiense*.

5. He thinks that the problem of human trypanosomiasis is impossible of solution if efforts are solely directed towards the elimination of the tsetse-fly. He attaches more importance to therapeutic measures in regions where the disease is endemic and not of a serious character, as was the case where he studied it; by such means human cases, which are the chief reservoirs of infection, can easily be sterilised.

6. According to DYE, "Bayer 205" administered in small doses ( $\frac{1}{8}$  grain) has the effect of considerably retarding in vitro the formol-gel reaction of sera of persons or

<sup>1</sup> DYE established a comparative table of the number of cases of trypanosomiasis and the percentage of children with hypertrophied glands in each place. He sees a close connection between them which does not, however, appear very clearly from the figures.

animals infected (or supposedly infected) with *T. brucei* or *rhodesiense*, a result which is not obtained with tryparsamide.

\* \* \*

I employed the formol-gel test in the case of 15 persons suffering from sleeping-sickness (*T. gambiense*) and of various persons apparently in good health; of 5 monkeys infected with *T. gambiense* and of three not so infected; and, lastly, of 38 ruminants (heifers, goats and sheep) employed for the Commission's experiments and inoculated with *T. brucei*, *T. rhodesiense* or *T. gambiense*.

I repeated the formol-gel test on each of the ruminants, two, three and four times respectively, making a total of 146 tests.

In many of these animals the test was made before inoculation with trypanosomes, and repeated a few weeks, and a few months, after infection.

In the case of 15 persons suffering from sleeping-sickness (*T. gambiense*) in whom the infection was of long standing, the sera became jellified after 10 to 60 minutes. Two became jellified only after 2 hours and two others after 6 hours.

The serum of patients having received from 24 to 30 grammes of tryparsamide, considered clinically cured and with normal or practically normal cerebro-spinal fluid, continued to give a positive formol-gel reaction, though rather more slowly (from 1 to 24 hours) than before treatment.

In the case of one patient, I carried out the test before treatment and 10 minutes after having given him an injection of 3 grammes of tryparsamide. The serum jellified in 15 minutes, both before and after the injection.

In the case of several clinically healthy persons, I obtained jellification only after one or more days.

\* \* \*

In the 23 formol-gel reactions carried out on animals before they had been inoculated with trypanosomes and when they were not suffering from any other disease, jellification of the sera did not occur before 24 hours. The majority of the sera solidified within two to ten days but some even remained liquid at the end of a month. In the case of three animals only, probably suffering from some other disease, jellification occurred in 24 hours.

After inoculation with trypanosomes and during the period of incubation and the early stages of infection, as long as the animals showed no symptoms of disease, jellification was always slow. As the infection progressed and pathological symptoms developed, the process became more rapid, (except in rare cases).

*T. gambiense* in domestic ruminants produced only very mild infections, with little or no effect on the general health of the animals. After having been inoculated, they were examined daily for months, and throughout all this time only occasional trypanosomes were found after one, two or three microscopic examinations, the animals apparently continuing in good health, as if they had been spontaneously cured without reappearance of trypanosomes. Sometimes it was only as the result of transmission experiments, carried out by Dr. DUKE with laboratory-bred glossinæ, that we found that infection, although apparently extinct, still persisted in a latent state, thus proving that the domestic ruminant may act as a healthy carrier of *T. gambiense*, just like the animals which are its natural reservoirs.

The sera of these ruminants, which carried the *T. gambiense* within their organism for months without exhibiting any symptoms of disease, generally jellified slowly, unlike that of persons suffering from sleeping-sickness also infected by *T. gambiense*, which, as we have seen, jellified very rapidly.

Monkeys of the genus *Cercopithecus* (*Lasiopyga*) are more sensitive to *T. gambiense* than ruminants. After a few months' infection, especially if they had exhibited morbid symptoms, their serum, unlike that of ruminants, jellified rapidly.

The serum of these same ruminants inoculated with *T. brucei* or *rhodesiense* produced jellification in the early stages of infection which differed very little from that obtained with the serum of the healthy animals; as symptoms developed, jellification became more and more rapid.

In various animals seriously infected with *T. brucei* or *rhodesiense*, I obtained rapid jellification, within from 15 minutes to 6 hours. Only in the case of heifers actually dying from East Coast fever have I found such intense jellification.

\* \* \*

While carrying out the reaction practically in conformity with the original Gaté-Papacostas technique (1 c.c. of clear serum in a tube 10 cm. by 0.10 cm., to which is dropped two drops of formalin from a pipette twenty drops approximately equal to 1 c.c.). I also placed in two other similar tubes a quantity of serum and formalin after the serum having been previously left in contact for an hour with about 1 c.g. of Bayer powder or tryparsamide.

In the case of the serum of animals infected with one of the polymorphic trypanosomes (*brucei*, *rhodesiense* or *gambiense*), the time required for jellification was generally four times as long when the serum contained "Bayer 205" and double the time when tryparsamide was added. Various other chemical substances (phosphates of lime) which I added to the sera also retarded the reaction.

\* \* \*

I also made use at the same time of the Fox-Mackie technique—one to three drops of blood serum on a slide inverted over a saucer or watch-glass containing formalin (Fig. 2).

The rate of jellification of these drops of serum on the slide was proportionate to that of the serum in the tube when the process was rapid, but even when it was slow in the tube, the serum on the slide invariably jellified in just over an hour. This led me to conclude that, unless the serum drops solidify within 60 minutes, the formol-gel test, carried out according to the Fox-Mackie technique, cannot teach us very much as regards the serum's capacity of jellification.

#### CONCLUSIONS.

1. As there exist in tropical regions, in addition to trypanosomiasis, various other diseases of man or animals which also give a positive formol-gel reaction, the test alone cannot be relied on in the diagnosis of any one of these affections.

2. When formol jellification is *negative* in a person or animal suffering from a chronic disease affecting the general health, it is unlikely to be a case of trypanosomiasis or any of the other chronic affections producing more or less rapid serum jellification such as syphilis, yaws, tuberculosis, leprosy, malaria, kala-azar, East Coast fever, etc.

3. In the absence of more certain data, the formol-gel test may furnish certain presumptive evidence of assistance in the diagnosis of advanced chronic diseases about which information is available as to the positive or negative formol-gel reactions generally associated therewith.

4. Human and animal trypanosomiasis and various other chronic tropical affections give negative formol-gel reactions only in exceptional cases, and this generally at the beginning of the disease.

5. The drugs which act most effectively on the disease affecting the organism from which the blood-serum has been taken exercise the greatest influence on formol-jellification, in as much as reactions which were previously positive become negative or at least greatly retarded.

6. I did not find that the serum of persons or animals infected with *T. gambiense*, *brucei* or *rhodesiense* to which had been added *in vitro* "Bayer 205" or tryparsamide showed any specific characteristics that could serve to differentiate between these trypanosomes.

7. The formol jellification of the serum appears to depend not so much on the intensity of the infection or infestation, as evidenced by the number of virulent microorganisms found, as on the gravity of the disease as determined by general symptoms, both objective and subjective, affecting the animal's general state of health.

8. Employed in clinical examination (examination of candidates for sickness insurance) or the examination of stock, a positive formol-gel reaction may serve to indicate the existence of certain chronic diseases.

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## CULTURES OF POLYMORPHIC TRYPANOSOMES (*BRUCEI*, *GAMBIENSE* AND *RHODESIENSE*).

The culture of non-pathogenic trypanosomes is so easy, that hæmoculture can be used for the diagnosis of these trypanosomes as successfully as in the case of the typhoid bacillus. Cultures of pathogenic trypanosomes—more especially those of the higher mammals—have not been so easy to obtain, although from time to time certain investigators have claimed brilliant results with media they have devised or modified; other investigators, even though they adhere strictly to the original techniques, do not obtain such satisfactory results.

NOVY AND MACNEAL (1904) succeeded for the first time in cultivating *T. lewisi* and *brucei* in a blood-agar medium very similar to that of Besançon and Griffon. They had no difficulty with *T. lewisi*, as regards either original cultures or subcultures. These *T. lewisi* cultures were found to be infective even after two years' successive subculture. By increasing the quantity of blood in proportion to the gelose in the medium in which they had cultivated *T. lewisi*, NOVY AND MACNEAL also obtained, though not without difficulty, the first cultures of *T. brucei*, discovered shortly before by Sir David BRUCE in Zululand and sent to European and American laboratories. Out of fifty blood cultures of animals suffering from "nagana", four only gave positive results. Subcultures were less difficult to obtain.

NOVY AND MACNEAL concluded from their studies that cultural characteristics are sufficient to differentiate *T. brucei* and *T. lewisi*, the latter growing readily on media containing less than half their volume of blood, whereas *T. brucei* requires a larger proportion of blood; this, according to NOVY AND MACNEAL, makes it possible to diagnose both these trypanosomes if co-existent in the blood of a rat.

The same investigators have shown that recent cultures of *T. brucei* are almost as virulent as the blood of animals suffering from "nagana". Old cultures or cultures heated to above 34° gradually lose their virulence until they cease to be infective.

NOVY AND MACNEAL also claimed that by means of repeated injections of attenuated cultures, employed for the purpose of vaccinating animals against the sanguicolous virus, they obtained if not complete at all events a very considerable degree of immunity.

THOMSON AND SINTON (1912), using the Novy-MacNeal medium slightly modified, succeeded at length in obtaining a few cultures of *T. gambiense* and *T. rhodesiense*, which could be kept little more than two or three weeks but which were avirulent for laboratory animals into which they were inoculated. In this respect, as well as morphologically, the cultured forms are very similar to those found in the intestine of glossinæ.

PONSELLE (1924) having first determined, in media prepared for the cultivation of trypanosomes of batrachians and fish, the importance of the sodium chloride and hydrogen ion content, and the absorption of the anti-bodies in the blood of the host, prepared a new medium on which, at the date of writing, he had had no difficulty in

cultivating *T. brucei*, *pecaudi*, *dimorphon* and *rhodesiense*; he simply had to modify the osmotic pressure for each trypanosome or group of trypanosomes with the right percentage of sodium chloride : 0.3 per cent for *T. brucei*, 0.6 per cent for *T. pecaudi* and 0.8 per cent for *T. rhodesiense* and *dimorphon*.

In PONSELLE'S view, this difference in the optimum osmotic pressure required by the various pathogenic trypanosomes may give indications of the receptivity of different species of glossinæ for various trypanosomes.

\* \* \*

Professor BRUMPT (1924), in a critical review of the facts and hypotheses concerning the individuality of *T. rhodesiense*, suggested that it would be interesting to obtain artificial cultures of *T. brucei* recently isolated from its natural hosts and belonging to strains of different origin, in order to see whether, as in the case of the NOVY-MACNEAL experiments, the cultures are always pathogenic. Again, if different strains of *T. rhodesiense*, with a genealogy similar to that of *T. brucei*, produced completely avirulent cultures, this would serve to differentiate the two trypanosomes. The progress achieved as the result of the use of Ponselle media in the technique of pathogenic trypanosome cultures may enable a solution of the problem to be found.

Our Commission had been instructed to study the relations between *T. gambiense* and *T. rhodesiense*, and had at its disposal strains of *T. gambiense* recently isolated from sleeping-sickness patients in Uganda and the Belgian Congo, and strains of *T. rhodesiense* and *T. brucei*, also recent, isolated from sick persons and animals in Tanganyika territory; there was every reason, therefore, to try the experiments suggested by Professor BRUMPT, a great authority on trypanosomiasis, so as to obtain ampler material, for the elucidation of the much-debated question of the identity or non-identity of these three trypanosomes.

\* \* \*

In these experiments I kept strictly to the Ponselle technique, as described by him in 1924. I occasionally modified it slightly, if the circumstances of the moment so required, but these modifications do not, in my opinion, account for the difference in the results obtained by PONSELLE and by myself.

The medium recommended by PONSELLE for the culture of the pathogenic trypanosomes of mammals consists of peptone and gelatine as absorbents of anti-bodies, in a sodium chloride solution the concentration of which varies in accordance with the optimum osmotic pressure required by the different species of trypanosomes.

Witte's peptone . . . . .	2 grammes.
Pure gelatine (Coignet, Gold Medal) . . . . .	2 grammes.
Sodium chloride, pure, crystallised. . . . .	0.3 to 0.8 gramme.
Normal solution of carbonate of soda <sup>1</sup> . . . . .	1 c.c.
Distilled water . . . . .	100 c.c.

<sup>1</sup> It is surprising that PONSELLE, after having shown in his previous studies the importance of "Ph" in the culture of trypanosomes, should in the end have given his formula for the pathogenic trypanosomes of mammals without indicating "Ph". He recommends the addition of simply 1 per cent of a normal carbonate-of-soda solution. I have frequently found "Ph" = 8 in distilled water at the Entebbe Laboratory perhaps because the water came from limewashed reservoirs or non-neutral glass bottles. If, as PONSELLE writes, it is proved that trypanosomes require an optimum "Ph" for each individual species or group of species, the Ponselle formula, with its fixed quantity of alkali, cannot reasonably be accepted as adequate for the purposes of so delicate an operation.

After rapid solution of the peptone and gelatine at 100° in a water-bath, the unfiltered medium is poured into big tubes, each one-third full. The tubes are then sterilised by heating in an autoclave for half-an-hour at 100° Centigrads. After cooling to laboratory temperature and while still liquid (the medium only solidifies when cooled below 15° Centigrads), an equal volume of rabbit serum is added, if intended for original cultures. For subcultures, a similar volume of defibrinated rabbit blood is added. The medium is then transferred with due precautions to culture tubes, 3 c.c. per tube, after which the medium is unactivated by heating to 56° Centigrads for half-an-hour. PONSELLE said that in order to obtain greater purity the gelatine should be washed in distilled water for six consecutive days. This I did when ice was available.

PONSELLE used infected mice, but as I had none I inoculated each culture tube with two or three drops of blood obtained by cardiac puncture from monkeys or guinea-pigs infected with trypanosomes. The culture was then kept for two weeks at laboratory temperature, which, be it noted, in Africa sometimes corresponds to that of an incubator-conditions which are known to be unsuitable for the culture of trypanosomes. After two weeks, I examined the tubes and subcultured those in which trypanosomes had developed; about half a cubic centimeter of the original culture was put into tubes containing medium prepared for subcultures, which were similarly kept at laboratory temperature.

Instead of employing rabbit's blood, as PONSELLE had done in preparing his medium, I sometimes used the serum and defibrinated blood of the *Cercopithecus*. My other experiments with the precipitin test as applied to the blood in the alimentary tract of tsetse-flies required a large number of rabbits, which I had to immunise against the blood of all the animals on which glossinæ would most probably feed so that the rabbit reserves of Entebbe and neighbouring regions were soon exhausted. The *Cercopithecus* was rather easier to procure and the blood of this monkey *in vivo* constitutes an excellent medium for all the polymorphic trypanosomes of mammals (*T. gambiense*, *T. rhodesiense* and *T. brucei*); accordingly there seemed to be no objection to its use. In the preparation of the Ponselle medium indeed it presented definite advantages. On one occasion, the native responsible for watching the thermometer in a water-bath which had been improvised to inactivate the Ponselle media must have allowed the thermometer to go up to about 80° Centigrads, for most of the tubes of a good series in process of preparation produced a medium of a jellylike consistency.

The Commission's work was nearing its end. I had to employ what time was left in other directions and could not spend it on these fruitless efforts. An African laboratory like that at Entebbe has only the personnel and material necessary for its regular work and researches. The good will which I invariably encountered was equalled only by my firm determination not to make demands on it except when absolutely necessary. Such occasions, however, presented themselves almost daily and I acknowledge most gratefully the readiness with which every effort was made to satisfy my requirements. My disappointment on finding that the Ponselle medium in process of preparation had coagulated in practically all the tubes made me abandon the idea of asking for extra ice again (manufactured 45 kilometers away) to wash the gelatine, or for more monkeys for purposes of cardiac puncture; this operation is generally harmless if it is carried out with due regard to asepsis and provided the animal does not move; otherwise, cardiac laceration may produce internal hæmorrhage, resulting in death.

I inoculated the liquid medium in the few tubes which had escaped this inactivation process, and as I could not afford to waste anything I also inoculated the tubes of gelatinised medium. I inoculated the blood of the same animal infected with

trypanosomes simultaneously into tubes containing the liquid and those containing the solidified medium.

It may have been simply coincidence ; but not only did the tubes containing the solidified medium give a higher percentage of positive cultures, but the cultures were richer than the few positive cultures in the liquid medium.

Films of the solidified-medium cultures stained by the Romanowsky method showed flagellates with well-marked differential staining. This same staining process when applied to films of cultures in the liquid medium produced less satisfactory results, although I still regard this process as being just as valuable as the special methods recommended by certain investigators for staining flagellates in cultures : I tried both without perceiving any appreciable advantages in either.

I used Pappenheim's panoptic stain for choice ; this gave excellent differentiation, especially if the staining reagents were left in contact two or three times as long as is generally recommended for fresh blood smears. In the staining of cultures a delay of an hour or two is never a matter of great importance. Sometimes by chance, or by way of experiment, I left the staining reagent in contact much longer than I have indicated. Rapid washing with dilute methyl alcohol, followed by washing under the tap, removes all excess staining and any precipitated stain that might affect the preparation. If the alcohol solution is too strong or is allowed to act too long, discoloration may go too far. When, on examination after alcohol treatment, it is found that the preparation is too pale, the only thing to do is to begin staining again, using Giemsa's stain, for as long as may be necessary.

\* \* \*

As the purpose of this investigation was to determine the differential characteristics of cultures of different trypanosomes, I began by cultivating, with the same concentration of sodium chloride, several strains of trypanosomes *T. gambiense*, *T. rhodesiense*, trypanosomes from Situtunga antelopes of Damba<sup>1</sup> origin, and *T. brucei*; details are given in the following table.

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<sup>1</sup> Damba is one of the islands in Lake Victoria belonging to the Sesse group, the population of which was evacuated as a prophylactic measure, after the terrible epidemic of sleeping-sickness which ravaged Uganda. Antelopes, which were very scarce on Damba as long as the island was inhabited, have multiplied enormously since the population left it. Shortly after the evacuation, Dr. DUKE (1912) isolated from these antelopes (*Tragelaphus spekei*) a trypanosome the biological and morphological characteristics of which are closely allied to those of *T. gambiense*. About ten years later (1921), he again isolated, from the same antelopes, trypanosomes which then exhibited the characteristics of *T. brucei*. Dr. DUKE's theory is that, before the evacuation of Damba, the *T. gambiense*, being regularly conveyed by the tsetse-fly from man to man and only in exceptional cases from man to antelopes, exhibited the characteristics of the human trypanosome; after the depopulation of the country, this trypanosome was forced for a number of years to live almost exclusively on the rapidly increasing herds of antelopes and finally adapted itself perfectly to its new vertebrate host, so that as a consequence the characteristics of the human trypanosome (*T. gambiense*) came to be replaced by those of the antelope trypanosome (*T. brucei*).

Some authorities are of opinion that the trypanosome found in the Damba (Situtunga) antelopes was always of the *brucei* type. For fuller details on this point the reader is referred to the reports of Drs. DUKE, BRUMPT, WENION, etc.

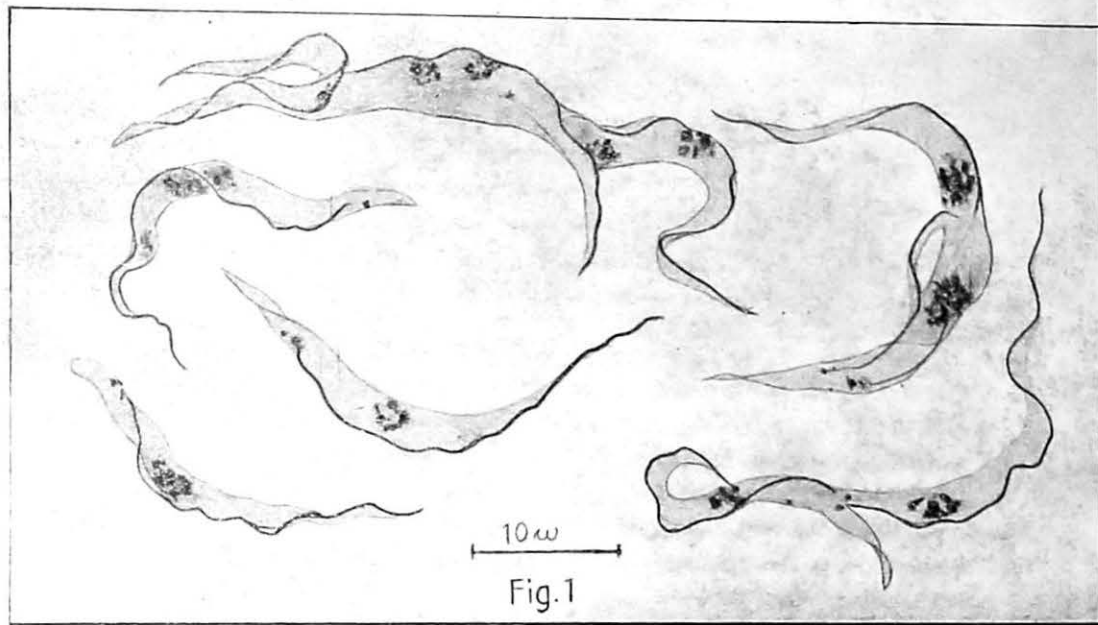


Figure 1. 22-day cultures of *T. brucei*.

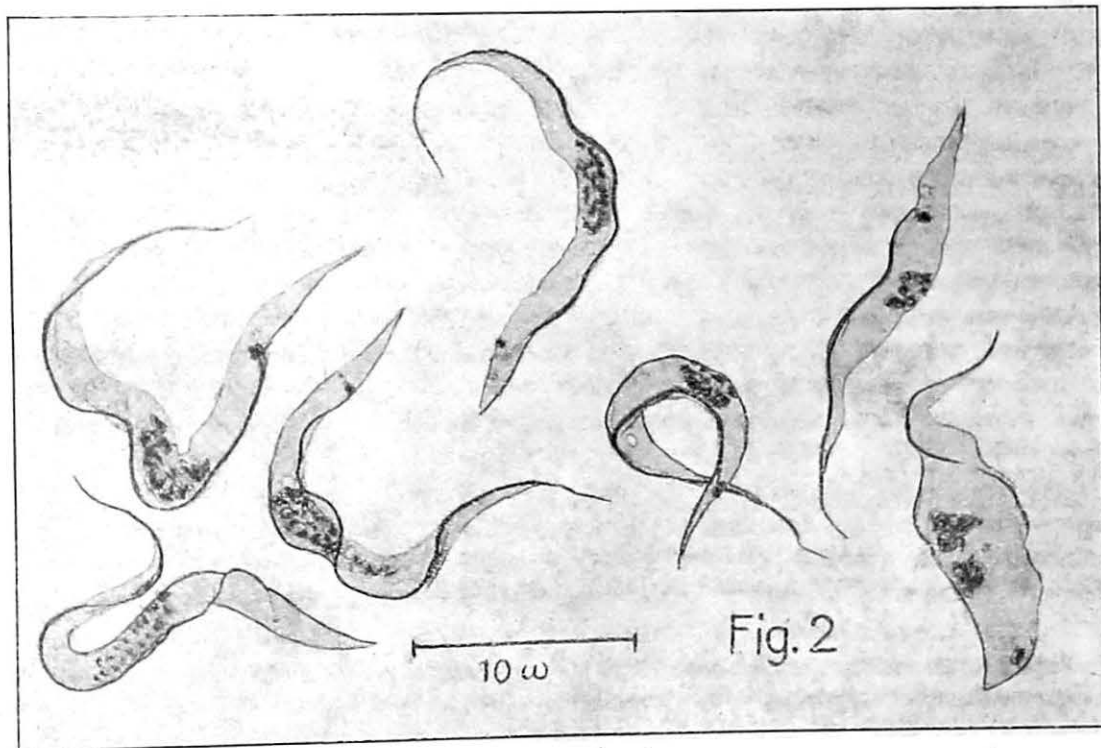
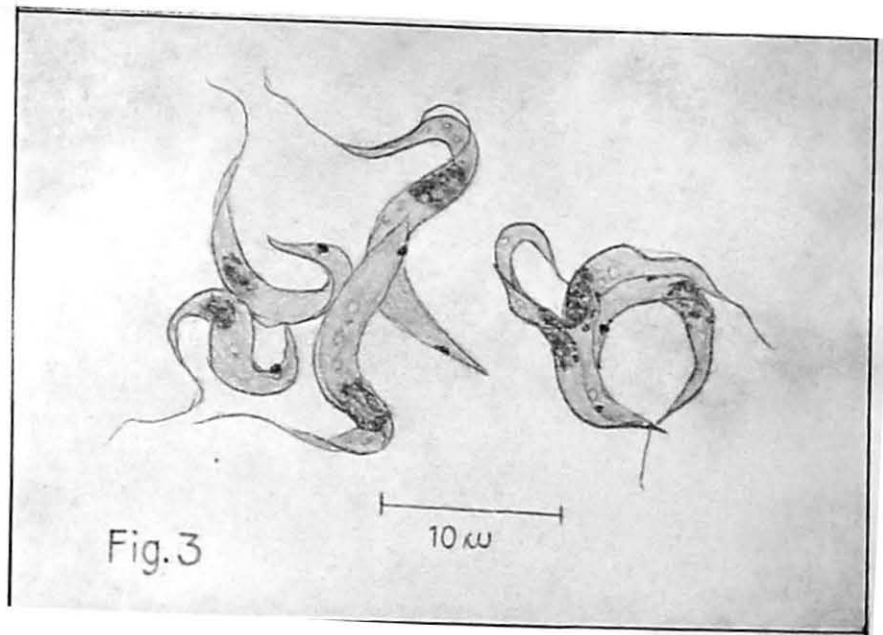
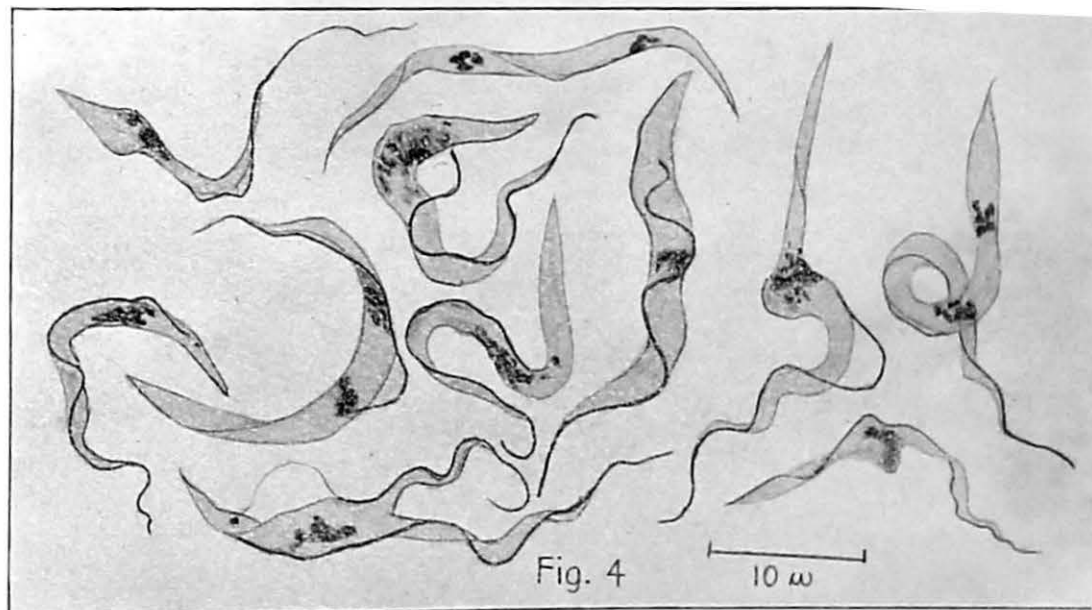


Figure 2. 19-day cultures of *Situtunga Trypanosoma*.



*Figure 3.* 45-day cultures of *Situtunga Trypanosoma*.



*Figure 4.* 62-day cultures of *Situtunga Trypanosoma*.

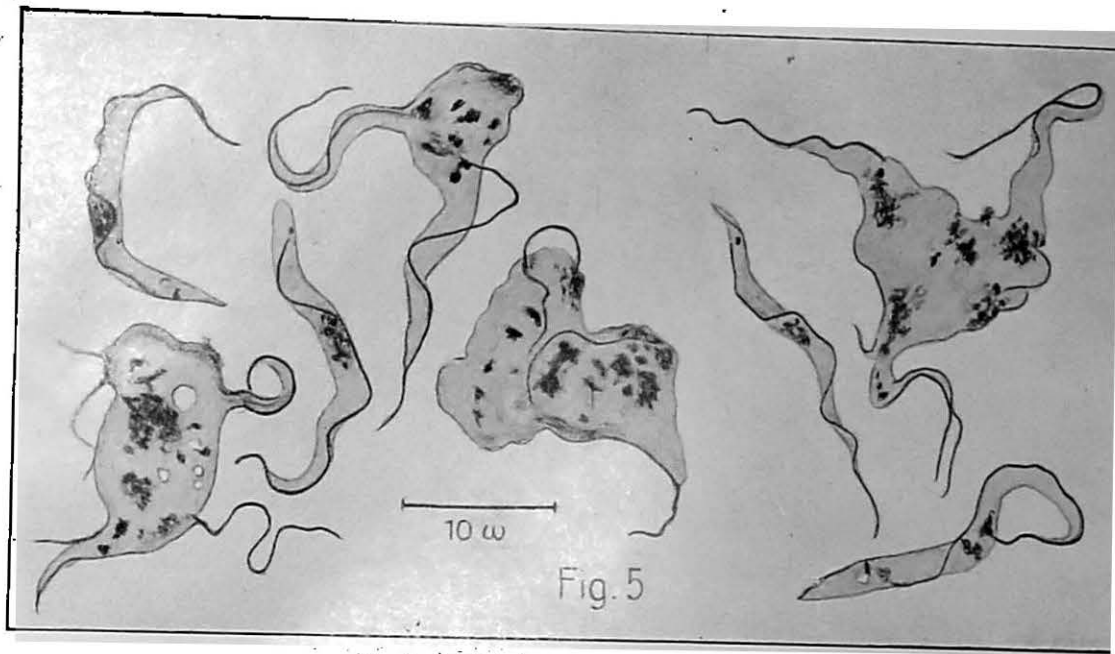


Figure 5. 14-day cultures of *T. rhodesiense*.

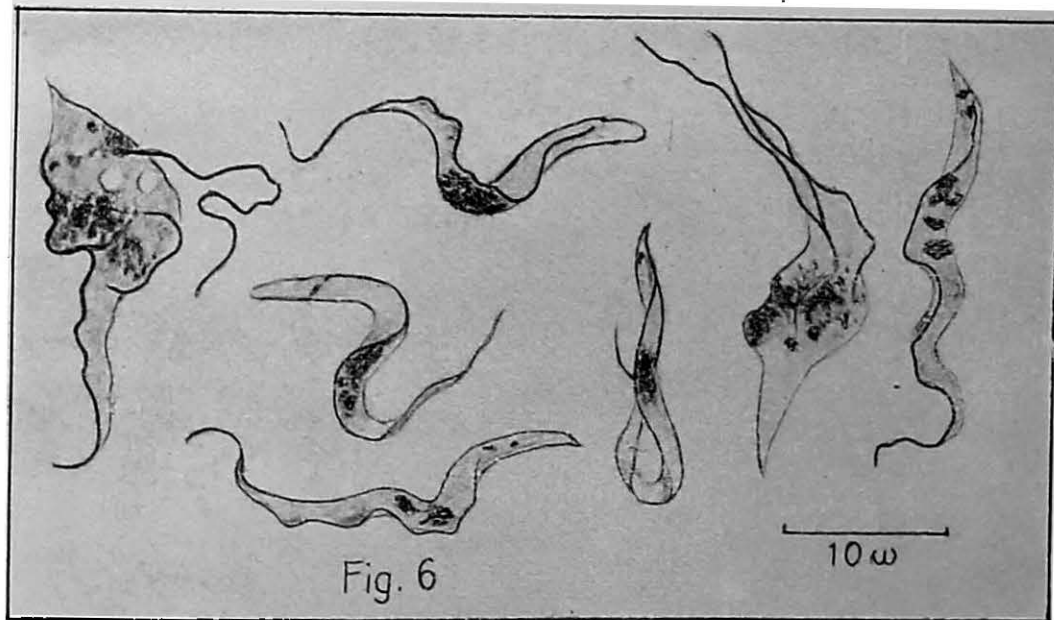


Figure 6. 22-day cultures of *T. rhodesiense*.



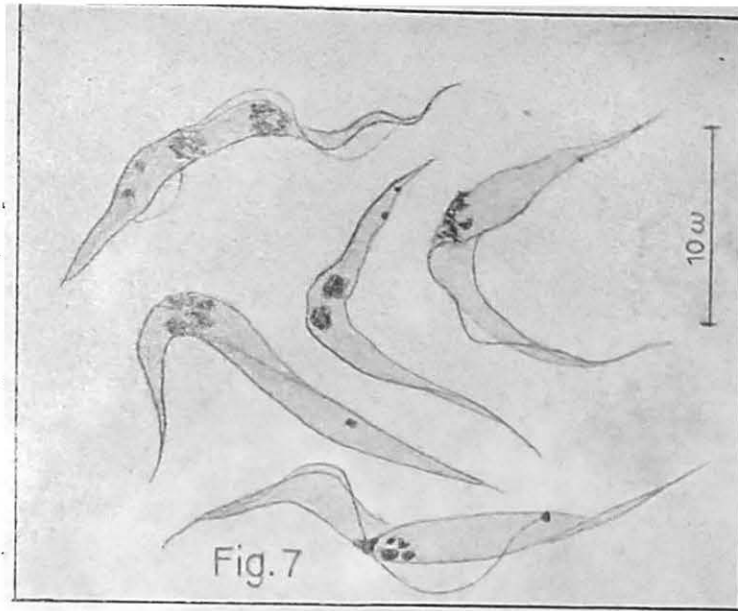


Figure 7. 31-day cultures of *T. gambiense*.

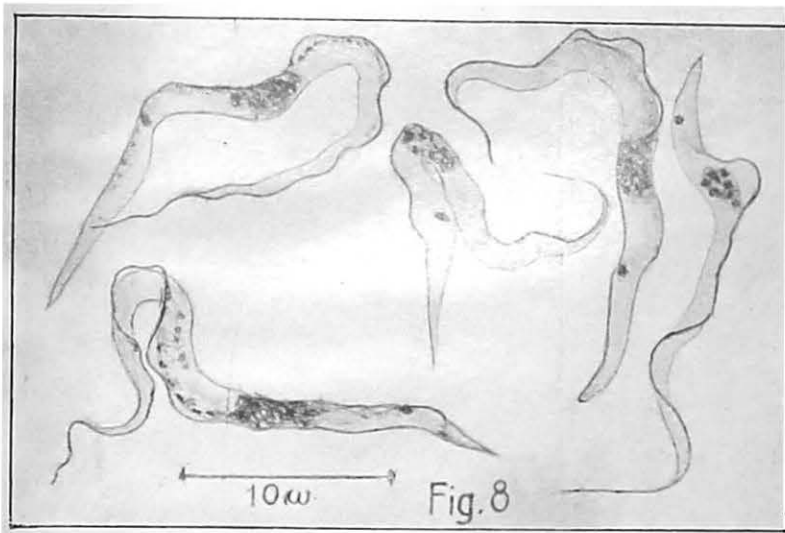


Figure 8. 54-day cultures of *T. gambiense*.

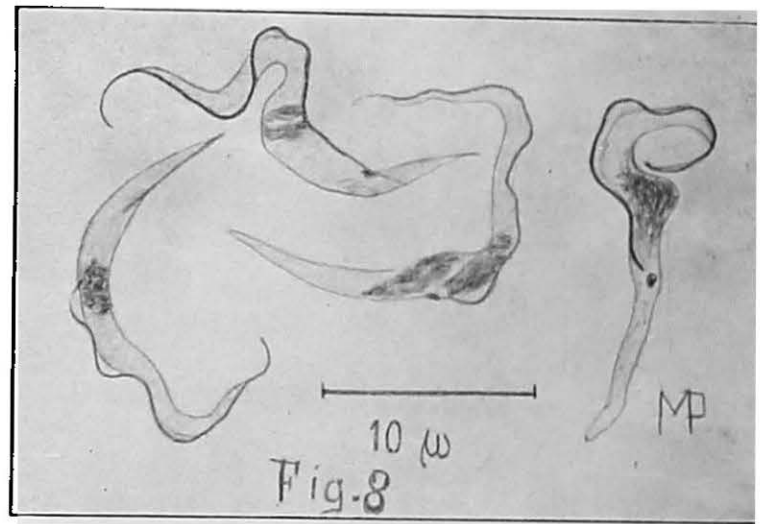


Figure 9. 65-day cultures of *T. gambiense*.

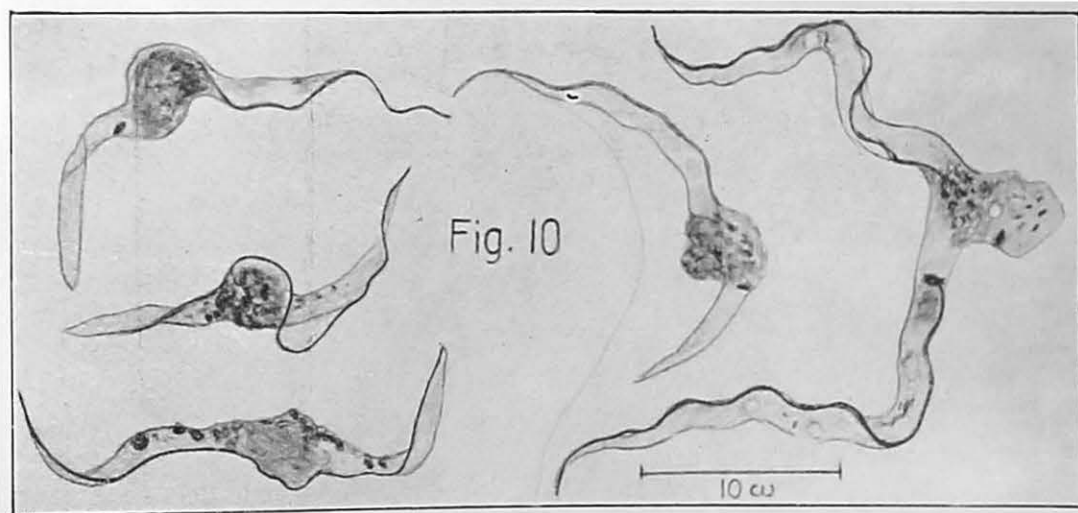


Figure 10. Abnormal forms of 65-day cultures of *T. gambiense* also found in old cultures of all the trypanosomes cultivated by me.



I. CULTURES IN PONSELLE'S MEDIUM WITH 0.3% SODIUM CHLORIDE.

No. of culture	Trypanosomes tried Isolated (date) by	Date of culture	Animal from which the trypanosomes inoculated were obtained	Number of trypanosomes per microscopic field	Liquid medium			Solidified medium				Observations
					Tubes inoculated	Positive tubes	Age of culture	Tubes inoculated	Positive tubes	Age of culture (days)	Total Positive tubes	
1.	<i>Gambiense</i> . DUKE. September 1926	26-10 1926	Monkey 131	1/30	5	1	28	5	2	35	3	
2.	<i>Brucei</i> . KLEINE. January 1927	9-3 1927	Rat 296	15/1	6	6	15	6	6	28	12	
3.	<i>Rhodesiense</i> . KLEINE. February 1927	25-3 1927	Guinea-pig 290	8/1	6	0	0	6	0	0	0	Infected
4.	<i>Ibid.</i>	8-27	Monkey 309	10/1	6	6	28	6	6	28	12	
5.	<i>Brucei</i> . HALL. April 1927	10-4 1927	Mbarara dog	30/1	6	0	0	6	0	0	0	Infected

II. CULTURES IN PONSELLE'S MEDIUM WITH 0.6% SODIUM CHLORIDE.

6.	<i>Rhodesiense</i> . CURZON. May 1926	27-10 1926	Monkey 132	15/1	6	1	30	0	0	0	1	
7.	<i>Rhodesiense</i> . CURZON. May 1926	18-9 1926	Rat 188	5/1	5	0	0	0	0	0	0	Infected
8.	<i>Ibid.</i>	<i>Ibid.</i>	Rat 189	20/1	5	0	0	0	0	0	0	Infected
9.	<i>Gambiense</i> . DUKE. September 1926	26-10 1926	Monkey 131	1/30	5	1	35	5	1	35	2	
10.	Damba strain. DUKE. September 1924	14-12 1926	Monkey 229	1/5	5	0	0	5	2	62	2	
11.	<i>Ibid.</i> November 1926.	24-12 1926	Rat 247	10/1	5	0	0	5	1	0	1	
12.	<i>Gambiense</i> . VAN HOOF. October 1926	"	Monkey 222	1/1	5	0	0	5	2	65	2	
13.	<i>Gambiense</i> . VAN HOOF. February 1926	"	Goat 338	0	5	0	0	5	0	0	0	
14.	<i>Gambiense</i> . DUKE. December 1926	"	Acholi, patient centrif. blood	1/50	5	0	0	5	0	0	0	
15.	<i>Ibid.</i>	"	Awassi <i>idem</i> centrif.	1/100	5	0	0	5	0	0	0	
16.	<i>Ibid.</i>	"	Boki <i>idem</i>	1/100	5	0	0	5	0	0	0	
17.	<i>Ibid.</i>	"	Falagela	1/100	5	0	0	5	0	0	0	
18.	<i>Ibid.</i>	"	Acholi C.S.F.	1/50	5	0	0	5	0	0	0	
19.	<i>Ibid.</i>	"	Awassi <i>idem</i>	0	5	0	0	5	0	0	0	
20.	<i>Ibid.</i>	"	Boki <i>idem</i>	0	5	0	0	5	0	0	0	
21.	<i>Ibid.</i>	"	Falagela	0	5	0	0	5	0	0	0	
22.	<i>Rhodesiense</i> . KLEINE. February 1927	25-3 1927	Monkey 309	10/1	6	6	28	6	6	35	12	
23.	<i>Brucei</i> . KLEINE. January 1927	9-3 1927	Rat 296	15/1	6	6	21	6	6	28	12	

III. CULTURES IN PONSELLE'S MEDIUM WITH 0.8% SODIUM CHLORIDE.

No. of culture	Trypanosomes tried Isolated (date) by	Date of culture	Animal from which the trypanosomes inoculated were obtained	Number of trypano- somes per micro- scopic field	Liquid medium			Solidified medium				Observations
					Tubes seeded	Positive tubes	Age of culture	Tubes seeded	Positive tubes	Age of culture (days)	Total Positive tubes	
22.	<i>Gambiense</i> . DUKE. September 1926	26-10 1926	Monkey 131	1/30	5	0	0	5	1	35	1	
23.	<i>Brucei</i> . KLEINE. January 1927	9-3 1927	Rat 296	15/1	6	6	28	6	6	35	12	
24.	<i>Rhodesiense</i> . KLEINE. February 1927	25-3 1927	Guinea-pig 290	8/1	6	0	0	6	0	0	0	Infected
25.	<i>Ibid.</i>	8-4-27	Monkey 309	10/1	6	6	28	6	6	28	12	
26.	<i>Brucei</i> . HALL. April 8th, 1927.	10-4 1927	Mbarara dog	30/1	6	0	0	6	0	0	0	Infected
					151	39		135	45		84	

Notes. — Centrif. blood = centrifuged blood ; C.S.F. = cerebro-spinal fluid.

SUMMARY.

Tubes inoculated, 286 ; positive, 84 = 29.3 per cent.

Tubes of liquid medium, 151 ; positive, 39 = 25.8 per cent.

Tubes of jellified medium, 135 ; positive, 45 = 33 per cent.

With the exception of Culture No. 10, all the cultures were inoculated with recently isolated trypanosomes.

All the cultures uncontaminated with foreign micro-organisms yielded some positive tubes, except the cultures of the blood of goat 338 and of the blood or cerebro-spinal fluid of patients suffering from sleeping-sickness, in which very few trypanosomes were present.

The culture tubes were only examined once a week. The room used was not appropriate for this kind of work, and despite every care, the media in tubes which had been opened often became contaminated.

Culture of the blood of various animals heavily infected with trypanosomes frequently resulted in contamination of all the media used, and we began to wonder accordingly if, in the final stage of trypanosomiasis, the presence of bacteria in the blood may not in many cases complicate the pathogenic action of the trypanosomes. Media which were sterilised and kept sterile for several days and then inoculated with the blood of certain heavily infected animals, produced abundant growth of bacteria in the course of the following days (with *T. rhodesiense*, rats 188 and 189 and guinea-pig 290 ; with *T. brucei* a dog from Mbarara, which arrived at the Entebbe Laboratory in a dying condition).

On the other hand, other animals gave cultures rich in trypanosomes in all the tubes inoculated, with both liquid and solidified media and with one or other of the three sodium chloride concentrations recommended by PONSSELLE : 0.3 to 0.6 and 0.8 per cent (rat 296 with *T. brucei* and monkey 309 with *T. rhodesiense*). All the tubes inoculated with these strains exhibited on the seventh day a large number of flagellates, separate, intermingled so as to form irregular rosettes or dividing longitudinally, both single and multiple, and all actively motile. At the second and third examination (fourteenth and twenty-first days) the cultures were not so rich, and flagellates were frequently found either motionless or very slightly motile. After the fourth week, the majority of the cultures were dead, only a few tubes still containing flagellates in full activity.

I endeavoured for each group of positive cultures to obtain subcultures in the media recommended by PONSSELLE. The results were not very encouraging : primary cultures always survived longer than subcultures.

I inoculated positive cultures several times, at different stages in their development, into animals which were susceptible to the trypanosomes in question ; not one of these cultures proved infective.

I tried by various means to make "fresh" laboratory-bred glossinæ absorb positive cultures. Instead of the Rodhain tube I used an ordinary small glass funnel, covering the wide opening with the fresh skin of a guinea-pig or rat, with the shaved part turned outwards.

I put into the funnel the liquid Ponselle medium, containing numerous flagellates, and placed the skin against the metal gauze of a Bruce box containing "fresh" glossinæ, recently hatched in the laboratory (Fig. 11).

By this means I succeeded in making four tsetse-flies, out of a box containing nearly fifty, absorb citrated fresh blood containing trypanosomes, though could not make a single glossina gorge itself on the culture medium.

I tried another method : I inoculated one of the little *Cercopithecus* under the skin, as far as possible into the skin, with several cultures rich in flagellates ; this

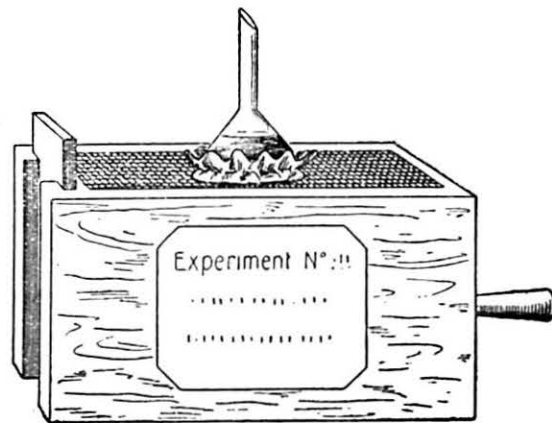


Fig. 11

Simple apparatus for carrying out the Rodhain experiment ; funnel containing citrated blood or liquid trypanosome culture media, covered with a fresh rat-skin placed against the metal gauze of a tsetse-fly box.

formed a big swelling to which I immediately applied boxes of glossinæ accustomed to feeding on this monkey.

I inoculated the cultures in the region of the sternum where there is little loose tissue and the application of tsetse-boxes is very simple.

With this process the flies gorged themselves rapidly, but although I applied the same glossinæ-boxes four or five times on the swellings resulting from injections of culture media, containing the same flagellates, no cultivated trypanosomes developed in any of the flies.

Animals inoculated a number of times, with cultures in this manner were immune to these cultural strains but continued to exhibit the same degree of susceptibility to blood forms of trypanosome inoculated subsequently.

\* \* \*

I tried several times to cultivate the trypanosomes found in the intestines and the salivary glands of experimentally infected tsetse-flies, but despite dissection, carried out with sterilised material, and despite repeated washing of the glands and intestine in normal serum, also sterilised, cultures were all contaminated and no flagellates developed.

#### SUMMARY AND CONCLUSIONS.

1. In Ponselle's medium, in which PONSELLE cultivated different pathogenic trypanosomes (*brucei*, *pecaudi*, *rhodesiense* and *dimorphon*) with absolutely constant results, "all the tubes inoculated giving abundant growth both in original cultures and in subcultures, which can be carried on indefinitely", I obtained cultures of *T. gambiense*, *T. rhodesiense* and *T. brucei* and also of the Damba-antelope trypanosomes, though with a lower percentage of positive results.

2. I cultivated all these trypanosomes in the same Ponselle medium, with the same sodium-chloride concentration.

3. I found no morphological difference between the various polymorphic trypanosomes at any stage of the cultures: all, no matter what the medium, exhibited the recognised morphology found in the gut of glossinæ. Nor did I observe any metacyclic or blood forms.

4. Rabbit serum may be replaced by monkey serum in Ponselle's media for the cultivation of the pathogenic trypanosomes of African mammals.

5. When inactivating Ponselle's media for the cultivation of pathogenic trypanosomes in the higher mammals, the temperature may be allowed to go up to from 70° to 75° (this is even desirable) when the medium assumes consistency of a viscous jelly.

6. I inoculated *Cercopithecus* monkeys and white rats with cultures containing an abundance of trypanosomes like flagellates—15, 30, 40, 45, 50, 60 and 65-day cultures—but did not succeed in infecting any of the animals. I repeated several inoculations, after keeping the trypanosome cultures in an incubator at 37° for 24 hours; the results, unlike those obtained by NÖELLER, were also negative.

7. Some of the animals which had been given repeated injections of cultures at different stages of development were subsequently inoculated with sanguicolous trypanosomes of the same species, and did not exhibit any appreciable degree of immunity.

8. I applied various boxes of "fresh" glossinæ to monkeys inoculated with cultures, and the flies which sucked them did not become infected.

9. The existence of bacteria in Ponselle's media and the cultivation of the polymorphic trypanosomes of African mammals appear to be incompatible; trypanosomes always disappear from contaminated cultures after a few days.

10. I have tried to cultivate the blood and cerebro-spinal fluid containing occasional *gambiense* trypanosomes, in order to see if the method could be used for the diagnosis of this form of trypanosomiasis. All the cultures were negative in cases in which microscopic diagnosis had been difficult and the percentage of positive cultures was so low even when the microscopic diagnosis had been easy, that, unless the cultural method should give better results in the hands of some other investigator very slight assistance—if, indeed, any—in the diagnosis of the trypanosomiasis in question can be looked for.

11. I found no confirmation of PONSELLE's suggestion that the differences in the osmotic pressure required for cultures of the various pathogenic trypanosomes may supply useful indications as to the receptivity of different tsetse-flies for the various species of trypanosomes. On the one hand, all the trypanosomes that I cultivated (*brucei*, *gambiense* and *rhodesiense* and those of the Damba antelope) completed the same evolutionary cycle in *Glossina palpalis*, at the Entebbe Laboratory (Dr. DUKES's experiments); on the other hand, I cultivated all these trypanosomes in the same Ponselle medium, prepared with no matter which of the sodium-chloride concentrations.

PONSELLE himself described *T. brucei* and *T. rhodesiense* (both transmitted in nature by the same glossinæ) as demanding very different osmotic pressures (0.3 per cent for *T. brucei* and 0.8 per cent for *T. rhodesiense*) and *T. rhodesiense* and *dimorphon*, on the contrary (transmitted by different flies) as requiring the same pressure (0.8 per cent).

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5.

THE PRECIPITIN TEST APPLIED TO THE BLOOD IN THE  
ALIMENTARY CANAL OF GLOSSINÆ

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## INTRODUCTION

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Owing to circumstances beyond my control, I did not arrive at the Entebbe Laboratory until four months after the Commission had begun its work. As my colleagues were already engaged on epidemiological studies which necessitated much time and effort before definite conclusions could be arrived at, I agreed to the suggestion of the President of the Commission that I should proceed as best I could with a study of the precipitin reaction applied to the blood in the alimentary canal of glossinæ.

I should mention that, in addition to being without any specialised serological training, I was faced with various other difficulties, chief among these being the short time at my disposal. During the eight months that the Commission still had before it to complete its work, an adequate supply of rabbits for purposes of immunisation had to be collected, the blood of numerous species of animals for antigens had to be procured and, once the antigens had been obtained and the anti-sera prepared, glossinæ had to be found with blood in the digestive canal, under conditions such as to make the application of the precipitin test possible.

When preparing any anti-serum, one must expect the death of a fairly large proportion of the rabbits inoculated, and that was why it was necessary for me to treat several rabbits simultaneously. Those at the Entebbe Laboratory—more than enough for routine work—would be used up in my first experiments and the neighbouring regions did not offer very adequate resources. Moreover, the Uganda rabbits are not of a vigorous constitution, as they must be if they are to respond to antigen inoculations by developing an abundant supply of anti-bodies. The annual reports of the Entebbe Laboratory, moreover, note the difficulty of obtaining hæmolytic sera, and this difficulty I also encountered in connection with the production of immunising sera with sufficiently marked precipitating properties.

A further difficulty, and by no means the least, lay in obtaining under satisfactory conditions the antigens from all animals liable to be bitten by glossinæ. Despite the diligent efforts of various persons, among whom I should like specially to thank Drs. DUKE and VAN HOOF, I only succeeded in obtaining the blood of some twenty odd species.

The short time at my disposal, the absence of suitable rabbits and the difficulty of preparing a sufficient number of precipitating sera precluded any attempt to deal with the question in a really adequate manner. I decided to do the best I could with such knowledge and material resources as I possessed.

### PART I.

#### GENERAL.

##### I. *History and Application.*

KRAUS (1897) was the first investigator to report the phenomenon of specific precipitins. He inoculated animals with virulent bacteria. The serum of these animals gave a precipitate when added to a filtered broth culture of the same bacteria. No precipitate was formed when he added to the filtered broth cultures bacteria different from those which had been inoculated.

BORDET (1899) and TCHISTOVITCH (1899) announced shortly afterwards that a precipitating serum could be obtained if, instead of bacterial cultures, some kind of albumin was injected. For example, if a rabbit was inoculated with the serum of an animal of a different species, its serum would acquire the property of precipitating not only solutions of the serum injected but also all the albumins belonging to the same animal species as that which had supplied the inoculated serum.

WASSERMANN, SCHUETZE and UHLENHUT next applied this specific precipitation to the medico-legal diagnosis of blood-stains. The serum of rabbits inoculated with human blood produced a strong precipitate only with solutions of human blood and remained inactive when added to solutions of blood of any other animal species. The blood of the higher monkeys, however, yielded a precipitate, though much less pronounced, with the same serum.

NUTTALL (1904) applied this reaction in his researches into the relationship between different zoological species. The principle is that a precipitating serum prepared by inoculating the blood of a given species produces a well-marked precipitate of the albumins of that species and a less marked precipitate of those of other species, further, the precipitate becomes less distinct the further off those species are in the zoological scale from the first species which furnished the antigen for the preparation of the anti-serum.

Precipitin tests have been employed not only for the identification of certain bacteria (meningococci, pneumococci, etc.) in the medico-legal diagnosis of blood-stains, sperm, etc., and to establish the relationship between different zoological species, but have also been used for the identification of meat (fresh or preserved), milk, eggs, honey and other substances containing proteids. They have been employed, further, for the identification of zoological species during successive phases of development. Certain species were wrongly believed to exist, which were proved in the end, as the result of this experiment, to be simply different morphological stages in the evolution of the same animal species.

The precipitin test has been recommended in the diagnosis of cancer, though the favourable results announced have not yet been confirmed. The test has also been used to determine serological differences in the human races, but has given no indication of precipitates peculiar to the several races.

The test has, lastly, been used for the identification of the blood in the alimentary canal of hæmatophagous insects.

## 2. *Nomenclature.*

Before going into this subject, it seems advisable to define the terms which will frequently recur in it.

By *antigens* are meant any proteid substances injected into rabbits or other animals for purposes of immunisation.

*Anti-bodies* appear in the serum of animals inoculated with antigens as a reaction against those antigens.

*Precipitins* are anti-bodies which produce a specific precipitate.

*Precipitinogens* are the antigens which produce the precipitins.

The term *phytoprecipitins* is used when the precipitinogen is formed of vegetable proteins (bacteria, fungi, etc.), and the term *zooprecipitin* when, on the contrary, it is formed of animal proteins (lactoprecipitins, hæmoprecipitins, erythroprecipitins, etc.).

The terms *thermoprecipitinogen* and *coctoprecipitin* are used to describe precipitinogens and precipitins that have been heated. By *precipitoids* are meant precipitins.

which have been inactivated by heat and have an inhibitive effect on precipitin reactions.

Antigens and anti-bodies are described as homologous or heretologous according to whether they belong to animals of the same species or of different species.

By *anti-sera* are meant sera containing antibodies.

*Co-precipitins* or *group precipitins* are those which react with antigens of a species other than that which supplied the antigen employed for their production.

Anti-sera take their name from the antigens which produce them. For example, the terms *anti-goat* serum, *anti-human* serum, *anti-crocodile* serum, etc., are employed when the antigens inoculated are composed respectively of the proteins of goat, man, crocodile, etc.

### 3. *Properties of Precipitin Anti-sera.*

Apart from their property of precipitating solutions of homologous precipitinogens, little is known concerning these precipitins. They lose their precipitating properties when heated to a temperature above 65°. Acids and powerful bases, digestive ferments (pepsin and more particularly trypsin) and many other chemical and physical factors also have a profound effect on their precipitating properties.

When we come to deal with precipitin tests applied to the blood in the alimentary canal of glossinæ, we shall see the difficulty of obtaining definite precipitin reactions if the blood has been subject for any length of time to the action of digestive ferments.

### 4. *Production of Precipitins.*

In practice, precipitin anti-sera are prepared by injecting the antigen into the rabbit by subcutaneous, peritoneal or intravenous inoculation.

Any substance containing proteins may be employed as an antigen: citrated whole blood, defibrinated blood, washed blood corpuscles, blood serum, ascitic fluid or pleural effusion, hydrocœle or cerebro-spinal fluid the extract of various organs, albuminous urine, etc.

The rabbit is generally chosen for purposes of immunisation, as it is easy to handle and can be made to react readily, producing a large quantity of anti-bodies for the antigens of almost every species except rodents.

The methods employed by different investigators for immunising rabbits vary as regards the number of inoculations, the amount inoculated and the intervals allowed to elapse between the injection.

Antigens can be inoculated by any one of the methods indicated above, but all investigators are agreed that intravenous injection produces prompter and completer immunisation, although more rabbits are killed as the result of traumatic and anaphylactic shock.

The amount of antigen injected intravenously must be smaller and this fact should be borne in mind when the antigen is scarce and difficult to obtain, as is the case with some antigens employed for this work.

The intervals between the injections vary, according to the method adopted by the different investigators, between two and seven days. The condition of the animal after each inoculation will best indicate the method to be followed. If it stops eating and loses weight or appears feverish, it must be given time to recover. The anti-body yield of sick animals is small.

5. *Technique of the Precipitin Reaction and Anti-serum Titration.*

I followed the recognised qualitative method of UHLENHUT. Quantitative methods are employed for the precipitate deposits. As will be seen later on, it seemed better, when applying the precipitin test to the contents of the alimentary canal of glossinæ, to estimate the results of the reaction not by the precipitate ultimately deposited but by the immediate appearance of opacity at the level where the anti-serum and the supernatant antigen are in contact with one another, following the Uhlenhut technique, as applied to the medico-legal diagnosis of blood-stains.

Precipitin tests give specific results not only with blood but with all the albumins belonging to the same species. In the case of a blood-stain, the precipitin reaction will indicate with a very great measure of probability not whether the stain contains blood but whether it contains proteins of a given zoological species. To determine whether these proteins are from the blood, the sperm etc., various processes must be employed—cytological, microcrystallographic, spectroscopic, chemical, etc. In forensic medicine, in diagnosing blood-stains, these preliminary processes are first used, in order to ascertain whether the stain is or is not a blood-stain, and only afterwards is the precipitin test applied to determine the animal species to which the blood belongs.

Before applying the precipitin test to the contents of the alimentary tract of glossinæ, I generally made a microscopic examination. After carrying out experiments on laboratory-bred glossinæ, fed on man or on known animals (cows, goats, hens), I realised that anti-sera rich in precipitins for homologous blood ceased to react or reacted only very slightly with such blood when it had been subject for any length of time to the action of the digestive ferments of the tsetse-fly. Finding that it was useless to carry out the test with blood that was almost completely digested, I employed it thereafter only when microscopic examination had shown that the red blood cells were but slightly modified.

Some investigators have succeeded in preparing specific precipitins not only for a given zoological species but also for the tissues of certain organisms. HEKTOEN prepared specific precipitins for the antigen composed of crystallin; these only precipitated solutions containing some kind of crystallin and proved inactive in the case of solutions of homologous blood. KLEINE, LEER and HEKTOEN, employing as antigens red blood corpuscles which had been well washed in order to separate them from the serum, produced anti-sera which only precipitated solutions of homologous hæmoglobin. Precipitin reactions simply indicate the animal species from which a specific protein is derived; erythro-precipitin reactions may indicate also whether these proteins are derived from blood corpuscles, without other chemical or microscopic examinations.

As I generally found it necessary to wash the blood found in glossinæ, so as to remove the numerous fatty and waste substances which would have clouded the solutions—absolute purity being essential if the precipitin reactions are to give reliable results—I decided, when immunising my rabbits, to adopt the practice of injecting washed blood corpuscles or at all events whole blood, instead of simply injecting blood serum separated from the clot; in other words, I tried to produce more erythro-precipitins than seroprecipitins.

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PART II.

THE PRECIPITIN TEST APPLIED TO THE BLOOD IN THE ALIMENTARY CANAL OF GLOSSINÆ.

In determining the origin of the blood found in the digestive canal of tsetse-flies I naturally followed the general technique employed for the medico-legal diagnosis of blood-stains, this technique having attained such a high degree of accuracy that there could be no question of my improving upon it or even of finding any other to equal it.

I encountered more difficulties than I had at first anticipated. Quite often the gut of the glossinæ caught in the bush contained incompletely digested blood corpuscles, distinctly visible on microscopic examination, and their solution did not form precipitates with any of the anti-sera prepared, either because my collection did not include the homologous anti-serum or because, if I did possess it, it failed to react properly on account of some inhibitive factor. In order to clear up these and other doubts, I carried out various experiments the description of which will supplement the present report. It might be useful to those who decide to apply the principle to the same or to other blood-sucking arthropods to learn how I surmounted these difficulties, so as to be saved from wasting as much time in fruitless attempts as I did.

To sum up, when applying the precipitin test to the contents of the alimentary tract of glossinæ, it is essential to procure a supply of animals for purposes of immunisation, to have a collection of antigens, to prepare precipitin anti-sera, and, lastly, to catch wild tsetse-flies with blood in their digestive canals to which precipitin tests can be applied. We shall deal in the following chapters with the several aspects of the question, in the order named.

I. ANIMALS TO BE IMMUNISED.

The animal selected should be of a species far removed from that for which the anti-serum is to be prepared. Thus, when preparing an anti-human precipitant serum, a monkey would not be suitable and similarly, when preparing an anti-hare serum, a rabbit should not be employed. For an anti-human serum, a rabbit, that is, a distant species would be suitable, just as a hen might be selected for an anti-hare serum.

The rabbit is generally used for the production of precipitin anti-sera, as it is easy to rear and to handle, and reacts to most antigens by producing plentiful anti-bodies. It is unsuitable only when preparing sera against other rodents. Nearly all my anti-sera were prepared from rabbits. Only when preparing anti-sera with reptile antigen, the effects of which on rabbits were manifestly toxic, I sometimes had to employ hens: I say sometimes, because, with very small doses frequently repeated, I did succeed with reptile blood in preparing from rabbits anti-sera sufficiently rich in precipitins.

The animal to be immunised should be well fed, mature, neither too old nor too young and in good health, so as to react to injections of foreign proteins by yielding a plentiful supply of anti-bodies.

The rabbits should be weighed at frequent intervals during the process of immunisation. Some do not stand antigen injections well. Several died and others lost weight to such an extent that I was obliged to suspend the injections and postpone the collection of the anti-serum until the animal had recovered. The thermometer

and the scales are the best means of ascertaining its condition. As a rule, all inoculations are followed by loss of weight. When this does not affect the animal's general condition to any appreciable extent, the inoculations may be continued and there is every chance that a good anti-serum will be produced; if, however, the scales show a considerable loss in weight, it is advisable to suspend the process of immunisation, as otherwise the animal may die or at all events supply only a poor anti-serum.

When an intensive method of immunisation (strong intravenous antigen injections), such as I employed, is used, it is only to be expected that many rabbits will succumb under such drastic treatment. It is desirable, therefore, when preparing an anti-serum, to treat a number of rabbits at the same time so that, even if one of them dies, it will still be possible to complete the process of immunisation with the survivors.

#### *Numbering of Rabbits.*

As I was treating dozens of rabbits of the same colour simultaneously, there was always the possibility that they might be mixed up by the natives in charge of the laboratory animals. The first animal with which this happened produced a serum precipitating two kinds of antigen. I shall show later what use I made of these polyvalent anti-sera. In order to avoid accidental polyvalence, I felt that it was essential to be absolutely sure about the identity of the animals inoculated. I therefore tattooed numbers on the rabbits' ears. My method was to write the figures in Indian ink inside each ear and while the ink was still wet to prick them out with a needle, making light intradermic punctures. I then washed the ear, so as to get rid of the surplus ink, and ascertained whether it had penetrated the skin, outlining the number. When numbered in this way, inoculated rabbits can go on living together, without its being necessary to isolate them in separate cages, and without any risk of their being mixed up by the natives.

## II. ANTIGENS.

Antigens are employed for two distinct purposes: they are injected into animals for the production of anti-bodies and are used for the preparation of precipitin test control solutions.

### *A. Antigens for Inoculation.*

These should be sterile and liquid. Any substance containing animal proteins can be used as an antigen in the preparation of zooprecipitins: fresh whole blood, citrated blood, defibrinated blood, hæmolysed blood, washed red corpuscles, serum without blood corpuscles, ascitic fluid, pericardial or pleural effusion, hydrocœle or cerebro-spinal fluid, milk, meat extract, albumen of eggs, or of urine, etc.

I succeeded in obtaining the following antigens for the preparation of my anti-sera: human, monkey, sheep, goat, ox, two kinds of antelope (*Tragelaphus spekei* and *T. scriptus*), hippopotamus, elephant, dog, domestic cat, wild cat, wild pig (*Phacocheirus*), snake (python), crocodile, monitor, hen, osprey, and various water birds frequenting the glossina-infested banks of Victoria Nyanza.

Whenever I had a live animal, in the Laboratory, I used to bleed it; I then injected part of the blood at once into a rabbit and allowed the rest to coagulate, in order to isolate the serum for subsequent injections. It is advisable to place the doses for each injection in separate tubes. It is extremely difficult in Africa to collect or keep sterile sera. I employed various methods, none of them satisfactory, to avoid the contamination of antigens obtained in the bush and to sterilise or make it possible to use those which were already contaminated or in a more or less advanced stage of putrefaction when they arrived.

I prepared little bottles with a rubber cap, sterilised and containing a small quantity of solution of carbolic citrate of sodium, which I used to send to people who were good enough to promise to supply me with antigens.

When they shot any game, they could fill a syringe with the serum or blood, which they squirted into the bottle without uncorking it, the needle passing through the rubber cap.

To suggest that a hunter should take all aseptic precautions when collecting blood in the bush would be to ignore the difficulties of this operation, and the antigens rarely reached the laboratory in a fit state for inoculation.

Despite the addition of antiseptics (in the small quantities which the rabbits could stand), infection and putrefaction of the antigens frequently occurred, and the heavy losses among the animals treated proved most discouraging.

I employed various means—enumerated below—so as to make it possible to employ infected antigens, if such antigens were difficult to obtain :

(a) The addition of big doses of chloroform, which I evaporated by heat before injection.

(b) Repeated heating to from 50° to 60° C., which produces coctoprecipitins, was tried ; the latter react well in the presence of thermoprecipitinogens, but are not very sensitive to non-heated antigens.

(c) I added "Bayer 205" and boiled the antigen<sup>1</sup>; this involves the drawbacks mentioned in the previous paragraph as regards thermoprecipitinogens.

(d) Filtration through a porcelain bougie. In the big water-filters—the only ones at my disposal—the little quantity of antigen, as a rule barely enough to immunise as many as two or three rabbits, would have been lost.

(e) The addition of phenol, tricrisol, etc.

(f) Drying the blood on a piece of cloth, sterilising it dry, and redissolving it aseptically.

To sum up, precipitin anti-sera prepared with heated antigens were not suitable for tests with the blood found in glossinæ ; filtration would have been the best means of making use of contaminated antigens, but not having a good filter, I added the antiseptics usually employed for preserving therapeutic sera. Chloroform, which is recommended by a number of investigators, and which I used a good deal, has one drawback, in that it is denser than the sera ; once it is covered by the serum, it is difficult to evaporate it and its action does not extend to the whole of the antigen. The contents of tubes in which the chloroform at the bottom was in permanent contact with the serum for a number of days still proved contaminated when inoculated into rabbits.

#### 1. *Notes on some of the Antigens employed.*

The following antigens were collected, after bleeding carried out at the Entebbe Laboratory or in its immediate neighbourhood : man, monkey, ox, sheep, goat, dog, domestic cat, wild cat, snake (python), lizard (monitor), crocodile, hen, osprey, various water birds, and the albumen of hens' eggs and crocodiles' eggs.

<sup>1</sup> FORNEAU, TRÉFOUEL AND VALLÉE (1924), "Recherches de chimiothérapie dans la série du 'Bayer 205'", *Ann. Inst. Pasteur*, Vol. 38, N° 2, p. 81. The "authors note that, if this drug be added to the serum, the latter does not coagulate when heated ; similarly, if 'Bayer 205' is added to the plasma and this is heated to 53°, no fibrinogens are formed. The quantities of the product required vary with each kind of albumin."

The small amount of antigen at my disposal did not permit of my carrying out very many experiments with a view to determining the optimum dose of "Bayer 205" to be added to each serum in order to prevent coagulation but without injuring its antigenic properties.

Other antigens were sent to me by friends: hippopotamus, elephant, crocodile, lizard (monitor), wild pig (*Phacocheirus*), two kinds of antelopes (*Tragelaphus spekei* and *T. scripticus*).

The last two, which arrived at the Laboratory more or less contaminated, resulted in the loss of most of my rabbits.

I had no difficulty in preparing anti-sera with the antigens collected and preserved aseptically, except in the case of reptile anti-sera, which proved highly toxic to rabbits. To begin with, I injected reptile antigens in accordance with the technique employed for the other antigens (strong intravenous injections), as this was quicker, required a smaller quantity of antigen and produced fewer co-precipitins. I soon realised that I must employ a different process with reptile antigens.

I succeeded in bleeding four snakes (pythons) and seventeen monitors, each animal furnishing sufficient blood to immunise three or four rabbits.

After two vain attempts at immunising with 5 c.c. of snake blood injected into the marginal vein of the rabbit's ear, I successfully injected two doses of 2 and 3.5 c.c. respectively into the peritoneum of a third rabbit. Before proceeding with the third injection, I titrated the rabbit anti-serum and, as its precipitin content appeared to be sufficiently high, took the precaution of drawing off a little blood so as to be sure of having a small supply of anti-snake serum. The rabbit succumbed to the third injection of 3 c.c.

I encountered equal difficulty with the blood of the monitor (*vide* photographs 1 and 2 at the end of the present report), a lizard which is fairly common at Entebbe. The blood of seventeen of this species furnished me with sufficient antigen to immunise more than fifty rabbits, but of the fourteen inoculated, only four supplied anti-sera that could be used. The first succumbed after the injection of big doses. Afterwards I injected smaller doses, but the rabbits still died. The blood of these big lizards exhibits one curious feature which may perhaps bear some relation to the toxic qualities observed. The animal was cut open and the blood, which was collected in sterilised Petri-dishes, began to coagulate rapidly; the serum also began to coagulate before becoming completely separated from the clot. All the monitors had the peritoneum thickly covered with long filariæ. The rapidity with which the monitor serum coagulated appeared to vary more or less according to the degree of infestation by filariæ; further, the coagulability of the blood serum bore a constant relation to its toxicity for the rabbit.

As the blood, including the serum, of these lizards coagulated rapidly even when citrated, I only obtained enough liquid antigen for the first injection, and then only if I had the rabbit to be immunised on the spot, so that the serum had no time to coagulate.

In the rare cases in which the rabbit stood the first injection, I did not succeed, using the monitor blood with a jellified serum, in collecting the small quantity of fluid necessary to complete the process of immunisation. I broke up in distilled water part of this jellified blood and filtered the resulting extract through a sterilised paper adding an equal volume of 1.8% sodium-chloride solution, in order to obtain a final solution in normal saline. I injected this solution into the peritoneum of the same rabbit, which always succumbed from one to twelve hours later, a result which would appear to be due to toxic rather than infective action.

This almost wholesale slaughter exhausted the rabbit supplies of the Entebbe Laboratory. Thus, one day, some natives appeared with a monitor for sale and I had not got a single rabbit with which to recommence the preparation of precipitin anti-serum. Some of the rabbits, however, which had been immunised with the antigens of other animals and had already been bled with the object of obtaining the corresponding



anti-sera, were still alive. We bled the monitor, it was bled and I injected 4 c.c. of fresh blood into the peritoneal cavity of a rabbit which had already produced *anti-human* serum; the animal stood this injection very well. I placed the remainder of the jellified blood in the sterilised centrifuge tubes and succeeded in this way in obtaining a small quantity of blood-stained serum for a second injection, which the animal also stood quite well.

Before attempting a third injection of antigen from the lizard, I first titrated the anti-serum of the rabbit and, having found it sufficiently active, tried to get a good supply of this serum, which precipitated solutions of both human and monitor antigens; I did not continue the injections. Later I succeeded with very small doses in immunising two rabbits (Nos. 46 and 47), exclusively *anti-monitor*. Dr. DUKE brought me back from one of his scientific "safari" eight of these enormous lizards alive, and I killed them on the days I was giving injections, so as to be able to inoculate fresh blood before it could coagulate. I injected at the same time big doses—5, 8 and 12 c.c.—of monitor blood into the peritoneum of a hen, which stood the operation, but only yielded a weak anti-serum.

I also encountered difficulties in connection with the *crocodile* antigen, difficulties due perhaps to septic rather than to toxic action.

Crocodiles, generally shot on the water's edge, have still time to dive, so that some hours, or even a day may elapse before they reappear, floating on the surface, or are washed up on the bank, in a more or less advanced state of putrefaction (*vide* photographs 3 and 4 at the end of the report).

I found in the cardiac cavity an ample supply of blood, more than enough to immunise half a dozen rabbits if it happened to be of good quality. Putrefactive organisms, which are easily visible on microscopic examination, are sufficient to explain the death of the rabbits from two to four days after the injections. I lost seven rabbits by using the blood of four big crocodiles and did not have a single success, although I varied the doses and employed different ways of administering the blood.

In the few glossinæ in which I found blood, I discovered a large percentage of big nuclear monitor or crocodile red blood cells. For the purposes of research into the precipitin test as applied to *Glossina palpalis* it is absolutely essential to obtain an anti-crocodile precipitant serum. I decided to get some crocodiles' eggs and to carry out inoculation with their albumen. This was how I came to prepare the first anti-crocodile serum (rabbit 37), which, I may add, possessed weak specific precipitating properties, adequate for the precipitin in test with crocodile blood, but giving uncertain results if the blood had been acted upon by the digestive ferments of the tsetse-fly.

I determined to continue the experiment on the same rabbits with stronger doses of albumen, so as ultimately to obtain a sufficiently active anti-serum, but the animals succumbed.

I decided to hatch the remaining eggs in the stove in order to obtain some live non-infested crocodiles, with the idea of immunising the rabbits with their blood or with an extract of their flesh. Six crocodiles were hatched (*vide* photograph 5). I fed them on fishguts. I intended to kill them when they appeared ready to supply sufficient blood to immunise one or two rabbits. Some natives, however, appeared at the Laboratory with a crocodile for sale, almost a meter and a half in length and still alive (*vide* photograph 6). The six little crocodiles were subsequently used for experiments with *T. grayi*. An account of these experiments will be given in a separate report.

The crocodile which we bought was soon found to be dying, as the result, I believe, of blows inflicted by the natives in order to keep it quiet, for its blood exhibited no bacteria either on culture or by direct examination. I only found a number of

extra-cellular hæmogregarines moving about rapidly in the plasma; these might easily have been mistaken for trypanosomes unless carefully examined<sup>1</sup>.

I succeeded finally in immunising two rabbits (Nos. 51 and 52), with smaller doses of the blood and serum of this last crocodile, and obtained a very active anti-crocodile serum, with which I was able to conclude my research into precipitin tests applied to blood in the digestive canal of *Glossina palpalis* found in the neighbourhood of the Entebbe Laboratory.

## 2. "Glossinated" Antigens.

The "glossinated" antigens, if one may so describe blood partially digested by glossinæ, exhibit certain peculiar characteristics which may be demonstrated by applying precipitin tests to the contents of the alimentary canal of tsetse-flies. As various investigators have shown, antigens changed by the action of a specific physical or chemical reagent produce precipitins which react better with antigens that have undergone similar modification. When attempting to determine the different kinds of meat which enter into the composition of sausages deliberately heated by fraudulent persons, the extracts of meat to be inoculated as antigens for the production of specific precipitins should be heated and subjected to the same physico-chemical treatment as the sausages have undergone.

Precipitin tests carried out with the blood found in glossinæ always gave such weak positive reactions that I decided to make a few experiments in order to determine whether the antigen or the anti-serum was responsible. I prepared an anti-serum with a well-marked precipitating action on solutions of goat's blood. I set a box of glossinæ to feed on a goat and every hour or so killed a few of these flies and applied the precipitin test with the strongly specific anti-goat serum. The first flies killed gave a precipitin reaction almost as definite as that obtained with the homologous antigen (goat); as digestive action continued, the solutions of blood extracted from the glossinæ gave less and less definite reactions, until (after thirty to fifty hours) the reactions were practically negative. I repeated the experiment, feeding two boxes of glossinæ on fowls. The nucleated red blood cells were digested more slowly: I found in one intestine nucleated cells which were recognisable, under the microscope, after five days' digestion and, in the sucking stomach, after ten days. Nevertheless, the results of precipitin tests carried out with blood which had undergone digestion in glossinæ for more than forty-eight hours could hardly be considered reliable.

These experiments would appear to show that the digestive process in glossinæ exercises a manifest inhibitive effect on the reaction. I endeavoured to remedy this defect by "glossinating" the antigen, that is, by subjecting the blood, before inoculation, to the digestive action of the tsetse-flies. For each inoculation, I fed with blood a hundred glossinæ contained in two boxes: these I dissected after an interval of twenty-four hours. I prepared two rabbits with such an antigen, using goat's blood for one and ox blood for the other, both "glossinated".

By this means I obtained a serum with a specific precipitating action on solutions of goat's blood and ox blood, after partial digestion by tsetse-flies. Although close upon six hundred glossinæ were used in this process of immunisation the quantities of

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<sup>1</sup> I frequently found in wild glossinæ containing blood with large nucleated red cells, hæmogregarines, both free and encysted, and began to wonder whether there might not be some connection between the hæmogregarines found in the crocodile and those found in tsetse-flies. Blood parasites might assist in the identification of blood in the glossinæ; for example, if I found in a glossina red corpuscles exhibiting the characteristics of human red cells and containing a crescent typical of *Plasmodium praecox*, I should infer the human origin of the blood with almost as much certainty as if I had carried out the precipitin reaction.

“glossinated” blood injected would have to be increased to obtain a very active anti-serum.

According to MACFIE (1912), a glossina, sucking hard, will absorb an average of two- to three-hundredths of a cubic centimeter of blood. When a tsetse-box is placed on an animal, a high percentage of the flies do not suck at all, or, if they do, do not gorge themselves. We shall not be far out if we assume that one hundred flies suck 1 c.c. of blood. After twenty-four hours' digestion, this amount is reduced by at least one-fifth. These figures give an idea of the number of glossinæ necessary to furnish the amount of blood required for the preparation of a precipitin anti-serum (20 to 30 c.c.).

Any attempt to generalise the method would involve a fresh difficulty. In order to “glossinate” the blood of all the zoological species required for the preparation of anti-sera, it is essential to have live animals. To fix glossinæ-boxes on crocodiles or other wild beasts would be a risky proceeding. I carried out experiments with a Rodhain tube (skin covering the wide opening of a funnel which is filled with blood through the narrow opening.) Even when I used freshly drawn blood and fresh thin skin of rats, it was only with difficulty and after prolonged and laborious attempts that I succeeded in making four flies gorge themselves out of the fifty in the box against which I placed the Rodhain tube. I should not get even four to do so with contaminated blood.

This method (“glossinated” antigens) appeared to me to be the only one capable of solving the principal difficulty attaching to precipitin tests, as applied to the blood in the digestive tract of tsetse-flies. The difficulties which arose in actual practice proved insurmountable, so that I had to give up all idea of employing the method for these particular researches.

#### B. *Antigens suitable for Precipitin Reaction Control Solutions.*

For precipitin tests carried out according to Uhlenhut's technique, various control tubes are employed containing homologous and heterologous antigen solutions. The substances which have been injected into the rabbits as precipitinogens can be employed for these control solutions.

The best way of keeping the antigen, for this purpose, is to use filter-papers, as NUTTALL recommends. When the antigen is being collected for purposes of inoculation, several sheets of filter-paper should be allowed to soak up the blood, simply leaving a small space blank, for the name of the animal and the place and date of collection to be written in in pencil.

An excellent way of keeping antigen papers is to use photograph negative-film albums consisting of a number of pockets of paraffined paper (*vide* photograph 8 at the end of the present study).

Antigen solutions can be more quickly obtained and will be clearer with this filter-paper method than with any other. The clearness of the solutions is of the first importance for precipitin reactions. Fatty substances have a tendency to cloud solutions made with blood found in glossinæ. Even when filtered, such solutions remain opalescent, and this makes it difficult to estimate the results of weak precipitin reactions. NUTTALL recommends, in such cases, that cloudy solutions be soaked up on filter-paper, which will give fresh perfectly clear solutions after drying in the incubator.

If precipitin tests are to be carried out under proper conditions, it is essential that the solutions should have the proper concentrations of sodium chloride, of hydrogen-ions and of the antigen itself.

### 1. *Hydrogen-ion Concentration.*

NUTTALL and other investigators had observed that powerful acids and alkalis interfere with precipitin reactions, which show up best in solutions neutral to litmus. HIRSCH (1923) studied the effects of hydrogen-ion concentrations on reactions between sheep antigen solutions and the homologous anti-serum, and found that precipitin reactions were clearly marked between "Ph" 6 and "Ph" 9.4, but that the closer the solutions approach the neutral point the more marked the reaction. I studied the "Ph" of the Victoria Nyanza water with which I prepared the antigen and sodium-chloride solutions and had no difficulty, with a weak soda solution, in obtaining a "Ph" between 7 and 7.5 for all these solutions. I proceeded, within these limits, to apply the precipitin test to the blood of *glossinæ*.

### 2. *Concentration of Sodium-chloride Solutions.*

GRAHAM-SMITH and SANGER (1903), at NUTTALL's suggestion, studied the concentrations of sodium-chloride solutions which produce the best precipitates, employing the quantitative method.

The presence of salts assists the precipitin reaction, as it does colloid reactions. Watery solutions cannot be used. The use of solutions containing more than 5 per cent sodium chloride, although they produce a precipitate, makes it difficult to estimate the results of the reaction when carried out according to Uhlenhut's technique. The anti-serum, instead of sinking to the bottom of the tube, rises to the surface or mixes with the antigen, so that the two substances are not definitely separated, as is necessary for precipitin tests carried out according to Uhlenhut's technique, the most suitable method of applying the test to blood in *glossinæ*. Such blood sometimes dissolves very slowly, thus giving bacteria time to develop. The method that I adopted consists in first making solutions with pure water, which hæmolyses rapidly, and then adding an equal volume of 18<sup>0</sup>/<sub>00</sub> sodium-chloride solution so as to obtain ultimately a 0.9% concentration.

### 3. *Concentration of Antigen Solutions.*

NUTTALL was the first to observe that precipitation does not occur with highly concentrated antigen solutions. The precipitate formed is broken up or dissolved in excess of antigen. The formation of co-precipitins is a further drawback; they are more frequent in high concentrations up to 1 in 500.

UHLENHUT and other investigators regard as specific all precipitin reactions which give positive results in higher dilutions than 1 in 1,000. This brings out the practical importance of antigen concentrations. In measuring them, any one of the methods employed for examination for albumens may be used. A further difficulty in applying precipitin tests to the blood absorbed by tsetse-flies is that only a small quantity of solution is obtained which cannot be wasted in preliminary chemical experiments.

I tried using the Sicard-Cantaloube rachi-albuminometer, specially constructed for the infinitesimal percentages of albumin in the cerebro-spinal fluid, but was obliged to give it up, as it wasted the greater part of the antigen solution, already insufficient for two or three precipitin tests.

Another way of estimating antigen concentration is more economical and suitable for experiments with the exceedingly small quantity of antigen obtained from *glossinæ*; this is Houser's method, which he employed in his capillary method for precipitin tests. It is based on the reaction of albumins to nitric acid and may be described as follows: a small quantity of the liquid to be examined is sucked up with a capillary

pipette ; a little air is allowed to enter the pipette to facilitate sealing over a flame ; the pipette is then plunged into boiling water and subsequently opened by breaking the point ; the heated liquid is placed on a glass slide and to it is added about one-quarter of its volume of 25% nitric acid. If the solution becomes opalescent, the proportion of albumin in the liquid is sufficient to give precipitin reactions. If the liquid is very cloudy, the original solution should be diluted and the test repeated. The slightest opalescence is a proof that the concentration is sufficient for the precipitin reaction.

NUTTALL, UHLENHUT and other investigators recommend a different method for determining the concentration of antigen solutions ; although less certain, it is quite adequate for all practical purposes, and for my particular purpose was specially valuable in that none of the small supply of antigen obtained from the tsetse-flies was spoilt by the action of chemical reagents. The method consists in shaking the antigen solutions, so as to form a froth on the surface. If even the smallest bubbles remain after thirty seconds, the antigen concentration is sufficient to give the precipitin reaction.

Some investigators recommend blowing through the antigen solution with a pipette, to form bubbles. I noted that the reaction of the liquid is perceptibly modified if carbonic acid is blown through. If one blows through a neutral solution containing a hydrogen-ion indicator, phenol red for example, changes in colour will be observed as the reaction of the solution becomes further removed from the neutral point.

To obviate the difficulty, it is best to take a small tube containing antigen solution, to place a finger over the opening and shake it until froth forms. If this is very thick, it is a sign that the solution may be too concentrated. Highly concentrated solutions, it has been found, have power to dissolve the precipitate without the reaction being perceptible and to form co-precipitations which may prevent a specific reaction from being obtained. If the froth is very thick, it is well to dilute the solution until the bubbles disappear in less than half a minute. The last dilution in which the bubbles remain for over half a minute is suitable for the precipitin reaction and the results of the latter will be practically specific, without any risk of co-precipitins forming.

If the antigen solution has been prepared from a paper soaked in blood, the froth reaction should not be carried out by shaking the tube of antigen solution which still contains that paper, as innumerable particles will detach themselves and cloud the solution, and unless the solution is clear it is impossible to obtain specific precipitin reactions. To prevent such particles from clouding the antigen solution, a small quantity of the latter should be poured out of the tube which contains the paper into a smaller tube, suitable for the precipitin test, which can be shaken quite freely and froth formed without any risk of the liquid becoming turbid.

To sum up, antigen solutions should be clear, should have a "Ph" between 7 and 7.5 and should be prepared with normal saline ; when stirred, they should form a froth the bubbles of which remain for at least half a minute ; the dilution should be not less than 1 in 500.

### III. METHOD EMPLOYED FOR PREPARING PRECIPITIN ANTI-SERA.

When asked to undertake this study, which requires weeks and months of preparatory work, I soon realised that I could not possibly complete it in the time that the Commission still had to finish its work.

Further, protracted methods of immunisation, with small and frequent injections, lead to the formation of co-precipitins even more frequently than when rapid methods are adopted. To save time and also to obtain more specific anti-sera, I decided to

adopt an intensive system very similar to that of FORNET and MULLER, consisting of a few (three or four) large doses (4, 8, 12 and 16 c.c.) of antigen injected into the marginal vein of the rabbit's ear. If the animals stood this drastic treatment, the anti-sera produced were highly specific.

The rabbits could not stand this intensive method of immunisation if the antigen was not aseptic or if, as in the case of reptiles, it possessed toxic qualities. For such cases I had to employ the intra-peritoneal method, using smaller doses, and even so I lost a large number of the animals inoculated. It was, as I mentioned, the hippopotamus and reptile antigens that killed most of my rabbits. But for the purpose of applying the precipitin tests to the blood found in *Glossina palpalis*, these were the animals most likely to prove suitable, as they were most likely to have been bitten by the tsetse-flies in question, so that I did not feel justified in relinquishing the attempt to prepare corresponding anti-sera.

When the thermometer or scales indicated any important change in the rabbit's general condition, I suspended the intensive process, leaving longer intervals between the injections and postponing bleeding; sick rabbits do not react satisfactorily and produce weak anti-sera.

The second week after the last injection, I collected from 2 to 3 c.c. from the ear of the rabbit just immunised, and proceeded to titrate provisionally the precipitin content of the serum. If the anti-serum was sufficiently active I bled the animal well, so as to collect as great a quantity of serum as possible. If, however, the precipitin content was low, I began the antigen injections again, with due precautions against anaphylactic phenomena.

With some rabbits I did not succeed in producing anti-sera at all, however persistently I went on with the antigen inoculations.

#### *1. Titration of Anti-sera.*

The titration of anti-sera should be carried out at frequent intervals—certainly before collecting the serum and before using it for tests. It was only when I was using the same serum every day that I did not take this precaution: I knew its titre, and when carrying out the test, used to prepare a control tube with a highly diluted homologous antigen, with which I could ascertain whether the precipitin content had undergone any appreciable change. If several weeks had elapsed since the last application of a particular anti-serum, I retitrated it systematically before employing it for a fresh precipitin test, to make sure that it was still active.

When titrating, I decided not to employ NUTTALL'S quantitative method, based upon the volume of the precipitate produced, but adopted instead the technique which I employed for the test. The blood in the alimentary canal of glossinæ is generally contaminated with intestinal microbes; these continue to grow rapidly in antigen and anti-serum solutions, which form a good culture medium for many bacteria. If the quantitative method is employed, the precipitates should be examined twenty-four hours after the test. During that time, the intestinal bacteria of the fly may have multiplied to such an extent as to make it difficult to determine how much of the deposit or opacity is actually caused by this bacterial growth.

To meet this difficulty, I followed Uhlenhut's recognised method for the titration of anti-sera, which is as follows: Increasing dilutions of the homologous antigen are made in normal saline (1/500, 1/1,000, 1/2,500, 1/5,000, 1/10,000, 1/15,000, 1/20,000); 1 c.c. of each solution is poured into one of a series of hæmolysing tubes, all the tubes being of the same size; 1 c.c. of 1/1,000 heterologous antigen is poured into each of two other similar tubes; finally, 1 c.c. of normal saline is poured into another tube; 0.1 c.c. of the

anti-serum to be titrated is added to each of the tubes, being dropped carefully to the bottom of the tube, below the antigen, so that the one liquid is superimposed over the other, the level of separation being clearly marked. The tubes should be examined at once, without shaking, by transmitted light against a dark background. The titre of the anti-serum corresponds to that of the highest dilution in which, after from three to five minutes, opacity persists at the junction of the antigen with the anti-serum; the opacity first takes the form of a ring, subsequently forming a small whitish deposit at the bottom of the tube. If, for example, at the end of three to five minutes a ring is visible in all dilutions up to 1/10,000 solution, this will be the titre of the serum under examination. The last three tubes containing heterologous antigens or normal saline should remain clear.

When on titration the precipitin content of the serum is found to be too low, the precipitinogen injections should be continued, with due precautions against anaphylactic phenomena.

If, on the other hand, the precipitin content of the anti-serum proves to be high, the animal should be well bled, so as to collect as much serum as possible.

## 2. *Collection of Anti-sera.*

Satisfactory precipitin reactions cannot be obtained unless the anti-sera are absolutely clear. To ensure this, certain rules must be followed, when bleeding the rabbits.

For titration, 2 to 3 c.c. of blood are sufficient and can be obtained from the marginal vein of the rabbit's ear, but when the moment comes to collect the anti-serum, the blood should be drawn from one of the big blood-vessels or from the heart.

The following are the principal causes of turbidity in sera: bleeding of the immunised rabbit while digestion is proceeding; bacterial contamination resulting from imperfect asepsis; and hæmolysis, or the presence of large numbers of red cells owing to imperfect separation of the serum from the clot.

I shall now explain the precautions I took in order to be sure of obtaining clear anti-sera.

(a) I isolated the rabbit the day before it was to be bled, and starved it for twelve to twenty-four hours. This prevents the milky colour frequently found in serum collected while digestion is in progress.

(b) The strictest aseptic precautions are essential in bleeding, in order to prevent the growth of bacteria in the serum.

When bleeding the rabbit from the ear for the purpose of anti-serum titration, I followed the technique recommended by WENYON: the ear is shaved, rubbed with xylol, washed with alcohol and covered with sterilised melted paraffin; one of the veins distended by the action of the xylol is then punctured, through the thin layer of paraffin, with a large sterilised needle. The blood only touches the sterilised paraffin and flows rapidly through a sterilised tube to the amount of from 2 to 4 c.c., which is more than enough to determine the precipitin content of the serum to be titrated.

When, on provisional titration, the precipitin content of the anti-serum was found to be sufficiently high, I bled the animal copiously with aseptic precautions, so as to obtain a good supply of it.

Some investigators prefer to give an anæsthetic and kill the rabbit, and, after opening the thorax, to draw up the blood aseptically, either direct from the heart or from the thoracic cavity, working quickly, before the blood has time to coagulate.

Others again prefer to shave and disinfect the rabbit's neck, to extend it and, with a sharp bistoury, to sever the big cervical blood-vessels, from which a stream of blood gushes forth, which is collected in a sterilised bottle with a wide mouth.

Lastly, some investigators dissect out one of the big blood-vessels in the neck, fix a ligature which is either tied or compressed : it is then cut across, the non-occluded end being held in such a manner that the blood flows into a sterilised receiver.

I always preferred to bleed the rabbits by puncturing the heart, without opening the thorax. This method has various advantages. If a good syringe with a sharp needle is used, it is quite easy to collect an abundant supply of blood aseptically. Further, the rabbits stand these punctures very well, a most important point. An animal that goes on living can be used as a reservoir for anti-serum, or can even be given fresh inoculations with the antigen of different animal species, for the preparation of polyvalent anti-sera or multi-precipitating sera. After collecting the anti-sera of rabbits 11 and 27, immunised with human and antelope antigens respectively, I injected monitor blood, and they furnished other anti-sera which precipitated monitor antigens simultaneously with the human and antelope antigens. With rabbit 3, I succeeded in preparing a serum which reacted strongly with goat, cow, sheep and antelope antigens.

As rabbits were becoming scarce in Uganda, and as my work on the precipitin test and on trypanosome cultures necessitated my having large numbers of them, I decided to carry out cardiac puncture again on the fifty-nine rabbits which I had managed to procure. Cardiac puncture is quite easy to perform after it has been practised once or twice, and does not appear to hurt the rabbit much if the needle is sharp ; otherwise, it is liable to displace the heart, without puncturing it or, if it does puncture it, produces serious lesions.

Most of my rabbits could stand three or four cardiac punctures, and some of them even underwent the operation six or eight times. I only lost three out of over a hundred ; these particular rabbits were not held firmly, and moved, so that the needle tore the myocardium, causing internal hæmorrhage. It was only in exceptional cases that I finally killed the animal, opened its thorax and drew out the remaining blood aseptically.

Cardiac puncture, then, is to be recommended for the collection of anti-sera. Asepsis is very simple ; the rabbits go on living and can be employed for the preparation of polyvalent anti-sera or simply kept for fresh punctures, thus constituting a living reservoir of the anti-serum ; this point is by no means negligible in tropical countries, where no ice is procurable and it is difficult to preserve the activity of the anti-sera in a high temperature.

(c) It is essential that bleeding should be carried out aseptically and when digestion is not in progress but this is not in itself sufficient to obtain clear sera. The serum separated from the clot must not be blood-stained.

To avoid the necessity of shaking the blood adherent to the sides of the tube and to facilitate the separation of the serum, the tubes in which the blood of the immunised rabbits was to be collected were moistened with a 0.9% sodium-chloride solution, and, whenever ice was available, the tubes were placed in the icechest directly the serum began to separate, so as to prevent hæmolysis.

To sum up, in order to obtain clear anti-sera, I starved the rabbits for twelve to twenty-four hours ; I then drew off blood aseptically by means of cardiac puncture, collecting it in tubes moistened with normal saline ; these tubes I placed in the icechest, to prevent hæmolysis and ensure the perfect separation of the serum.



### 3. *Preservation of Anti-sera.*

After separation from the clot, the serum is carefully decanted into a short wide sterilised tube with strict aseptic precautions; in the tube are placed sterilised ampoules, preferably of dark glass, sealed at one end and open at the other, the latter being completely covered with anti-serum. The ampoules are then filled by the use of a vacuum apparatus. The ampoules should then be sealed with a flame without heating the serum, which must be protected from heat and light until required for use.

These operations involve a number of small but not unimportant technical details, a description of which, although it might appear superfluous, may nevertheless be of assistance to anyone lacking experience who undertakes similar work.

*Decantation* should be most carefully carried out, to avoid clouding the serum. The last portion in contact with the clot contains a number of red blood corpuscles. Even if there is only a small quantity of serum, it is advisable to have at least two sterilised tubes at hand into which to decant it: one for the upper layer, which is almost sure to be clear, and the other—preferably a conical centrifuge tube—to receive the lower layer, which may be stained with blood, and should if necessary be clarified by centrifugalisation.

It is better to sacrifice the small amount of anti-serum which may be difficult to separate from the clot rather than try to procure a larger quantity at the cost of obtaining a cloudy anti-serum.

*Filling of Ampoules.* — The anti-serum could be kept aseptically in a single sterilised bottle. At the same time, as blood serum is an excellent culture media for many bacteria, it would be inadvisable to keep it all in one receptacle, since, if it became contaminated, as may easily happen in tropical countries, the whole supply would be spoilt.

If the serum is stored in small ampoules, each large enough to hold the volume required for one precipitin test, then, should the contents of one of these ampoules become contaminated, there will be no risk of the whole supply being wasted as would be bound to happen if all the serum were kept in a single bottle which had to be opened for each test.

Suitable ampoules can easily be made with a gas or spirit stove or of the Bunsen type or even with a small Primus stove. Glass tubing should be used, if possible coloured, of somewhat smaller bore than the tubing used for making capillary pipettes.

The kind of ampoule that I found best for keeping anti-sera is the one shown in Fig. 2: the body of the tube is from 0.5 to 1 c.c. in capacity, the short end being just under 1 cm. and the other from 3 to 4 cm. in length. The short end should be closed and the long one left open. Ampoules of this capacity hold sufficient serum for one complete precipitin test, including all control tubes.

I found ampoules with two capillary extremities to possess definite advantages. I kept my first sera in capillary tubes like those used for anti-smallpox vaccine or in ordinary ampoules with one capillary extremity and one wide round end. When I came to seal the capillary extremity over the flame, after it had been moistened by the anti-serum, the heat coagulated the film of serum, producing a collection of small whitish particles, which might be confused with the specific precipitate formed in precipitin tests. This drawback can easily be remedied by using the ampoules mentioned above, subject to certain precautions which I will describe; these may appear superfluous to very experienced investigators but may save novices a certain amount of disappointment.

If no vacuum apparatus is available, the ampoules may be filled by leaving the two extremities open and drawing up the serum. When there are a number of ampoules, this process takes time and bacterial contamination may result.

Anti-serum asepsis, although not absolutely essential, since infected sera do not lose all their activity, is nevertheless highly desirable, not only because the precipitin content remains more constant but chiefly because bacterial contamination makes the serum turbid, so that it is sometimes difficult to distinguish between such turbidity and the opacity characteristic of precipitins. It is important, therefore, to avoid anything that might result in the contamination of the anti-sera.

There are different forms of small apparatus for filling ampoules by a vacuum process; the container can be sterilised very easily, thus combining the advantages of asepsis and rapidity.

Any form of vacuum apparatus can be used. An apparatus with a jar provided with a third opening, in addition to the two necessary for the admission and exit of air, is a great advantage. This third aperture is hermetically closed with a cork to which is attached a greased metal clamp by means of which the ampoules can be raised or lowered in the interior of the jar without admission of air (*vide* Fig. 1, and photograph 9).

When sealing the ampoule in the flame, the heated part should not contain any serum, as the latter will coagulate, clouding the anti-serum with a kind of precipitate and producing steam, which may distend the molten capillary end, leaving it still wider open. It is thus important that the end of the ampoule to be sealed should be completely empty; any one of the following methods may be used to ensure this.

1. If the jar has no metal rod passing through a third opening, only a small quantity of anti-serum should be placed inside the vacuum apparatus, not enough to fill the ampoules placed in it. When the vacuum has been created and the aperture for admitting air is opened again, the serum will pass straight into the ampoules; and as there is insufficient serum to fill them, the air will drive it to the far end of the ampoule, leaving the capillary extremity empty.

2. If this process does not produce the desired results (*i.e.*, if the serum fills the ampoules completely, including the capillary extremity to be sealed), the ampoules may be placed in centrifugalising tubes with the open ends upwards. A few turns of the centrifuge will bring down the anti-serum drop and drive air bubbles towards the capillary end to be heated.

3. The following process is, in my view, the quickest and most reliable: the jar of the vacuum apparatus should, in addition to the taps for the admittance and exit of air, possess a third opening through which a metal rod is inserted in such a way as to preclude the entrance of air, so that at a given moment the ampoules may be raised until their extremities, at first covered by the serum, stand clear above it. The advantages of this particular process are as follows; when the vacuum has been created, the air tap should be very slightly opened, so that the serum is forced *slowly* into the ampoules by compression; when the ampoules are three-quarters full, the air-aperture should be closed again and the metal rod pulled until the open ends of the ampoules are raised above the surface of the anti-serum. If the air-aperture is then reopened, air will enter the ampoules and cause the anti-serum to rise, leaving the capillary extremities empty, so that there is nothing to prevent their being heated and sealed (*vide* Fig. 1).

4. In order to obviate difficulties dependent upon the heat necessary to seal the ends of the ampoules I tried to close them by plunging the ends into melted paraffin. This, however, after cooling and contracting, frequently became brittle and dropped off, and the contents of some of the ampoules consequently became mouldy.

As the Entebbe Laboratory possessed a good apparatus for filling tubes with vaccine (*vide* photograph 9 at the end of the present report), I employed the third of the methods described above for filling the ampoules with anti-serum.

When the serum has passed through the extremity of the ampoule, it is bound to leave a certain residue which, insignificant as it may appear, will coagulate under the influence of heat. Further, with all sera, even without the action of heat, a sediment is formed at the bottom of the receptacles.

If, as I suggest, the capillary ends of the ampoules to be sealed by heat are 3 or 4 cm. long and if the ampoules are stored in a vertical position with this extremity downwards, the precipitates formed by heating the serum still clinging to the sides of the ampoules and those which appear in all blood sera kept for any length of time, even when protected from heat and light, will all collect in this extremity.

When clear anti-serum is required for carrying out a precipitin test, the shorter upper end of the ampoule should be opened with a small file (such as is supplied with boxes of ampoules containing drugs). After opening, it should be held over the open end of a small sterilised tube; preferably one with a conical end. The long extremity of the ampoule should then be cut across so as to get rid of any sediment which may have collected there. The serum will enter the small conical tube through the shorter extremity without any debris.

After sealing the ampoules, care should be taken not to place them at once in a horizontal position or to reverse them, since otherwise the serum would come into contact with the heated glass and its precipitin content would be seriously impaired. Once precipitins have become converted into precipitoids, precipitin tests are no longer possible.

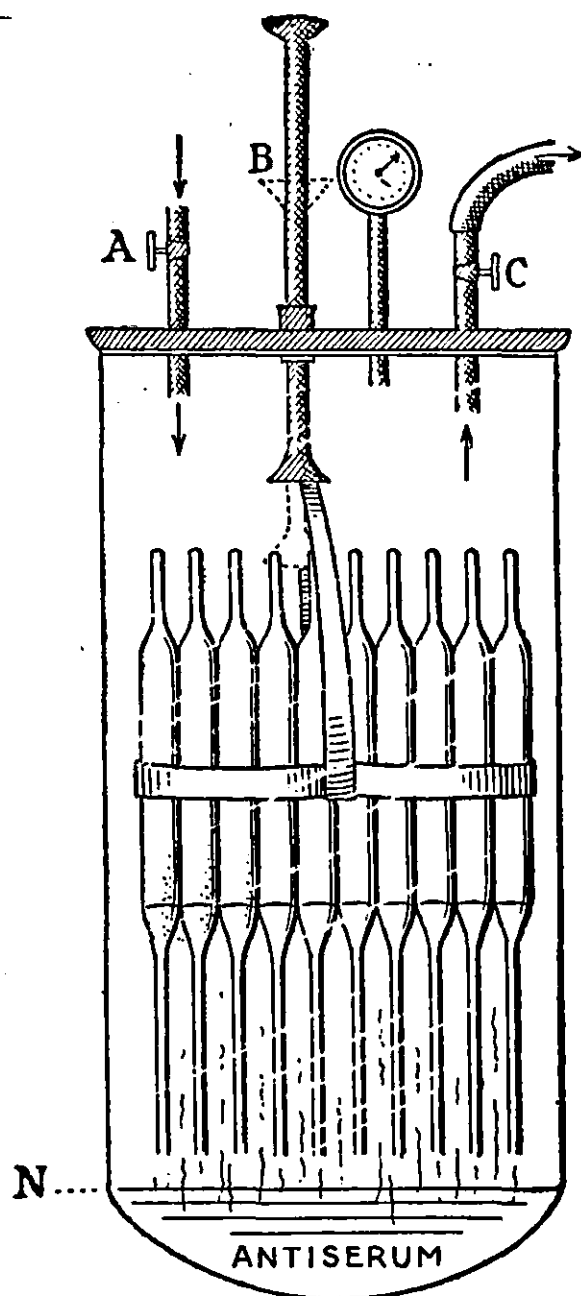


Fig. 1

Sketch showing method of introducing the anti-serum into the ampoules: After creating a vacuum through tube C, open slowly tap A, allowing air to enter only when the serum has filled a little more than three-quarters of each of the ampoules. Then close rapidly tap A and pull rod B until the lower extremities of the ampoules are suspended above the surface of the anti-serum N. When tap A is opened again to admit more air, the latter, pressing on the anti-serum, will penetrate into the capillary extremities of the ampoules, which can then be sealed without any risk of coagulation or of inactivation of the anti-serum by heating.

After filling and sealing, the ampoules should be placed in bamboo holders (*vide* photograph 9) and kept in an ice-chest, which, even without ice, can still be used to regulate the temperature.

The discouraging results obtained in a large proportion of these tests are generally due to slight technical mistakes, which I have preferred to describe perhaps at inordinate length rather than risk not being explicit enough.

To sum up, anti-sera should be enclosed in ampoules, but without being subjected to the injurious action of heat; they should be kept in a vertical position, with the extremity which has been heated turned downwards, so that the deposits to be eliminated, when the ampoule is opened and the anti-serum drawn off, may accumulate there.

#### 4. *Co-precipitating or Multi-precipitating Sera.*

In order to obtain a highly specific anti-serum, I always selected a healthy rabbit, to which I administered frequent intravenous injections of large doses of a good antigen.

Progressive and prolonged immunisation produces a less specific anti-serum, containing co-precipitins or group precipitins. The formation of co-precipitins has drawbacks, but may present certain advantages.

More than once I immunised a rabbit with the antigen of a certain animal, and then, after having bled the rabbit and kept the first anti-serum, carried out immunisation a second, a third, or even a fourth time, against the antigens of animals of *very close* or *very distant* species.

With a multi-precipitating serum, prepared with the antigens of *very closely allied* species—for example, that obtained with the blood of various ruminants (goat, sheep, ox and antelope)—I obtained valuable information concerning practical details of the precipitin test applied to blood found in the alimentary canal of tsetse-flies. By adding a single dose of this polyvalent serum, I knew at once whether the blood in the glossina belonged to one of the species of the group from which the multi-precipitating serum had been prepared. Thus, by means of a single test, I could eliminate a whole group of species, if the reaction was negative. If it was positive, I simply had to try again with more specific sera, free

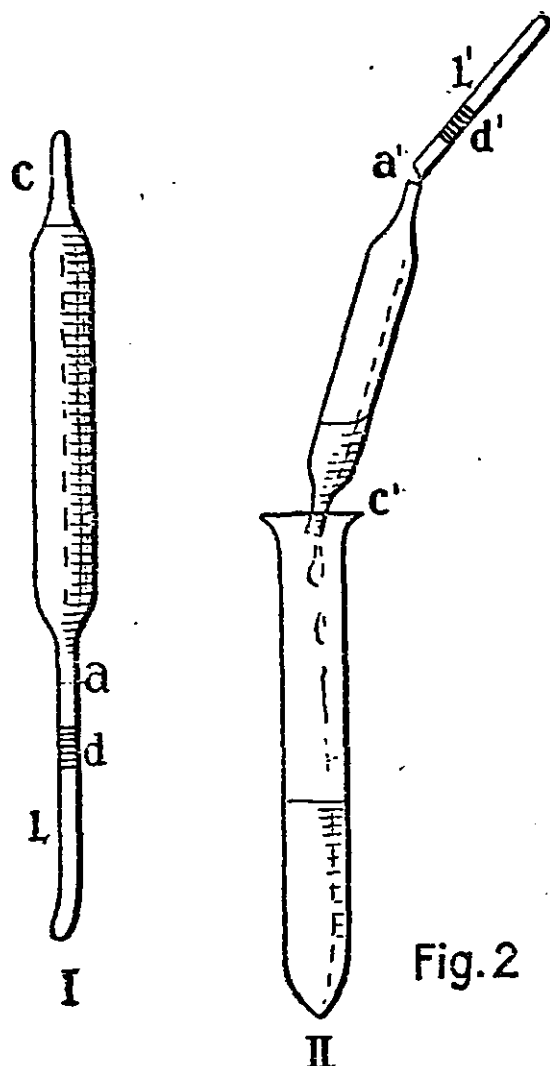


Fig. 2

Ampoule suitable for keeping anti-sera :

I. Ampoule in the vertical position in which it should be kept in the ice-chest; *c*, short (upper) extremity; *l*, long (lower) extremity; *d*, sediment accumulated in the long extremity, and *a*, point at which the extremity should be cut to eliminate sediment.

II. Method of opening ampoules containing anti-serum without clouding the serum: first cut extremity *c'* and hold it over the opening of a small tube with a conical end; next cut long extremity *l'* to exclude sediment *d'* and the serum will drop into the tube without carrying the sediment with it. If, despite these precautions, the sediment is carried into the tube, the latter should be centrifugalised; hence the advantage of a tube with a conical end.

from co-precipitins, for the several species which had contributed towards the multi-precipitating serum. In this way, I narrowed the enquiry into the species whose blood had been found in the tsetse-fly.

I also found a use for multi-precipitating sera prepared with antigens of *distant* species. If, for example, I obtained a positive precipitin reaction with anti-human-monitor or anti-antelope-monitor bi-precipitating sera, and if I could at the same time determine by microscopic examination the morphological characteristics of the red cells (nucleated or non-nucleated), I was near to a diagnosis. If the anti-human-monitor serum precipitated the blood found in a glossina, and the microscopic examination showed that the red cells in this blood were non-nucleated, I could infer, with the highest degree of probability, that the blood was that of man, ultimately confirming this opinion by one more test carried out with an exclusively anti-human serum.

5. *Table showing Immunisation of Rabbits and Fowls.*

The following table gives a summary of my work on the immunisation of rabbits and hens and the results obtained.

IMMUNISATION OF RABBITS <sup>1</sup>

Rabbit No.	Antigen	Inoculations												Date of bleeding or death; titration.	
		1		2		3		4		5		6			
		Date	c.c.	Date	c.c.	Date	c.c.	Date	c.c.	Date	c.c.	Date	c.c.		
1	ox sheep	3-5-26 blood v	5	14-5-26 blood v	10	19-5-26 1		19-5-26 blood v	12	27-5-26 blood v	15				B. 4-6-26 1/1500 B. 4-6-26 1/1000
2	ox	3-5-26 blood v	5	14-5-26 blood v	10	20-5-26 blood v	15	27-5-26 blood v	20						B. 4-6-26 1/2500
3	ox	4-5-26 blood v	5	14-5-26 blood v	10	21-5-26 blood v	17								B. 4-6-26 1/2500
4	monitor	5-5-26 blood v	5												D. 6-5-26
5	man	6-5-26 blood v	5	8-5-26 blood v	10	10-5-26 serum v	15								D. 14-5-26
6	python	12-5-26 blood v	5												D. 13-5-26
7	goat	12-5-26 blood v	6	14-5-26 serum v	10	16-5-26 serum v	12								B. 26-5-26 1/2000
8	python	13-5-26 blood v	5	15-5-26 serum v	10										D. 15-5-26
9	python	19-5-26 serum v	2	23-5-26 serum v	3½										B. 30-5-26 1/1500
10	sheep	19-5-26 blood v	5	21-5-26 serum v	10	24-5-26 serum v	15								B. 4-6-26 1/5000
11	man	19-5-26 blood v	5	21-5-26 serum v	10	24-5-26 serum v	10								B. 4-6-26 1/2000
12	Hen	25-5-26 serum v	6	31-5-26 serum v	10	7-6-26 serum v	15								B. 21-6-26 1/5000
13	man	7-6-26 blood v	6	9-6-26 serum v	10	11-6-26 serum v	12	13-6-26 blood v	15						B. 22-6-26 1/2500
14	ox (glossinated)	21-6-26 tsetse v	50	25-6-26 tsetse v	50	29-6-26 tsetse v	50	4-7-26 tsetse v	50	8-7-26 tsetse v	50	14-7-26 tsetse v	50		B. 22-7-26 1/500
15	goat (glossinated)	21-6-26 tsetse v	50	25-6-26 tsetse v	50	29-6-26 tsetse v	50	4-7-26 tsetse v	50	8-7-26 tsetse v	50	14-7-26 tsetse v	50		B. 22-7-26 1/500
16	hippota- mus	29-6-26 blood v	6												D. 30-6-26
17	hippota- mus	29-6-26 blood p	8												D. 30-6-26
18	hippota- mus	30-6-26 blood p	5												D. 1-7-26
2a	hippota- mus	2-7-26 blood v	2½	5-7-26 blood v	5										D. 8-7-26
19	monitor	3-7-26 blood v	5												D. 4-7-26
20	phaco- chærus	7-7-26 serum p	4	9-7-26 serum p	6	13-7-26 serum p	8								B. 23-7-26 1/1500
21	hippota- mus	7-7-26 serum p	5	9-7-26 serum p	6	13-7-26 serum p	8	the antiserum gave a precipitin even with the physio- logical solution							
22	hippota- mus	7-7-26 serum v	5												D. 9-7-26
23	monitor	7-7-26 blood v	2	12-7-26 serum p	4	15-7-26 blood p	8								D. 16-7-26
24	man	13-7-26 blood v	5	15-7-26 serum v	10	18-7-26 serum v	12	21-7-26 C.S.F. p	9						B. 30-7-26 1/5000

<sup>1</sup> v = Intravenous injection ; p = Intraperitoneal injection ; B. = bled ; D. = died.

IMMUNISATION OF RABBITS (*continued*)

Rabbit No.	Antigen	Inoculations								Date of bleeding or death; titration.
		1		2		3		4		
		Date	c.c.	Date	c.c.	Date	c.c.	Date	c.c.	
25	hippopotamus	22-7-26 serum v	5	24-7-26 serum v	10	26-7-26 serum v	12			B. 3-8-26 1/2500
26	water fowl and hen	1-8-26 blood p	10	3-8-26 serum p	15	9-8-26 serum p	15			D. 14-8-26
3a	goat, sheep, ox	6-8-26 blood of goat p	4	11-8-26 blood of sheep p	10	16-8-26 serum of goat p	8	16-8-26 serum of sheep p	8	B. 28-8-26 1/1500 goat 1/1500 sheep 1/2000 ox
27	antelope	13-8-26 serum p	5	16-8-26 serum p	10	19-8-26 serum p	10	21-8-26 serum p	8	B. 3-8-26 1/5000
28	monkey	20-8-26 blood v	5	23-8-26 serum v	10	26-8-26 serum p	15			B. 2-9-26 1/5000
29	water fowl and hen	23-8-26 blood cor- morant v	5	25-8-26 serum cor- morant v	10	27-8-26 blood of hen v	10	29-8-26 serum of hen p	12	B. 6-9-26 1/2000
3b	antelope, (goat, sheep, ox)	25-8-26 serum of ante- lope p	3	28-8-26 serum of ante- lope p	6	31-8-26 serum of ante- lope p	4			B. 10-8-26 1/2000
15a	albumen of hen's egg	26-8-26 saturated solution p	5	28-8-26 saturated solution p	10	30-8-26 saturated solution p	10			B. 10-9-26 1/1000
30	dog	26-8-26 blood v	8	1-9-26 serum v	10	4-9-26 serum p	12			B. 14-9-26 1/1000
31	Monitor and hippopotamus	1-9-26 blood of monitor p	3	3-9-26 blood of hippo- potamus p	6					D. 5-9-26
32	albumen of crocodile's egg	6-9-26 saturated solution p	5	8-9-26 saturated solution p	10					D. 11-9-26
33	monitor	6-9-26 blood p	5							D. 7-9-26
27a	monitor (antelope)	7-9-26 blood of monitor p	2	9-9-26 blood solution p	3	11-9-26 blood p	6			B. 21-9-26 1/2000
15b	albumen of hen's egg	21-9-26 saturated solution p	5	27-9-26 saturated solution p	8	6-10-26 saturated solution p	10			D. 9-10-26
10a	albumen of crocodile's egg	21-9-26 saturated solution p	8							D. 23-9-26
34	albumen of crocodile's egg	21-9-26 saturated solution p	4	25-9-26 saturated solution p	8					D. 27-9-26
35	albumen of crocodile's egg	21-9-26 saturated solution p	5	25-9-26 saturated solution p	8					D. 26-9-26
36	osprey and hen	2-10-26 blood of osprey v	5	4-10-26 blood of osprey p	6	9-10-26 blood of hen v	10	15-10-26 serum of hen p	10	B. 25-10-26 1/2500
30a	monitor (dog)	8-10-26 blood of monitor p	2	11-10-26 blood p	3	14-10-26 blood p	4	18-10-26 blood p	5	D. 18-10-26

IMMUNISATION OF RABBITS (conclusion)

Rabbit No.	Antigen	Inoculations								Date of bleeding or death ; titration.
		1		2		3		4		
		Date	c.c.	Date	c.c.	Date	c.c.	Date	c.c.	
37	crocodile, blood and albumin	11-10-26 blood p	5	14-10-26 albumin solution p	5	18-10-26 albumin solution p	10			B. 28-10-26 1/500
24a	man	13-10-26 blood p	5	18-10-26 serum v	10					D. 19-10-26
38	crocodile	13-11-26 serum p	5	16-11-26 serum p	6					D. 19-11-26
39	Situtunga <i>T. spekei</i>	13-11-26 serum p	4	16-11-26 serum p	7	20-11-26 serum p	8	24-11-26 serum p	10	B. 1-12-26 1/5000
40	monitor	13-11-26 blood p	5	16-11-26 blood p	8					D. 16-11-26
41	monitor	16-11-26 blood p	5							D. 17-11-26
42	monitor	16-11-26 blood p	5							D. 16-11-26
43	monitor	16-11-26 blood p	2	20-11-26 blood p	4					D. 21-11-26
44	monitor	18-11-26 blood p	2	20-11-26 blood p	3	25-11-26 blood p	5			D. 26-11-26
45	crocodile	18-11-26 serum p	2	24-11-26 serum p	3	29-11-26 serum p	5			D. 4-12-26
46	monitor	22-11-26 serum p	2	24-11-26 serum p	3	26-11-26 serum p	3½	30-11-26	4	B. 8-12-26 1/2000
47	monitor	24-11-26 serum p	2	26-11-26 serum p	2½	30-11-26 serum p	3	3-12-26 serum p	4	B. 12-12-26 1/2000
48	Situtunga	29-11-26 serum p	4	3-12-26 serum p	7					D. 5-12-26
49	crocodile	16-12-26 serum p	3	18-12-26 serum p	4	20-12-26 serum p	5			D. 22-12-26
50	monitor	18-12-26 serum p	3	20-12-26 serum p	4	24-12-26 serum p	4			D. 27-12-26
37a	crocodile	21-2-26 serum p	2	24-12-26 serum p	3					D. 26-12-26
51	crocodile	31-12-26 serum p	1½	5-1-27 serum p	2½	12-1-27 serum p	3	21-1-27 serum p	3	B. 3-2-27 1/2500
52	crocodile	31-12-26 serum p	1½	5-1-27 serum p	2½	12-1-27 serum p	3	21-1-27 serum p	3	1/2000
53	sheep	4-1-26 blood corpuscles v	5	12-1-27 blood corpuscles v	5	18-1-27 blood corpuscles p	8	25-1-27 blood corpuscles p	10	B. 3-2-27 1/3000
54	wild cat	17-1-27 blood v	5	24-1-27 serum v	10	31-1-27 serum v	15			9-2-27 1/3000
55	wild cat	17-1-27 blood v	5	24-1-27 serum v	10	31-1-27 serum v	15			B. 2-2-27 1/5000
56	elephant	10-3-27 sol. blood p	5	14-3-27 sol. blood p	5	18-3-27 sol. blood p	5	23-3-27 sol. blood p	8	B. 5-4-27 1/1000
57	elephant	10-3-27 sol. blood p	5	14-3-27 sol. blood p	5	18-3-27 sol. blood p	5	23-3-27 sol. blood p	8	B. 5-4-27 1/1500
58	monkey	15-3-27 blood v	5	17-3-27 serum v	10	19-3-27 serum v	15			B. 30-3-27 1/3000
59	man	16-3-27 blood v	8	18-3-27 serum v	10					D. 19-3-27



IMMUNISATION OF HENS

Hen No.	Antigen	Inoculations						Date of bleeding
		1		2		3		
1	monitor	7-12-26 blood p	6	14-12-26 blood p	10	21-12-26 centr. blood p	14	B. 30-12-26 1/500
2	crocodile	31-12-26 blood	5 v	5-1-27 serum	10 v	8-1-27 serum	15 p	B. 20-1-24 1/500

IV. PREPARATION OF SOLUTIONS OF THE BLOOD FOUND IN TSETSE-FLIES, FOR USE IN THE PRECIPITIN TEST.

1. *Capture of Glossinæ.*

My study was concerned only with *Glossina palpalis*, caught in the forest on the banks of Lake Victoria, in the neighbourhood of the Entebbe Laboratory<sup>1</sup>.

The fly-boys were generally told to bring back about fifty wild glossinæ. They sometimes caught a few gorged with blood, but as a rule the quantity of blood obtained from the flies was small.

Having chosen a clearing in the trees at the water's edge, each fly-boy, armed with a net like a butterfly-net, set about catching any tsetse-flies that happened to rest on his companions (*vide* photographs 14 and 15).

Like all the Uganda natives, the fly-boys wear long white tunics on which the dark glossinæ stand out clearly. I accompanied them on several occasions and did not find, as various observers appear to have noted, that the flies have any marked preference for khaki, such as I was wearing, rather than the fly-boys' light-coloured garments. The tsetse flies, however, showed a very definite preference for human flesh, whether black or white.

All the tsetse-flies found in the forest are in search of food. The only ones the fly-boys can see and catch are hungry, and instead of being gorged with blood, are in search of it. It is a difficult matter, therefore, to obtain suitable glossinæ for the purposes of the precipitin test. When one does chance to find a small quantity of blood in a tsetse-fly, it has been acted upon so long by digestive ferments in the fly that a clearly marked precipitin reaction is difficult to obtain.

R. B. LLOYD (1925) applied the test to a number of *Phlebotomus*, under much more favourable conditions. He had no difficulty in procuring in a cattle-shed specimens of *Phlebotomus* full of blood recently sucked to which he applied the precipitin test. In this limited area, into which only two animal species (man and cattle) ever penetrated, he merely had to prepare anti-human and anti-ox sera.

The problem of identifying the blood found in tsetse-flies is far more complex. It is practically impossible to find and catch, in the bush, a tsetse-fly gorged with

<sup>1</sup> *Vide* note, page 224.

freshly drawn blood, and when applying the precipitin test not two or three but a large number of anti-sera are required, prepared with the antigens of all the animals in any given region which are likely to be bitten by the species of glossina in question. If the researches are extended to all the tsetse species living in the various regions of Africa on very different kinds of fauna, the problem becomes still much more complicated.

## 2. *Dissection of Glossinæ.*

The glossinæ should be dissected directly they are caught, so that the few flies that happen to contain remains of blood may not be allowed to go on digesting it. To begin with, the fly-boys used to bring me live glossinæ. Later on, they killed them as soon as they caught them, crushing the head, so as to stop digestion. If this was done, dissection could be postponed. It could not be left, however, till the following day, as putrefaction would have produced further modifications in the blood found in the gut.

No special dissection technique is required to collect blood from the tsetse-fly. All that is required is three snips with the scissors, two to remove the wings and legs and a third to sever the abdominal extremity. The fly, thus prepared, is placed on a slide on which a drop of normal saline has been placed. Slight pressure with a dissecting needle on the abdominal surface will force out the gut at the posterior extremity. By putting the point of the needle through one of the loops in the intestines the whole gut can be extracted and laid in the drop of normal saline; the gut is easily discernible with the naked eye, when it contains blood, owing to its bright red colour. It is always advisable to continue pressure from the abdominal ring towards the posterior part, so as to force out the sucking stomach, which can also be recognised with the naked eye, by the gas-bubbles it contains; bright blood is sometimes found in the sucking stomach several days after the blood in the gut has been digested.

I carried out experiments to determine how long the blood absorbed by tsetse-flies resists digestive action in the intestine; the results were as follows:

After three days, *non-nucleated* red cells are no longer found in the intestine. Tsetse-flies which have only sucked a little blood digest it rapidly, while those which have absorbed a large quantity (some go on sucking until they burst) naturally take longer to complete the process. The digestion of blood containing nucleated red cells is much slower. In my experiments with clean glossinæ fed on hens, I still found after five days a number of nucleated red cells, some of them surrounded with remnants of cytoplasm tinged with hæmoglobin.

It sometimes happens that blood containing nucleated, or non-nucleated red cells in the sucking stomach of the glossina may retain a very bright red colour such as is not generally found in the intestine. Accordingly, when looking for blood in the tsetse-fly, a point should be made of examining the sucking stomach where exceptionally it may be found even after it has disappeared from the intestine.

The abdominal cavity of all tsetse-flies, particularly the females, contains a large quantity of fat bodies; these, when crushed in the blood, form a milky-looking emulsion which makes it difficult to detect the opaque ring characteristic of precipitin reactions, especially if the latter are not well marked, as is generally the case with those obtained with more or less digested blood from the intestine of tsetse-flies caught in the bush. Before proceeding to dissolve the blood, it is well to remove these

fat bodies from the intestine by dissecting it with two needles or by shaking it gently in a watch-glass or a Petri-dish containing normal saline.

3. *Microscopic Examination of the Blood found in Tsetse-flies.*

Before carrying out the precipitin test with blood occasionally found in tsetse-flies, I always endeavoured, by means of a preliminary microscopic examination, to obtain some information concerning the structure, form and dimensions of the red cells, so as to have a guide as to the anti-sera with which it was best to begin experimenting. The small amount of blood found in occasional glossinæ was not sufficient for many experiments. Being unable to apply to it each one of the anti-sera that I possessed, I needed some criterion for determining which antiserum it was desirable to try first as being most likely to be homologous with the blood in the tsetse.

If, with the naked eye, I saw that the intestine contained blood to which the precipitin test could be applied, I placed it with a dissecting needle or a small pair of forceps in the opening of the small tube in which I proposed to carry out the test. With a dissecting needle I squeezed out the blood into the opening of the tube, and with the same needle with blood still adhering to it I made a minute blood film. Microscopic examination provided valuable data, enabling me to simplify the precipitin test. The form and dimensions of a complete red cell or of a nucleus, if found, indicated the procedure to be followed.

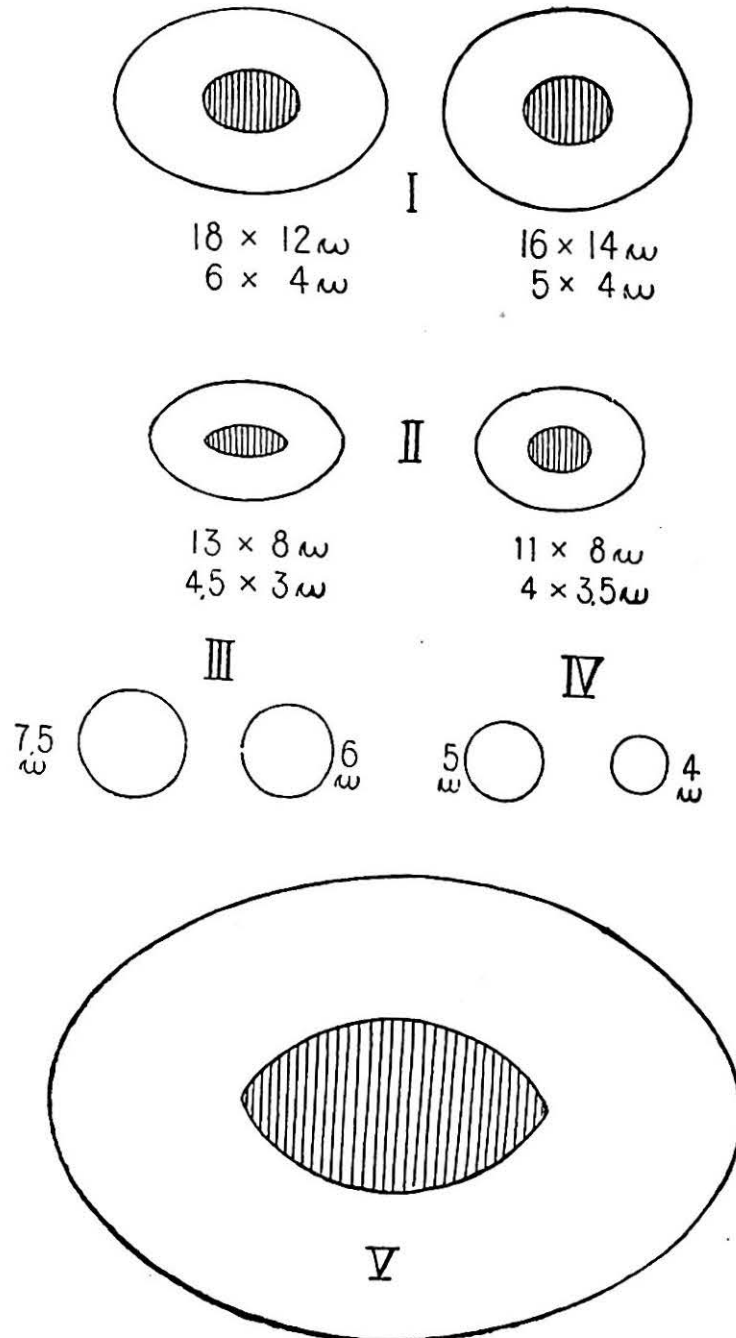


Fig. 3

Showing the relative dimensions of the four blood-cell groups which I established to facilitate the selection of homologous anti-sera.

- I. Group of nucleated red cells of reptiles ;
- II. Group of nucleated red cells of birds ;
- III. Group of non-ruminant mammals ;
- IV. Group of ruminant mammals ;
- V. Shows the relative size of the red cells of the lung fish (*Proptopterus*) ("mamba" in the Uganda tongue), which comes up to the surface of Lake Victoria to breathe, sometimes close to the place where glossinæ are found.

For the purposes of this report, I have drawn up a table of four groups of red cells (*vide* Fig. 3) :

- |                                  |   |  |
|----------------------------------|---|--|
| <i>Nucleated red cells :</i>     | } | I. Approximate average dimensions of the elliptical axes of the cytoplasm and the nucleus respectively : $18 \times 12$ and $6 \times 4 \mu$ .   |
|                                  |   | II. Approximate average dimensions of the elliptical axes of the cytoplasm and the nucleus respectively : $13 \times 8$ and $4.5 \times 3 \mu$ . |
| <i>Non-nucleated red cells :</i> | } | III. Average diameter from $7.5$ to $6 \mu$ .  |
|                                  |   | IV. Average diameter from $5$ to $4 \mu$ .   |

Group I includes the blood of reptiles on which the tsetse-flies were most likely to feed.

Group II includes the blood of birds and certain fishes.

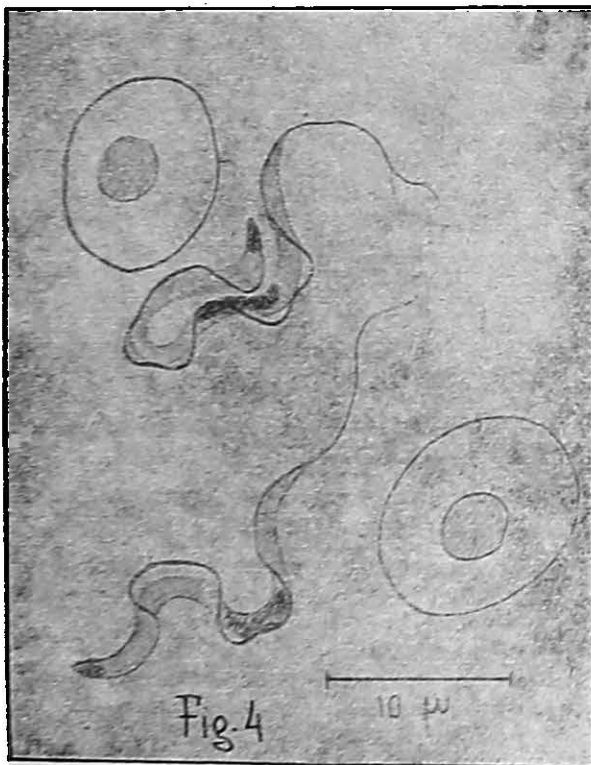
Group III includes the blood of most mammals.

Group IV includes the blood of most ruminant mammals.

Whenever the microscopic examination enabled me to classify the red cells in one or other of these groups, I naturally began by trying the anti-sera of that group when applying the precipitin test. Failing such preliminary information, I ran the risk of using up all the blood obtained from the tsetse-flies with heterologous anti-sera.

In the same figure, I have shown a nucleated red cell of the following huge dimensions:  $46 \times 32$  for the cytoplasm and  $20 \times 12$  for the nucleus. This is the red cell of a large fish, of the genus *Proptopterus*, provided with lungs (*vide* photograph 7), which is sometimes as much as 1 meter 50 in length and has attracted the attention of a number of observers. As this fish comes up to the surface of the water or mud to breathe, on the banks of the lake on which the tsetse-flies live, the question arose as to whether the flies feed on it or not. These red blood corpuscles are not as big across as the canal formed by the upper and lower lips of the glossina proboscis, but are a good deal broader than the hypopharynx ; at the same time, the compressible nature of blood corpuscles enables them to go through passages much narrower than themselves.

These considerations led me to carry out blood examinations on the fish given



Trypanosomes found in a "semutundu", a fish caught in Lake Victoria Nyanza.

to the sleeping-sickness patients in the charge of the Commission. Of the four kinds of fish examined, two, known in the Uganda vernacular as "semutundu" and "malekalende", harboured trypanosomes exhibiting the typical characteristics of fish trypanosomes; this may be seen from Fig. 4, drawn with a camera lucida.

Repeated microscopic examination failed to reveal any trypanosomes in the lung-fish. None of the fish trypanosomes have the glossina as an intermediate host, nor have I ever found in tsetse-flies red blood cells of dimensions in any way approaching those of the "mamba" fish, as it is called in the Uganda vernacular.

#### 4. *Preparation of Solutions of the Blood found in Tsetse-flies.*

After making films of the blood first emulsified in a drop of normal saline to prevent any modification of the red cells and to permit of their classification, I added to the tube containing the tsetse gut about one cubic centimeter of distilled water, which dissolved the red cells. I waited half-an-hour before applying the test to this solution, so that hæmolysis might be complete. The precipitin test has to be carried out in a saline solution; I accordingly added an equal volume of 1.8% sodium-chloride solution, producing a 0.9% dilution.

To begin with, I diluted the blood in the gut of the tsetse-flies straight away with normal saline. It dissolved very slowly. Distilled water hæmolyses and extracts the blood more rapidly, and the addition of an equal volume of 1.8% sodium-chloride solution produced the concentration required for the precipitin test.

It is best to be sparing as regards the quantity of liquid employed for dissolving the blood. I learned from experience that with obviously blood-stained liquids the precipitin reaction is not as clearly marked as the appearance of the solution might lead one to expect. The reaction with the blood from glossinæ experimentally fed by myself on man, goat and a cow was much less well marked than when the "non-glossinated" blood of these was used.

#### 5. *Filtration of Solutions of the Blood found in Tsetse-flies.*

The blood solutions were sometimes clouded by the large amount of intestinal detritus found in glossinæ; this should be got rid of before attempting the test. To clarify the solutions I employed both centrifugalisation and filtration. The former necessitated the loss of a part of the solution, which was difficult to separate from the deposit. If filtration is employed, the filter-paper and the residue can be used to make another solution. NUTTALL recommends, when there is difficulty in clarifying the antigen solutions, that a filter-paper should be soaked in them and then left to dry. If, after drying, the paper is dropped into normal saline, a perfectly clear solution can be obtained. This is why I prefer filtration to centrifugalisation.

As only a very small quantity of solution can be prepared with the blood found in glossinæ, it is important that none be lost during the process of filtration. I employed the following method: I soaked a small paper filter with normal saline and passed through it a solution of blood from a glossina in distilled water. I washed out the tube which had contained this blood with an equal volume of 1.8% sodium-chloride solution, which I then passed through the same filter-paper, thus obtaining a 0.9% solution, sufficiently clear for the purposes of the precipitin test. The filter-paper, when dried, served as a small reserve for use later.

## 6. *Hydrogen-ion Concentration.*

It remained to give the solutions a Ph favourable for the precipitin reaction. When examining solutions of blood extracted from tsetse-flies, I found that, although prepared with water and sodium-chloride solutions with a Ph of 7.5, the Ph was 6.6, the lowest point indicated by the only hydrogen-ion indicator then available (phenol red). It was necessary to add about 0.16 c.c. of N/1000 caustic-soda solution per cubic centimeter of blood solution, to obtain a Ph between 7 and 7.5. In actual practice, for every cubic centimeter of the fly-blood solution, I added three drops of N/1000 soda solution, with a pipette releasing about twenty drops per cubic centimeter. After repeated experiments, I realised the advantages of correction of the hydrogen-ion concentration. Litmus-paper, if sufficiently sensitive, proves fairly satisfactory: the blood solution should be neutral to litmus. Phenolphthalein cannot be used; with solutions of this substance all anti-sera form precipitates: there are no negative precipitin reactions.

## 7. *Concentration of Solutions of Blood extracted from Tsetse-flies.*

After clarifying the blood solution and correcting the hydrogen-ion concentration the tube containing the liquid was shaken. If bubbles remained about thirty seconds, this showed the antigen concentration to be sufficient for the purpose of the test.

## V. METHOD OF APPLYING THE PRECIPITIN TEST TO BLOOD FOUND IN GLOSSINÆ.

### I. *Apparatus and Material (vide photograph 8).*

- (a) Small glass tubes in stand;
- (b) Distilled water and 0.9 and 1.8 % sodium-chloride solutions;
- (c) Phenol red or sensitive litmus;
- (d) Funnels and small filter-papers;
- (e) Capillary pipettes with rubber teats;
- (f) Antigen papers;
- (g) Anti-sera;
- (h) Films and solutions of blood found in tsetse-flies.

(a) The glass tubes should be quite small (about  $7 \times 0.7$  cm.), equal in size and of the same thickness throughout.

When the result of the precipitin test is read by the precipitates deposited, it is best to have tubes with a conical end, so that the sediment may be concentrated and more clearly visible. In applying the precipitin test to the contents of the alimentary canal of glossinæ, I found it best to judge the results solely by the opacity which appears immediately at the level where the anti-serum and the antigen are in contact, irrespective of the precipitate deposited later; the blood extracted from glossinæ generally contains numbers of bacteria, which multiply in the reaction tubes, clouding the solution and producing precipitates which it is difficult to distinguish from specific precipitates. Except when the precipitate is taken as a criterion of results, it is unnecessary to use tubes with a conical bottom: such tubes actually have certain disadvantages. Anti-sera are denser than the antigen solutions generally employed and drop to the bottom of the tubes, so that often it is not easy to detect the faint opacity indicative of a feebly positive precipitin reaction when this forms where the sides of the tube begin to converge to the conical end. Errors due to refraction

may arise. In this study, therefore, in which no attention was paid to the precipitates deposited, I always preferred to use small cylindrical tubes uniformly transparent and rounded at the bottom.

(b) The distilled water or rain-water and the sodium-chloride solutions must be perfectly clear and their hydrogen-ion concentration at the time of use should be between Ph 7 and Ph 7.5.

(c) The phenol red scale may be used as an indicator in determining the hydrogen-ion concentration of the antigen solution. In practice, litmus-paper would answer quite well but for the fact that damp tropical heat may impair its sensitiveness.

(d) The blood extracted from glossinæ contains a quantity of fat bodies and detritus, which must be separated by filtration before adding the anti-serum. Only a small quantity of blood is generally obtained from a tsetse-fly, and the funnels and filter-papers should be proportionately small, so that the liquid may not be wasted by adhering to the paper or the funnel. Before filtering, it is well to moisten the filter-paper with normal saline to prevent the small quantity of blood solution from soaking into it.

(e) There should be plenty of capillary pipettes, one for each anti-serum and antigen solution. A pipette taken out of a highly concentrated solution and put into a less concentrated one, any part of the first still clinging to it, is enough to modify low concentrations such as 1/1000, 1/10,000, 1/20,000.

(f) Antigen papers. — The precipitin test always necessitates the use of a number of control tubes with homologous and heterologous antigen solutions.

If protected from chemical action of any sort, antigens may preserve their precipitin content in the presence of the homologous serum for many years. After tens of centuries, the blood of mummies still reacts specifically with anti-human serum.

Nuttall's process for the preservation of antigens on filter-paper is very practical. When carrying out the test, 2 square centimeters or so of the paper soaked in antigen should be cut up, with forceps and scissors, and dropped into an ordinary test-tube containing 2 to 3 c.c. of distilled water or of quite pure ordinary water. After half-an-hour or an hour, an equal volume of a 1.8% sodium-chloride solution is added and in this way a solution of antigen in normal saline is readily obtained. Some bloods do not dissolve readily in normal saline. It is always simplest and quickest to hæmolyse first in ordinary water and then to add an equal volume of 1.8% sodium-chloride solution.

The tubes containing the antigen paper should not be shaken to make the antigen dissolve more rapidly, as the solution might thus be made turbid with minute particles of the paper.

It is best not to employ too highly concentrated antigen solutions; the froth test can be employed to determine the best concentrations suited to the precipitin reaction.

(g) Before carrying out the test, the anti-serum should be transferred from the ampoule to a small tube with a conical bottom. If, despite precautions, the serum has become clouded by precipitates forming in the ampoules, rapid centrifugalisation will make these precipitates collect in the conical extremity of the tube, and thus facilitate the decantation of the supernatant anti-serum. In the chapter on anti-sera, I have described the measures which should be taken to ensure perfectly clear sera.



(h) It has been shown in the preceding chapter how solutions of the blood found in tsetse-flies should be prepared for the application of the precipitin test under the best possible conditions, and how blood films can be used to determine which anti-sera should be tried first.

## 2. *Technique employed.*

I have described in detail, in the earlier chapters, the method of preparing the reagents and the material and apparatus required to obtain clearly marked reactions when applying the precipitin test to blood found in tsetse-flies. I now propose to give a summary of the method which I adopted, enumerating in order the technical details described above.

The glossinæ were caught and killed by crushing the head; they were dissected immediately, separating the blood in the gut from the fatty bodies and other abdominal viscera: blood films were prepared and examined under the microscope, the red cells being classified according to groups. The blood was dissolved first in ordinary water, an equal volume of a 1.8% sodium-chloride solution being added after an hour; the solution was then passed through a small filter-paper moistened with normal saline. N/1000 soda was used for neutralisation and dilutions of blood higher than 1/1000 were prepared, taking advantage of the froth test (bubbles lasting at least thirty seconds).

A similar method was employed in preparing the antigen solutions; about 2 square centimeters of filter-paper that had been soaked in homologous antigen and of two other papers soaked in heterologous antigens of the anti-serum to be tested; these pieces of paper were placed in an ordinary tube containing from 2 to 3 c.c. of water; an equal volume of a 1.8% sodium-chloride solution being added after half-an-hour or an hour. N/1000 caustic-soda solution was employed for neutralisation. The liquid was then separated from the paper and the froth test applied, concentrations over 1/1000 producing bubbles lasting at least thirty seconds.

The anti-serum was then carefully decanted from the ampoules into small sterilised conical tubes in the following manner. The shorter end of the ampoule, which is uppermost in the box, was carefully cut across, and held over the open end of the conical tube. The longer end of the ampoule, the one that has been kept downwards, was next removed to eliminate the precipitates accumulated in it. The serum drops into the conical tube through the shorter clean end of the ampoule without bringing with it any of the deposit that has accumulated in the other extremity (*vide* Fig. 2, II). If, despite these precautions the serum was cloudy, it was centrifuged.

Five small tubes of the type described in the chapter on apparatus were then placed in a stand. Into Tube I, about 0.5 c.c. of the tsetse blood solution to be identified was dropped with a pipette; into Tube II an equal volume antigen solution homologous with the anti-serum about to be employed; and into Tubes III and IV, solutions of heterologous antigens, for control purposes. Tube V contained normal saline.

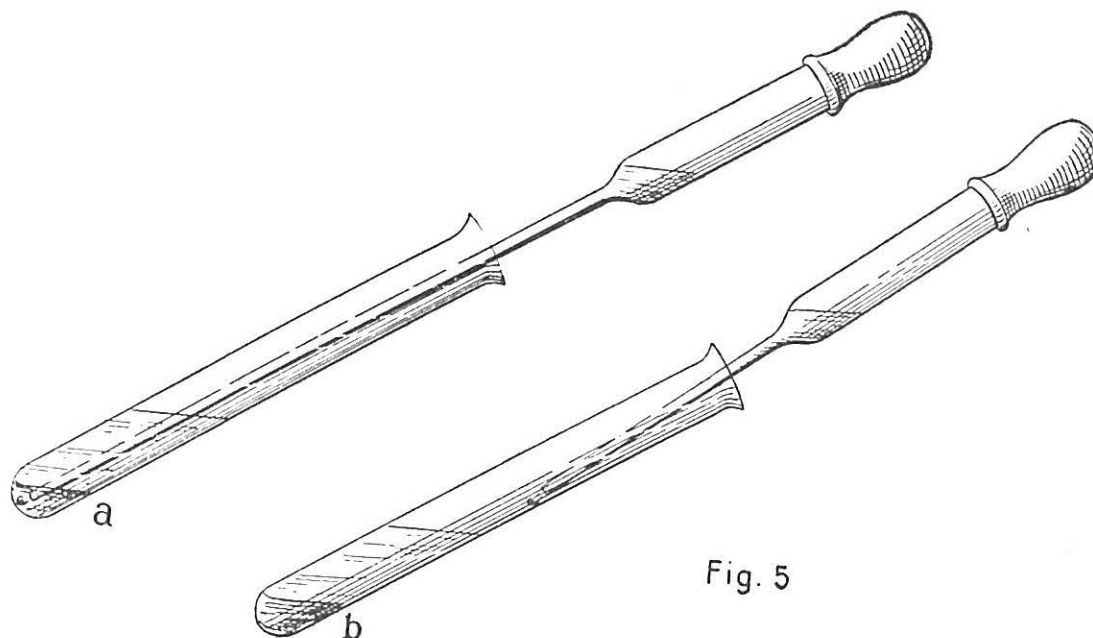
To each of the tubes about 0.1 c.c. of the recently titrated anti-serum considered after microscopic examination of the red cells in question most likely to be homologous was added.

Those who prefer to read the results of the precipitin test by the precipitates deposited add the anti-serum to the antigen solutions, shake, and leave the precipitates for about twenty-four hours to settle. I have explained why, in applying the precipitin test to blood from the gut of glossinæ, it is safer to judge the result not by the precipitates ultimately deposited but by the opaque ring which forms at the junction of serum and antigen soon after the two liquids have come into contact with one another.



In order that the line of separation of these two liquids may be clearly seen the anti-serum should be at the bottom of the tube below the antigen solution without mixing with it.

One method recommended for this purpose is to incline the tube containing the antigen solution and to allow 0.1 c.c. of anti-serum to flow slowly along its side; the serum, being denser, will flow to the bottom of the tube and form a layer distinctly separated from the antigen above it (*vide* Fig. 5, *b*).



Methods recommended for pouring the anti-serum over the antigen solution, so as to separate the two liquids distinctly :

*a.* As in the nitric acid-albumin reaction, introduce the anti-serum carefully so that it forms a layer under the solution.

*b.* Incline the tube at an angle which will allow the anti-serum to flow along its sides.

I frequently found in my experiments that the anti-serum did not sink sufficiently slowly. Only a very small quantity of these reagents is available, so that small narrow tubes should be employed for the test. The anti-serum deposited at the opening of the tubes is sometimes retained there by capillary attraction, and, when it eventually drops, does so so suddenly that it mixes with the antigen solution, so that the two liquids are not distinctly separated, as is essential for an absolutely definite precipitin reaction.

To avoid any risk of wasting these solutions, it is best to deposit the anti-serum at the bottom of the tube by means of a pipette with a fairly long capillary end, instead of allowing it to flow by its own weight down the sides of the tube (*vide* Fig. 5, *a*). By squeezing the teat of the pipette gently the two liquids can be clearly separated, provided that the pipette is not completely emptied as this would release little air-bubbles which would penetrate the anti-serum and antigen and cause them to mix. This is immaterial if the reaction is to be determined by the precipitates deposited during the first twenty-four hours, but it makes it impossible to detect the opacity formed at the level of separation if the reactions be positive, the only criterion that I would recommend in applying the precipitin test to blood found in the alimentary canal of tsetse-flies. This blood generally contains bacteria which multiply quickly in serum solutions,

with characteristic turbidity which makes it difficult to appreciate the results of a feebly positive precipitin reaction, for example, reactions obtained with blood which has been acted upon by digestive ferments in a glossina for any length of time.

The precipitin test should be carried out at laboratory temperature and not at incubator temperature ; the reaction should be manifest soon after the anti-serum and the antigen come into contact, that is, from two to five minutes in tubes I and II. The reaction is characterised by the appearance of a whitish opacity, in the form of a fine ring, which increases during the first ten or fifteen minutes, and is subsequently deposited at the bottom of the tube in the form of a flocculent precipitate.

In order that this precipitin ring may be more easily seen, it is well to place the tubes against a dark background, and the light should descend obliquely between the tubes and this background, on the principle employed in microscopic examinations by dark ground illumination. This makes the faint rings produced by feebly marked precipitin reactions more clearly visible.

### 3. *Interpretation of Results.*

Tube I, if the blood under examination is homologous with the anti-serum employed, should show a specific opaque ring.

Tube II, containing the homologous anti-serum and antigen, should always exhibit definite opacity.

Tubes III and IV, with heterologous antigens, should not exhibit the opacity of specific precipitates.

Lastly, tube V, containing normal saline and anti-serum only, should not show any opacity.

Anti-sera do not preserve their activity indefinitely, though certain investigators have noted cases in which they appear to increase in activity as time goes on, instead of decreasing, as is generally the case. It is advisable, therefore, to titrate anti-sera systematically every time they are employed for a test, or at all events to place in a control tube a dilution of the antigen corresponding to the highest titre last noted, as this will furnish evidence whether the precipitin content is still the same.

This control tube, containing the homologous anti-serum and antigen in the highest dilution permitting a clearly marked positive reaction, should be prepared first. If the precipitin content of the anti-serum is found to have changed, it must be retitrated before carrying out the test.

If no opacity is visible in tubes III and IV it is a proof that the precipitate rings in tubes I and II are due to the specific action of the anti-serum.

Certain anti-sera become cloudy when brought into contact with water or even with the normal salt solution and should be rejected. I had to give up using one of my anti-hippopotamus sera on that account (No. 21, page 202).

I have often received on the same day a number of tsetse-flies containing blood which gave a positive reaction with the same anti-serum. For example, when a crocodile had remained on the previous day in a place where it was exposed to the bites of numbers of these flies, it was not surprising that, among the flies caught, the nucleated red cells of Group I should predominate and repeated positive reactions be obtained with anti-crocodile serum.

If one anti-serum was to be applied the same day to different blood specimens which looked the same under the microscope, it was not considered necessary to repeat control tubes II, III, IV and V, as will be seen from the table below in which I have summed up the results obtained by me with the precipitin test applied to blood found in the alimentary canal of the *Glossina palpalis* in the neighbourhood of the Entebbe Laboratory.

#### 4. *Presentation of Results of Precipitin Tests.*

If the reaction was only feebly positive, I always tried to intensify it and show it up more clearly.

When the anti-serum was in contact with antigen, I inclined the tube at a considerable angle to increase the surface of contact of the two liquids, which do not mix. After twenty or thirty seconds, I turned the tube in a different direction, at an angle of 90 degrees with the first. After waiting a further twenty or thirty seconds, I placed the tube in a vertical position. The light precipitates formed over a wider surface of contact, when the tube was inclined, became concentrated on a smaller surface when it was placed upright again. In this way, I managed to detect feeble or indefinite precipitin reactions, which would otherwise have passed unobserved.

#### 5. *Houser's Capillary Method.*

This method has one advantage, namely, that a smaller quantity of the various substances is required for the test, a point which should, in my view, be taken into consideration, since only a very small quantity of blood is found as a rule in tsetse-flies caught in the bush. It differs from other methods mainly as regards the diameter of the tubes and the way in which the reagents float one above the other. The tubes, which are quite easy to prepare over a flame, should be about 6 cm. long and 2 mm. in diameter. A capillary pipette is employed to half-fill the tube with anti-serum and above this serum is carefully placed the antigen to be identified or used for control.

Otherwise, the capillary method is very like Uhlenhut's, by which it is undoubtedly easier to obtain a clear line of demarcation between the anti-serum and the antigen, a most important point in the technique of the precipitin test.

Even with this classic method, however, it is necessary to tilt the test-tubes, in order to increase the surface of contact between the anti-serum and the antigen for the purpose of showing up feebly positive precipitin reactions, for example, the reactions obtained with blood found in tsetse-flies which is totally or partially modified by the action of the digestive ferments in the fly. Houser's capillary method is only satisfactory with more definitely positive precipitin reactions.

For the same reasons, I preferred Uhlenhut's recognised method to the microscopic method, which is as follows: instead of mixing in the tubes the anti-serum and the antigens to be identified or to be used for purposes of control, the reagents are mixed in a hanging-drop preparation. The results of the precipitin test are read under the microscope, as in agglutination tests.

#### 6. *Tabulation of Results of Precipitin Tests.*

In the following table, I have condensed the results of the precipitin tests applied to tsetse-flies, showing the date on which the flies were caught, the number of glossinæ per capture, the number of glossinæ containing blood, the microscopic classification of the red blood cells, the number of times (one, two or three) the precipitin test was applied to each specimen of blood found, with the serial numbers of the anti-sera employed in these tests, and the results obtained with solutions made with the blood extracted from tsetse-flies and with control antigens; lastly, I have noted a number of micro-organisms in blood found in the digestive canal of the tsetse-flies in question.

At the end of the table is a note explaining the abbreviations employed. The results shown in this table are summed up in another table, in which I have enumerated, in order of preparation, all the sera the precipitin content of which on titration was found to be sufficiently high to warrant its use (*vide* page 220).

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PRECIPITIN REACTIONS

Date of capture	Number of tsetse-flies per capture	No. of tsetse-fly containing blood	Red blood cell groups	FIRST			SECOND			THIRD			Micro-organisms found in gut of tsetse-flies
				No. of serum employed	Results with		No. of serum employed	Results with		No. of serum employed	Results with		
					Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly	
10-8-26	50	1	III	11	P.2	—	20	P.2	—	2	P.2	—	
		2	I	11a	P.2	—	12	P.3	—	9	P.1	—	<i>T. grayi</i>
		3	I	11a	P.2	—	12	P.3	—	9	P.1	—	
14-8-26	50	4	I	11a	P.2	—	12	P.3	—	9	P.1	—	
		5	I	11a	P.2	—	12	P.3	—	9	P.1	—	<i>T. grayi</i>
		6	I	11a	P.2	—	12	P.3	—	9	P.1	—	
		7	III	11	P.2	—	20	P.2	—	1	P.2	—	
20-8-26	50	8	I	11a	P.2	—	12	P.3	—				<i>Hæmogregarinæ</i>
		9	III	13	P.3	P.1	3a	P.2	—				
		10	I	11a	P.2	—	12	P.3	—	9	P.1	—	
		11	I	11a	P.2	—	9	P.1	—				<i>Bacil. long.</i>
		12	I	11a	P.2	—	9	P.1	—				<i>T. grayi</i>
14-9-26	50	13	I	11a	P.2	—	9	P.1	—				<i>T. grayi</i>
		14	II	29	P.2	—	12	P.3	—	11a	P.2	—	
		15	II	29	P.2	—	12	P.3	—	—	—	—	
24-9-26	50	16	I	11a	P.2	—	12	P.3	—	9	P.1	—	<i>Bacil. long.</i>
		17	I	11a	P.2	—	12	P.3	—	9	P.1	—	
6-10-26	50	18	I	11a	P.2	—	12	P.3	—				
		19	I	11a	P.2	P.1	12	P.3	—	13	P.3	—	<i>T. grayi</i>
		20	I	11a	P.2	—	12	P.3	—	9	P.1	—	<i>T. grayi</i>
		21	III	13	P.3	P.2	20	P.2	—				
8-10-26	50	22	I	11a	P.2	—	9	P.2	—				
		23	III	13	P.3	—	20	P.2	—	2	P.3	—	
		24	III	13	P.3	P.2	20	P.2	—				
		25	I	11a	P.2	—	9	P.1	—	12	P.3	—	<i>T. grayi</i>
		26	III	13	P.3	—	20	P.2	—	2	P.3	—	<i>T. grayi</i>
12-10-26	50	27	I	11a	P.2	—	9	P.1	—	29	P.2	—	
		28	I	11a	P.2	—	9	P.1	—				
		29	III	13	P.3	—	20	P.2	—	25	P.3	P.1	
		30	III	13	P.3	—	20	P.2	—	2	P.3	—	<i>Bacil. long.</i>
		31	III	13	P.3	—	20	P.2	—	25	P.3	—	<i>Bacil. long.</i>
		32	III	13	P.3	—	25	P.3	—				
15-10-26	50	33	I	11a	P.2	—	9	P.1	—	29	P.2	—	
		34	I	11a	P.2	—	9	P.1	—	29	P.2	—	<i>T. grayi</i>
		35	III	13	P.3	—	25	P.3	—	3b	P.2	—	
		36	III	13	P.3	—	25	P.3	—	3b	P.2	—	
		37	III	13	P.3	—	25	P.3	—	3b	P.2	—	
		38	I	11a	P.2	—	9	P.1	—	29	P.2	—	<i>T. grayi</i>

PRECIPITIN REACTIONS (continued)

Date of capture	Number of tsetse-flies per capture	No. of tsetse-fly containing blood	Red blood cell groups	FIRST			SECOND			THIRD			Micro-organisms found in gut of tsetse-flies
				No. of serum employed	Results with		No. of serum employed	Results with		No. of serum employed	Results with		
					Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly	
15-10-26	50	39	I	11a	P.2	—	9	P.1	—	29	P.2	—	Bacil. long.
		40	I	11a	P.2	P.1	12	P.3	—	13	P.3	—	Hæmogregarinæ
		41	I	11a	P.2	—	9	P.1	—	29	P.2	—	<i>T. grayi</i>
21-10-26	50	42	I	27a	P.3	—	9	P.1	—				
		43	I	27a	P.3	—	9	P.1	—				
		44	III	13	P.3	—	25	P.3	P.2				
		45	I	27a	P.3	P.1	9	P.1	—	27	P.3	—	
		46	I	27a	P.3	P.2	9	P.1	—	27	P.3	—	
		47	I	27a	P.3	—	9	P.1	—	29	P.2	—	<i>T. grayi</i>
25-10-26	50	48	II	12	P.3	—	29	P.2	P.1	27a	P.3	—	
		49	I	27a	P.3	—	9	P.1	—			—	Hæmogregarinæ
		50	III	13	P.3	—	30	P.2	—	3b	P.2	—	
		51	I	27a	P.3	—	9	P.1	—				
27-10-26	40	52	I	27a	P.3	P.1	9	P.1	—	27	P.3	—	Bacil. long.
		53	I	27a	P.3	—	9	P.1	—	29	P.2	—	<i>T. grayi</i>
30-10-26	60	54	I	27a	P.3	—	12	P.3	—				
		55	IV	13	P.3	—	3b	P.2	—	27	P.3	—	
		56	IV	13	P.3	—	3b	P.2	P.1	27	P.3	—	
		57	III	13	P.3	—	30	P.2	—				
		58	I	27a	P.3	—	12	P.3	—				<i>T. grayi</i>
		59	III	13	P.2	—	25	P.3	—	3b	P.2	—	
4-11-26	50	61	I	27a	P.3	—	36	P.3	—	29	P.2	—	
		62	III	13	P.2	—	25	P.3	—	30	P.2	—	
		63	I	27a	P.3	—	36	P.3	—	29	P.2	—	<i>T. grayi</i>
11-11-26	50	64	I	27a	P.3	—	36	P.3	—				
		65	IV	13	P.2	—	25	P.3	—	3b	P.2	—	
		66	I	27a	P.3	—	36	P.3	—	29	P.2	—	
16-11-26	50	67	I	27a	P.3	—	36	P.3	—				
		68	I	27a	P.3	—	36	P.3	—				<i>T. grayi</i> Hæmogregarinæ
		69	III	13	P.2	—	25	P.3	—				
		70	III	13	P.2	—	25	P.3	—	3b	P.2	—	
		71	III	13	P.2	—	25	P.3	P.2				Bacil. long
		72	III	13	P.2	—	25	P.3	P.2				
		73	I	27a	P.3	—	36	P.3	—	29	P.2	—	<i>T. grayi</i>
		74	I	27a	P.3	—	36	P.3	—	29	P.2	—	<i>T. grayi</i>

PRECIPITIN REACTIONS (continued)

Date of capture	Number of tsetse-flies per capture	No. of tsetse-fly containing blood	Red blood cell groups	FIRST			SECOND			THIRD			Micro-organisms found in gut of tsetse-flies.
				No. of serum employed	Results with		No. of serum employed	Results with		No. of serum employed	Results with		
					Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly	
22-11-26	50	75	III	13	P.2	—	25	P.3	—	3b	P.2	—	
		76	I	27a	P.3	—							
24-11-27	50	77	I	27a	P.3	P.1	36	P.3	—	27	P.3	—	
		78	I	27a	P.3	—	36	P.3	—	29	P.2	—	
		79	I	27a	P.3	P.1	36	P.3	—	27	P.3	—	
		80	I	27a	P.3	P.2	36	P.3	—	27	P.3	—	
28-11-26	50	81	I	27a	P.3	—	36	P.3	—	29	P.2	—	Hæmogregarinæ
		82	I	27a	P.3	—	36	P.3	—				Bacil. long.
		83	III	24	P.3	—	25	P.3	—	3b	P.2	—	Bacil. long.
2-12-26	50	84	I	27a	P.3	—	36	P.3	—	29	P.2	—	
		85	III	24	P.3	—	25	P.3	—	10	P.2	—	<i>T. grayi</i>
		86	III	24	P.3	—	25	P.3	P.1				
		87	I	27a	P.3	—	36	P.3	—	29	P.2	—	
7-12-26	50	88	I	27a	P.3	P.1	36	P.3	—	27	P.3	—	
		89	I	27a	P.3	P.1	36	P.3	—	27	P.3	—	
		90	I	27a	P.3	—	36	P.3	—				
		91	I	27a	P.3	—	36	P.3	—	29	P.2	—	
		92	I	27a	P.3	P.1	36	P.3	—	27	P.3	—	
10-12-26	50	93	I	46	P.2	—	36	P.3	—				
		94	I	46	P.2	—	36	P.3	—	29	P.2	—	
		95	II	12	P.3	—	36	P.3	—	29	P.2	—	
		96	I	36	P.2	—	36	P.3	—	29	P.2	—	<i>T. grayi</i>
		97	I	36	P.2	—	36	P.3	—				
		98	II	12	P.3	—	36	P.3	—	29	P.2	—	
		99	III	24	P.3	—	25	P.3	—	3b	P.2	—	
15-12-26	50	100	I	46	P.2	—	36	P.3	—	29	P.2	—	
		101	I	46	P.2	P.1	36	P.3	—				
		102	I	46	P.2	—	36	P.3	—	29	P.2	—	Bacil. long.
		103	III	24	P.3	—	25	P.3	—	7	P.2	—	
		104	I	46	P.2	P.1	36	P.3	—				
17-12-26	30	105	I	46	P.2	—	36	P.3	—				Bacil. long.
		106	I	46	P.2	—	36	P.3	—	29	P.2	—	
19-12-26	45	107	I	46	P.2	—	36	P.3	—				
		108	I	46	P.2	—	36	P.3	—				
23-12-26	50	109	IV	3a	P.2	P.1	27	P.3	—	39	P.3	—	
		110	IV	3b	P.2	—	27	P.3	—	39	P.3	—	
		111	I	46	P.2	—	36	P.3	—				
27-12-26	50	112	I	46	P.2	—	36	P.3	—				
		113	I	46	P.2	—	36	P.3	—				<i>T. grayi</i>

PRECIPITIN REACTIONS (continued).

Date of capture	Number of tsetse-flies per capture	No. of tsetse-fly containing blood	Red blood cell groups	FIRST			SECOND			THIRD			Micro-organisms found in gut of tsetse-flies
				No. of serum employed	Results with		No. of serum employed	Results with		No. of serum employed	Results with		
					Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly	
27-12-26	50	114	I	46	P.2	—	36	P.3	—				<i>T. grayi</i>
		115	IV	39	P.3	—	3b	P.2	P.1	27	P.3	—	
		116	III	24	P.3	—	3b	P.2	—				
29-12-26	50	117	I	46	P.2	—	36	P.3	—				
		118	IV	3a	P.2	—	39	P.3	—	27	P.3	P.1	
		119	III	24	P.3	—	27	P.3	—				
		120	III	24	P.3	—	25	P.3	—				Bacil. long.
4-1-27	35	121	I	46	P.2	—	36	P.3	—				<i>T. grayi</i>
		122	I	46	P.2	P.1	36	P.3	—				Hæmogregarinæ
		123	I	46	P.2	—	36	P.3	—				Bacil. long.
8-1-27	40	124	I	46	P.2	—	36	P.3	—				<i>T. grayi</i>
		125	I	46	P.2	—	36	P.3	—				
		126	I	46	P.2	—	36	P.3	—				Bacil. long.
12-1-27	50	127	I	46	P.2	—	36	P.3	—				<i>T. grayi</i>
		128	I	46	P.2	—	36	P.3	—				<i>T. grayi</i>
		129	I	46	P.2	—	36	P.3	—				
		130	IV	39	P.3	—	3b	P.2	—	7	P.2	—	
		131	III	24	P.3	P.3	25	P.3	—	+		+	Bacil. long.
		132	I	46	P.2	—	36	P.3	—				Bacil. long.
18-1-27	50	133	I	46	P.2	—							<i>T. grayi</i>
		134	IV	39	P.3	P.1?	3b	P.2	—	7	P.2	—	
		135	I	46	P.2	—	12	P.3	—				
		136	I	46	P.2	—	12	P.3	—				
25-1-27	50	137	I	46	P.2	—							Bacil. long.
		138	I	46	P.2	—							
		139	I	46	P.2	—							<i>T. grayi</i>
1-2-27	50	140	I	46	P.2	P.1							Hæmogregarinæ
		141	I	46	P.2	P.1							
		142	I	46	P.2	P.1							
		143	I	46	P.2	P.1							
6-2-27	35	144	I	46	P.2	—	51	P.3	—				
		145	I	46	P.2	—	51	P.3	—				Hæmogregarinæ
		146	III	24	P.3	P.2	25	P.3	—				
		147	III	24	P.3	P.3	25	P.3	—				
7-2-27	60	148	III	24	P.3	—	25	P.3	—	3b	P.2	—	
		149	I	46	P.2	—	51	P.3	—				<i>T. grayi</i>
		150	IV	39	P.3	—	3b	P.2	—	10	P.2	—	
		151	I	46	P.2	—	51	P.3	—				
		152	IV	39	P.3	—	3a	P.2	—	10	P.2	—	

PRECIPITIN REACTIONS (conclusion).

Date of capture	Number of tsetse-flies per capture	No. of tsetse-fly containing blood	Red blood cell groups	FIRST			SECOND			THIRD			Micro-organisms found in gut of tsetse-flies
				No. of serum employed	Results with		No. of serum employed	Results with		No. of serum employed	Results with		
					Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly		Homologous antigens	Blood from tsetse-fly	
7-2-27		153	I	46	P.2	—	51	P.3	—				<i>T. grayi</i>
		154	I	46	P.2	—	51	P.3	—				
9-2-27	50	154	I	46	P.2	—	51	P.3	—				
		155	I	46	P.2	—	51	P.3	—				
		156	I	46	P.2	—	51	P.3	—				
		157	I	46	P.2	—	51	P.3	—				
		158	I	46	P.1	—	51	P.3	—				
12-2-27	50	159	I	46	P.2	—	51	P.3	—				
		160	I	46	P.2	—	51	P.3	—				Hæmogregarinæ
		161	III	54	P.3	—	24	P.3	—				
15-2-27	40	162	I	46	P.2	—	51	P.3	P.2				
		163	I	46	P.2	P.1	51	P.3	P.2				Bacil. long.
		164	I	46	P.2	P.1	51	P.3	P.2				
		165	IV	39	P.3	—	3a	P.2	—	7	P.2	—	
23-2-27	50	166	I	46	P.2	—	51	P.3	—				<i>T. grayi</i>
		167	I	46	P.2	—	51	P.3	—				
		168	III	24	P.3	—	25	P.3	—	54	P.3	—	
		169	III	24	P.3	—	25	P.3	—	—	P.3	—	
17-3-27	45	170	I	47	P.2	—	51	P.3	P.1				
		171	I	47	P.2	—	51	P.3	P.1				<i>T. grayi</i>
		172	I	47	P.2	—	51	P.3	—				
		173	III	54	P.3	—	24	P.3	—				
28-3-27	30	174	I	47	P.2	—	51	P.3	—				<i>T. grayi</i>
		175	I	47	P.2	—	51	P.3	P.1				<i>T. grayi</i>
		176	I	47	P.2	—	51	P.3	—				<i>T. grayi</i>
9-4-27	50	177	I	47	P.2	—	51	P.3	—				
		178	III	54	P.3	—	56	P.2	—	30	P.2	—	Bacil. long.
		179	I	47	P.2	—	51	P.3	P.1				
11-4-27	40	180	I	47	P.2	—	51	P.3	P.1				<i>T. grayi</i>
		181	I	47	P.2	—	51	P.3	—				
		182	I	47	P.2	—	52	P.2	—				
14-4-27	50	183	I	47	P.2	P.1	52	P.2	—				
		184	III	54	P.3	—	56	P.2	—	3a	P.2	—	Bacil. long.
		185	I	47	P.2	—	52	P.2	—				<i>T. grayi</i>
		186	I	47	P.2	—	52	P.2	—				

NOTE. — Red cell groups: I = reptiles; II = birds; III = non-ruminant mammals; IV = ruminants.  
 Nos. of anti-sera, *vide* table on following page.  
 P. 1 = feebly positive; P. 2 = positive; P. 3 = strongly positive.  
 — = negative precipitin reaction with blood found in glossinæ.



SUMMARY.

Glossinæ dissected . . . . .	2150
Glossinæ found, on microscopic examination, to contain blood . . . . .	186
Glossinæ found, on microscopic examination, to contain blood (percentage) . . . . .	8.6
Glossinæ containing blood of Group I (reptiles) . . . . .	127 = 68.3%
Glossinæ containing blood of Group II (birds) . . . . .	5 = 2.7%
Glossinæ containing blood of Group III (non-ruminant mammals) . . . . .	42 = 22.5%
Glossinæ containing blood of Group IV (ruminants) . . . . .	12 = 6.5%
	<hr/> 186 = 100.0

APPLICATION OF PREPARED ANTI-SERA

Nos. of anti-serum employed	Number of times employed	Number of positive precipitin reactions obtained with each serum	Percentage
1 Ox-sheep	1	0	0
2 Ox	4	0	0
3 Ox	0	0	0
7 Goat	4	0	0
9 Python	30	0	0
10 Sheep	3	0	0
11 Man	2	0	0
11a Man-monitor	26	2	7.6
12 Hen	23	0	0
13 Man	29	3	10
20 <i>Phacochærus</i>	9	0	0
24 Man	14	3	21
25 Hippopotamus	28	4	14
3a { Ox Sheep } Goat }	6	1?	16?
3b { Ox Sheep } Goat } Antelope (bushbuck) }	19	2	10
27 Bushbuck	17	1?	5?
28 Monkey	0	0	0
29 { Hen Water-birds }	24	1?	4?
30 Dog	4	0	0
27a Bushbuck-monitor	35	10	28
36 Hen-osprey	52	0	0
39 Situtunga (antelope)	9	1?	11?
46 Monitor	44	8	18
47 Monitor	14	1	7
51 Crocodile	27	6	22
52 Crocodile	4	0	0
53 Sheep	0	0	0
54 Wild cat	6	0	0
55 Wild cat	0	0	0
56 Elephant	2	0	0
		<hr/> 43	

VI. OTHER CRITERIA WHICH MAY BE OF ASSISTANCE IN THE IDENTIFICATION OF THE BLOOD FOUND IN TSETSE-FLIES.

1. We have shown what a useful adjunct the *microscopic examination of the red cells* may be in selecting the anti-serum most likely to be homologous.

2. The *crystallographical system* in which blood pigments precipitate varies in different zoological species and may also furnish certain indications.

3. *Anaphylactic phenomena*, regarded as valuable by certain investigators, are not very practical, owing, among other things, to the large number of guinea-pigs that have to be sacrificed.

4. Examination should be made for the *co-existence of hæmoparasites*. The above table and another of my studies on the *T. grayi* lead me to believe that this flagellate is associated with the blood containing Group I red cells (reptiles). I am not quite so sure, though it is extremely probable, that there is also an association of blood of this type with hæmogregarines, either free or encysted, in the intestine of tsetse-flies.

5. I also tried the *miostagmin* and the *epiphanin tests*, with improvised apparatus, using both homologous and heterologous antigens. Perhaps owing to the inadequacy of the material, the results were not always in accordance with the actual facts.

6. *Complement fixation test*. — The Commission's programme covered only the application of the precipitin test to blood found in the alimentary canal of glossinæ. In view, however, of the difficulty of preventing the inhibitive effect of digestive ferments on these reactions—already noted by NUTTALL and other investigators—I tried immunising rabbit 53 with washed sheep's red blood cells, in order to obtain a hæmolytic serum for the complement fixation test.

The technique is the same as for the Wassermann reaction, except that, in the latter, the antigen is known and the anti-bodies of the syphilitic sera have to be determined, whereas, in the fixation test for identifying blood, the anti-bodies are prepared and known beforehand, and it is the antigen that has to be determined by complement deviation.

The fixation test for identifying blood involves a more delicate and more complex technique than the precipitin test and requires all the apparatus employed for Wassermann reactions, with electrical centrifuge, ice-chest, etc., in proper working order.

I succeeded, not without difficulty, in carrying out some experiments with known and unknown antigens. The results seemed to me reliable, even when precipitin reactions ceased to be well-marked owing to turbidity of antigen solutions or the prolonged action of digestive ferments which the blood had undergone in tsetse-flies.

## VII. COMMENTS.

The bush on the shores of Lake Victoria, in the neighbourhood of Entebbe, where the glossinæ for the purposes of this study were caught, is frequented by tsetse-flies, crocodiles and monitors, large African lizards. It is not surprising, then, that the blood found in the flies which I dissected exhibited a high percentage (71 per cent) of nucleated red cells, belonging mostly (68.3 per cent) to Group I (reptiles).

Nucleated red cells have been proved by experiments to offer greater resistance than non-nucleated red cells to digestive action in glossinæ. When the latter contain blood with nucleated red cells, microscopic examination generally reveals certain facts which will probably be of assistance in selecting the homologous anti-serum, whereas non-nucleated red cells, whose form and dimensions are more easily modified by digestive action, give no definite indications for making a choice out of the few anti-sera that can be tried with the small quantity of blood found in glossinæ.

The fact that non-nucleated red cells are more liable to be modified and the corresponding difficulty in selecting the homologous anti-sera probably partly account for the low percentage of glossinæ containing non-nucleated blood and the correspondingly small number of positive precipitin reactions obtained in such cases, shown in the above table.

Next in decreasing order of frequency appear the nucleated red cells of Group III (22.5 per cent), with which anti-hippopotamus and anti-human sera gave a relatively high proportion of positive reactions.

The hippopotamus, which as a rule only leaves the water at night to feed, nevertheless spends its days close to the shore, within reach of tsetse-flies. The percentage frequency of hippopotamus blood in the table is therefore not surprising.

Human beings, however, go very little into the bush where the fly-boys catch the glossinæ. It may be assumed, then, that it is the blood of the fly-boys themselves that gives the positive reactions obtained with anti-human serum. With this anti-serum I obtained a number of positive precipitin reactions with the blood extracted from tsetse-flies and these reactions were as markedly positive as those given by the homologous antigen. The flies were probably caught just as they were biting the boys. The blood, which had only just been drawn and had barely undergone the action of digestive ferments in the fly, still preserved all its antigen properties intact and could thus react strongly to the homologous anti-serum.

The fly-boys select a clearing in the bush at the water's edge. It is not worth while going into the thick bush with its tangle of creepers to catch tsetse, for they soon come swarming about the fly-boys and settle on them, trying to bite any part of their bodies that happens to be exposed.

The bite of a tsetse-fly, which is very like that of the *Stomoxys* fly, may pass unnoticed at first, unless close to the extremity of a nerve, and not be felt until it begins to itch and the glossina has sucked a fair amount of blood.

Wishing to try the precipitin test with known elements (anti-human serum, with human blood extracted from glossinæ during successive and specific stages of digestion), I decided to apply fly-boxes containing a few tsetse-flies to myself. I sometimes found more swellings than there were flies, though I had not felt the bites.

For several days, however, the itching reminded me rather unpleasantly of the bites.

When at work the fly-boys, keep an eye on one another, but the tsetse-flies may rest on them without being seen and begin to suck blood without their noticing it at once. This probably explains why in many of the boxes received I found such a high proportion of glossinæ with blood belonging to Group III, some of which produced abundant precipitates with anti-human serum.

As may be seen from the above tables, I was not successful in the majority of cases in finding sera which gave a positive precipitin reaction with blood found in glossinæ. These negative results must be due to the fact that I did not employ homologous anti-sera, or that, if I did, some inhibitive action inherent in the digestive juices of the fly prevented the precipitin reactions from being well marked.

Obviously, the fact that I obtained more positive precipitin reactions with certain anti-sera does not necessarily imply that the tsetse prefer those particular animals; it simply means that I had greater facilities for preparing and employing these anti-sera, or was better able to select sera homologous to the blood found in the glossinæ.

To determine which animals the tsetse-flies prefer, it would be necessary to apply to all the different specimens of blood found in the alimentary canal of these flies the anti-sera prepared with the blood of all animals that they might have fed upon; such an experiment is impossible on practical grounds: it would be difficult to obtain the blood of all these animals, which vary in different regions, with the different species of glossinæ, and again, even if anti-sera could be prepared with the antigens of all these animals, the small quantity of blood generally found in the glossinæ would be insufficient for a series of experiments with so many anti-sera.

I decided, therefore, in this study simply to examine the reaction of the blood found in *Glossina palpalis* in the neighbourhood of Entebbe<sup>1</sup> with the anti-sera which I had succeeded in preparing and which are noted in the tables on pages 216-221.

The precipitin test opens up a vast field of investigation, covering not only glossinæ but also mosquitoes, ticks and, generally speaking, all blood-sucking arthropods.

My study simply represents a small contribution to the solution of a vaster problem, namely, to determine the animals whose blood the tsetse-flies prefer, with a view to discovering which of these animals constitute natural reservoirs of the virus of trypanosomiasis.

### VIII. CONCLUSIONS.

1. When applying the precipitin test to blood found in the alimentary canal of *G. palpalis* caught on the banks of Lake Victoria, near the Entebbe Laboratory, I found, out of 2,150 tsetse-flies dissected, 186 (8.6 per cent) containing blood. With these 186 flies I obtained 43 (23 per cent) positive precipitin reactions: 6 with anti-human serum (out of 45 reactions), 21 with anti-monitor (lizard) serum (119 reactions), 6 with anti-crocodile serum (31 reactions), 4 with anti-hippopotamus serum (28 reactions), 2 with anti-ruminant serum (19 reactions), and 4 doubtful reactions, one with anti-ruminant serum, a second with anti-bushbuck, a third with anti-Situtunga (antelope) serum, and, lastly, a fourth with an anti-serum prepared with hen's blood and the blood of various water-birds.

The results would presumably not be the same in another fly-belt, where the fauna would be different from those studied at Entebbe. If, for example, the anti-Situtunga serum were applied to the blood in the gut of tsetse-flies on the islands in Lake Victoria, where the blood of this antelope constitutes the chief food of *G. palpalis*, I am convinced that a higher number of positive precipitin reactions would be obtained than I got with the flies caught near the Entebbe Laboratory.

2. There is reason to believe that, whenever they have any choice, tsetse-flies show a preference for certain animals; but my experiments on the precipitin test and the experimental application of wild and laboratory-bred glossinæ to numbers of zoological species, whose blood they finally sucked more or less readily, point to the fact that tsetse-flies, essentially blood-sucking insects, can feed on the blood of any animals that are found habitually in or may happen to visit places in which they themselves find a suitable environment for living and breeding (temperature, humidity, shade, nature of the soil, etc.).

3. The first observers to study the precipitin test noted that the action of pepsin, and particularly of trypsin, had a marked effect on the intensity of precipitin reactions.

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<sup>1</sup> I suggested to the President of the Commission that I should visit Tanganyika Territory—after concluding my experiments on the precipitin test applied to *Glossina palpalis*—in order to try this test on *G. morsitans*.

I still had to complete other work at the Entebbe Laboratory, however, and Mr. SWYNNERTON was expecting his collaborator, who was to observe my experiments on *G. palpalis*, with a view to carrying out similar work on *G. morsitans* in Tanganyika Territory, so that the idea of my visiting a *G. morsitans* zone was definitely abandoned.

In May 1927, when my laboratory work was finished, I again suggested to the President of the Commission that I should visit Tanganyika Territory, to study Mr. SWYNNERTON'S work on the prophylaxis of human and animal trypanosomiasis. The rains made the roads impassable, and I was unable either to satisfy my curiosity or to carry out the third recommendation adopted by the London Conference in May 1925, when proposing the appointment of the International Commission.

HEKTOEN, FANTUS AND PORTIS (1919) proved that extracts of the fæces of healthy persons living on an unrestricted meat diet rarely gave positive reactions with anti-sera prepared with the antigen of the animals (ox, goat, sheep, hen, etc.) on which man generally feeds, showing that, ordinarily, foreign proteins which enter the stomach are not found in the same state in the fæces.

When engaged in comparative tests with sera with a high precipitin content and homologous antigens, before and after digestive action in the glossinæ, I myself found that digestion has a very marked effect on the intensity of the precipitin reaction. With blood containing red cells clearly recognisable under the microscope, taken from glossinæ experimentally fed on specific animals, I often failed to obtain positive reactions, though I had employed sufficiently active homologous anti-sera, producing well-marked positive reactions in the control tubes.

4. In practice, the fly-boys only catch tsetse-flies that have come out for the purpose of biting them, and these do not contain any blood but are, on the contrary, in search of it. Only in 8.6 per cent of the flies caught did I find any vestiges of blood still recognisable on microscopic examination. This blood, however, was so modified by the digestive ferments in the fly that I only obtained 2 per cent feebly positive precipitin reactions, despite the special technical precautions I took to overcome the difficulties encountered and to make sure of the modest results obtained with specimens of blood less modified by digestive action in the glossinæ. The few well-marked *positive* reactions were obtained with anti-human serum, probably because the blood in the glossinæ had been quite recently sucked from the fly-boys themselves, without the flies' digestive ferments having had time to modify its antigenic properties to any great extent.

The expedients that I adopted to overcome the various difficulties I encountered constitute the only original part of my contribution to this work, which I have been obliged to describe at greater length than I could have wished.

5. The complement fixation test, in a laboratory suitably equipped for Wassermann reactions, should furnish more fruitful results than the precipitin test in identifying the blood found in the alimentary tract of glossinæ.

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6.

STUDY ON THE PRESENCE OF HÆMOPARASITES  
IN GLOSSINÆ WITH A VIEW TO THE IDENTIFICATION  
OF THE BLOOD IN THEIR INTESTINES.  
BRIEF NOTES ON THESE MICRO-ORGANISMS.

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When dissecting glossinæ for the purpose of applying the precipitin test to the contents of the alimentary canal, I made a note of any material that might serve to corroborate the results of that test or that appeared for other reasons to be of scientific interest.

Apart from a few data obtained at Busitema, this study was entirely carried out at the Entebbe Laboratory.

I. TRYPANOSOMA GRAYI.

As I mentioned in my article on the precipitin test, *T. grayi* is generally to be found in association with the nucleated red blood cells of reptiles. The fact of such an association is most valuable in helping to determine the animal species whose blood the tsetse-fly has sucked.

1. *Percentage of Glossinæ infested with T. grayi.*

When dissecting wild glossinæ for the purposes of the precipitin test, I observed the following phenomenon : whenever lack of time prevented the immediate dissection of all the tsetse-flies caught in the neighbouring bush, not only did I find the following morning that there was less blood in the gut of the remaining flies (this is satisfactorily explained by the process of digestion during the night) but *T. grayi* itself had disappeared, as if this flagellate had found in the glossina gut only a possible medium for multiplying (like any of the artificial culture media prepared in the laboratory) but one not suitable for its complete cyclic evolution.

Is therefore *T. grayi*, present in glossinæ, to be compared to other species of flagellates found in the gut of other flies ?

\* \* \*

I realised, after drawing up the following table, that it confirmed the various conclusions which had at once occurred to me.

Place and date of capture of glossinæ	Interval between capture and dissection of glossinæ	Total number caught	With nucleated red cells	With nucleated red cells and <i>T. grayi</i>	With non-nucleated red cells	With non-nucleated red cells and <i>T. grayi</i>	Without red cells	Without red cells and with <i>T. grayi</i>	Percentage of glossinæ with <i>T. grayi</i>
Entebbe 16-12-1926	22 hours	50	5	4	1	0	44	2	12%
„ 30-12-1926	5 „	50	3	1	2	0	45	10	22%
„ 3-1-1927	4 „	50	4	0	3	0	43	9	18%
„ 5-1-1927	5 „	45	6	2	2	1	37	4	16%
„ 1-2-1927	72 „	30	0	0	0	0	30	1	3%
„ 22-2-1927	4 days	50	0	0	0	0	50	0	0%
„ 27-2-1927	5 hours	50	9	5	2	0	41	6	22%
„ 14-3-1927	24 „	24	3	1	0	0	21	1	8%
„ 15-3-1927	48 „	18	0	0	0	0	18	0	0%
„ 16-3-1927	72 „	8	0	0	0	0	8	0	0%
„ 23-3-1927	5 „	50	7	5	3	1	40	6	24%
„ 24-3-1927	5 „	50	9	4	3	0	38	5	18%
„ 30-3-1927	72 „	50	0	0	0	0	50	1	2%
„ 5-4-1927	4 days	40	0	0	0	0	40	0	0%
Kami 26-5-1927	8 hours	112	0	0	8	0	104	0	0%

The above table shows that :

(a) In glossinæ containing nucleated red cells *T. grayi* is fairly frequently found, but this flagellate is rarely found together with non-nucleated red cells.

(b) The percentage of wild glossinæ containing *T. grayi* drops from 16 to 24 for those dissected four or five hours after being caught, to 3 and 0 for those which die of starvation during the course of the days following (from one to five days).

(c) *T. grayi* is fairly frequently found in tsetse-flies in which it may no longer be possible to diagnose the red blood cells. In these cases, the glossina gut is generally full of a dark green mass of blood, in process of digestion, which probably consists of nucleated red blood cells.

Possibly, in the rare cases in which non-nucleated red blood cells and *T. grayi* are found in the same glossina, the fly has sucked the blood of a mammal before digesting and completely evacuating this dark green mass of nucleated blood containing *T. grayi*.

(d) I found no flagellates in the glossinæ I dissected on the Kami river, near Buteba, where there were no crocodiles. It is true that I did not examine a great many tsetse-flies, but on the shores of Lake Victoria, where crocodiles abound, I should certainly have found, among the same number, some glossinæ infested with *T. grayi*.



2. *T. grayi* Cultures.

Despite the fact that I dissected the tsetse-flies with instruments recently sterilised in an autoclave and a Pasteur steriliser and that I washed the glossina gut thoroughly in sterilised normal saline, and tried various Ponselle media, all attempts to culture *T. grayi* were frustrated by the rapid and prolific development of bacteria in the media inoculated. I noted, in my study on cultures of pathogenic trypanosomes, the incompatibility between trypanosomes and bacteria.

3. *T. grayi* Inoculations.

While dissecting glossinæ with the object of applying the precipitin test to the contents of the alimentary canal, I collected a large number of flagellates, to be used for inoculation. The so-called metacyclic forms of *T. grayi* are found, however, in the posterior extremity of the gut, so that I was obliged to adopt a different method of dissection, from that employed for the purposes of the precipitin test, when I simply had to extract the middle part of the gut, containing the recently absorbed blood.

In order to be sure of not losing these transmissible metacyclic forms, I employed abdominal pressure without cutting the posterior extremity of the fly, using the microscope to find the terminal portion of the intestine, from the entrance of the Malpighian tubes to the papillæ of the rectal extremity. The whole of the intestinal contents were diluted with normal saline and inoculated into young crocodiles hatched out from eggs in the laboratory incubator. One crocodile was inoculated in the subcutaneous tissue, and a second in the peritoneal cavity, while the flagellates were placed on the conjunctiva of a third and on the mucous membrane in the mouth of a fourth. I carried out from five to eight inoculations on each of these reptiles. I examined their blood repeatedly for weeks and even months without discovering a single flagellate.

I afterwards applied boxes of laboratory-bred flies, for fifteen to twenty-five days, to the inoculated crocodiles and not one of the glossinæ became infected, although by patient effort I succeeded in making a fair percentage of them suck the crocodiles' blood on each application.

4. *Action of the Blood of Certain Animals on T. grayi in the Gut of Glossinæ.*

A number of investigators had noted the fact that in glossinæ with a heavy natural infection of *T. grayi* the flagellates disappeared after the flies had been experimentally fed on goats.

Having the necessary material at my disposal, I decided to repeat these experiments, and proceeded as follows: I sent out into the bush every day for enough wild glossinæ to fill two boxes each containing about 50; I dissected the flies in one of the boxes at once, and applied the other box to goats or monkeys, not dissecting these flies until the following day, so that the blood absorbed by them might have time to act on such flagellates as they might contain.

From the flies dissected immediately on arrival at the Laboratory, before being fed, I found anything from 12 per cent to 22 per cent *T. grayi* infestation.

Only twice did I find *T. grayi* in the gut, out of 400 flies which died and were dissected eighteen hours after being fed on goats.

No flagellates were found in the gut of glossinæ (close on 250) dissected eighteen hours after being fed on monkeys.

These results led me to the conclusion that goat's blood has no specific sterilising action on *T. grayi*, as monkey's blood produced the same effect.

As already mentioned, simple starvation is sufficient to cause the disappearance of intestinal flagellates from wild glossinæ.

5. *Action in vitro of Blood Serum of Certain Animals on T. grayi.*

MINCHIN AND GRAY (1905) added *T. grayi* to goat serum, and observed that the flagellates died rapidly; human serum had no such trypanocidal effect on *T. grayi*.

I had the necessary material to repeat these experiments, and decided to proceed as follows: I dissected the tsetse-flies at once in goat or human serum. If flagellates were present in the preparation, I covered a drop on a slide with a cover-slip, ringed it with neutral vaseline, and examined it every half-hour.

I dissected the glossinæ directly they were brought in from the bush, generally in the afternoon, and observed the preparations repeatedly during the afternoon and part of the night, having brought the necessary laboratory material home with me. The flagellates continued their normal movements all the time, both in the human serum preparations and in those containing goat serum.

The difficulty of reconciling my results with those obtained by other investigators suggested that we might not have experimented with the same flagellates. In order to facilitate the identification of those experimented with by other observers and those examined by myself, I drew some of the latter with a camera lucida. Fig. 1, opposite, though far from perfect, will be of more use than any description to determine its identity.

Photograph 24 shows a fly-boy working on the Kami river near the bridge on the Busia-Tororo road; we did not find any crocodiles there, and the intestines of none of the tsetse-flies dissected contained *T. grayi*.

## II. HÆMOGREGARINES.

When dissecting wild glossinæ with the object of identifying the blood in them by means of the precipitin test, I quite often found the gut full of hæmogregarines.

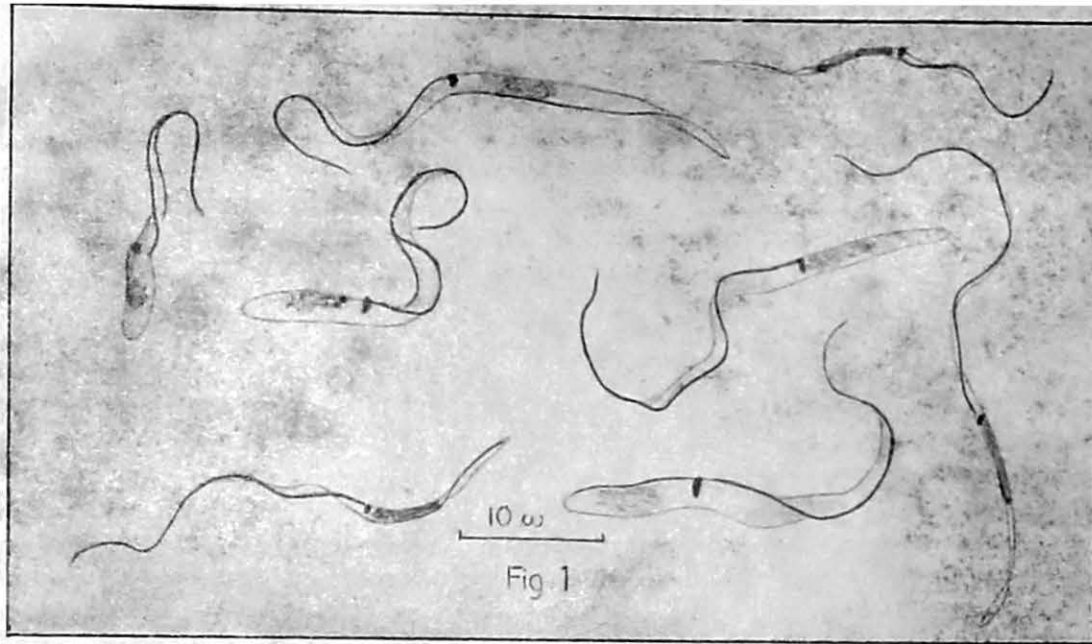
These were usually present together with nucleated red cells, either free or enclosed in the blood corpuscles, only rarely were hæmogregarines and non-nucleated red cells found in the same gut. I have never seen hæmogregarines in non-nucleated red cells, and this made me wonder if the presence of these which I sometimes found along with these cells might not indicate that the fly harboured the hæmogregarines at the time when it fed on blood with non-nucleated red cells.

Hæmogregarines are sometimes found together with *T. grayi*. Unlike the latter which disappear with the blood, they persist in a fair number of glossinæ whose gut does not contain any blood.

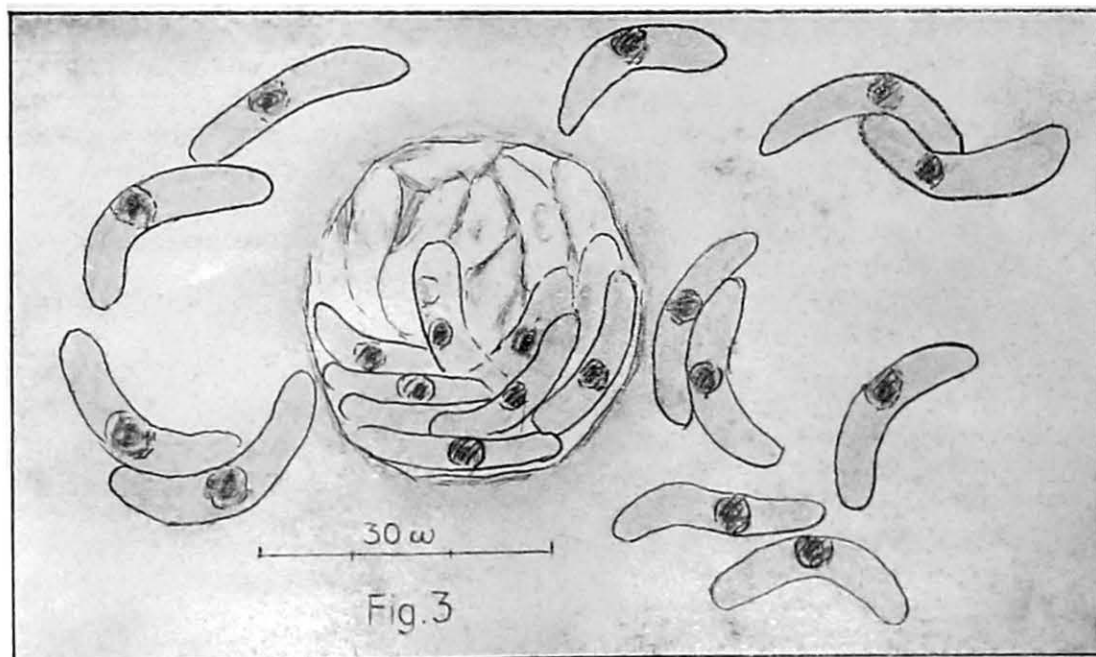
The frequent association of hæmogregarines and nucleated red cells was of assistance in determining which anti-sera to try first for the precipitin test; so were tsetse-flies with blood containing indeterminate red cells and hæmogregarines or *T. grayi*. The former was an indication to try anti-sera prepared with reptile antigen. Before attempting the precipitin test, I used systematically to prepare a film of the intestinal contents of the tsetse, in order to ascertain the morphological features of the red cells. In films stained with Leishman or Giemsa stain, the hæmogregarines were found either: (a) free, between the red cells; (b) inside the nucleated red cells; or (c) encysted.

As many of the cysts were open when the films were made, it was not possible to determine whether the free forms were naturally free or had escaped from the ruptured cysts.

I never succeeded in determining the number of hæmogregarines in each cyst. If the cysts were already open, the hæmogregarines released from one mixed with



**Figure 1:** Flagellates found in the gut of *Glossina palpalis*, described as *Trypanosoma grayi*. (Neighbourhood of Entebbe.)



**Figure 3.** Hæmogregarines, free and encysted, in a film of the intestinal contents of another wild glossina. (Neighbourhood of Entebbe.) (Cysts resembling those of coccidia.)

those released from others. When they were closed, the Romanowsky stains used for the films penetrated so imperfectly that it was impossible to make an exact count of the parasites enclosed within the cyst wall.

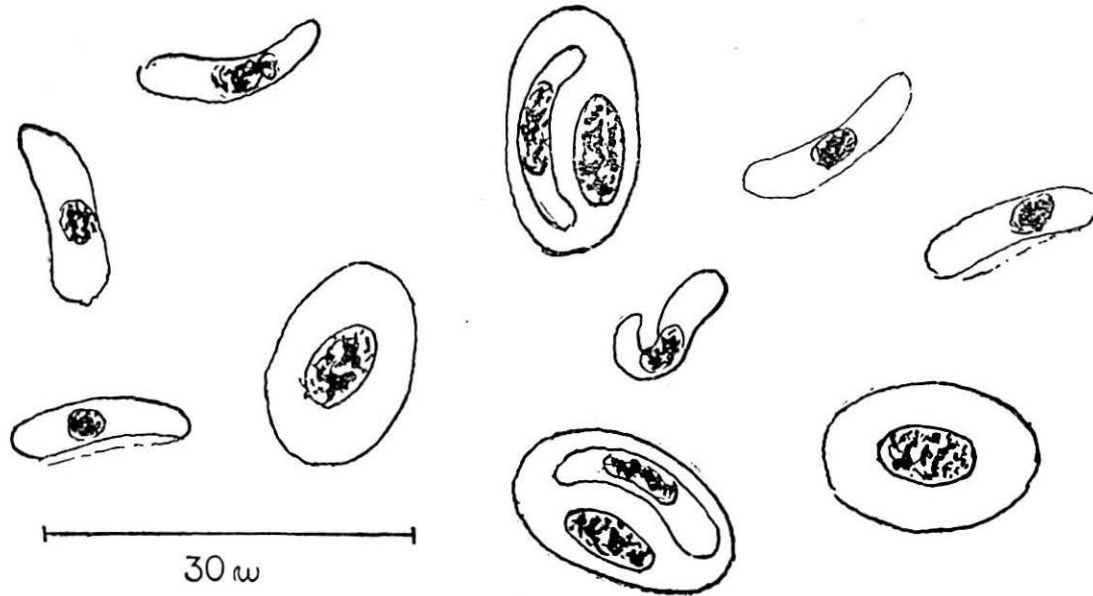


Fig. 2

Camera-lucida sketch of haemogregarines, both free and enclosed in reptilian red cells, seen in a smear made of the intestinal contents of a wild glossina.

I found in the blood of a crocodile nearly  $1\frac{1}{2}$  meters long (photograph 6), which some natives had caught and brought alive to the Entebbe Laboratory, numbers of haemogregarines swimming freely between the blood corpuscles, and so motile that, when examined in a fresh preparation with a low-power objective, they might have been taken at first sight for trypanosomes.

May there not be some relation between the haemogregarines (free, intra-cellular or encysted) which I found in the gut of *G. palpalis* in association with reptile red cells and those which I observed in the blood of the reptile itself? Further research should determine this point. The task which was then absorbing most of my attention was Item IV of the Commission's programme: the identification of blood found in glossinae. As the study of this problem might be facilitated to some extent by a knowledge of the micro-organisms which have a preference for various kinds of blood, the publication of these notes in the Commission's report is justified.

\* \* \*

With the object of preparing anti-sera, I inoculated two rabbits with fresh crocodile blood containing living haemogregarines. The blood was examined repeatedly, but no haemogregarines were ever found.

I had not yet hatched out any crocodiles in the incubator (photograph 5), or I should have inoculated this blood containing haemogregarines into other crocodiles, with the idea of subsequently applying laboratory-bred flies, and thus obtaining some information as to the part played by glossina in relationship to this haemogregarine:

### III. LONG BACILLI IN THE GUT OF GLOSSINÆ.

A considerable percentage of wild and laboratory-bred glossinæ have the gut full of bacilli (?) so long as to suggest mycelia.

While working on the identification of the contents of the alimentary canal of tsetse-flies, the President of the Commission suggested that I should note the glossinæ that harboured these long bacilli, as he had an impression that trypanosomes were not found in association with them. I therefore showed him my notes on the subject, since my own observations had given me a similar impression.

We heard afterwards that Dr. CARPENTER had also observed the incompatibility between these long bacilliform organisms and flagellates in the glossina gut.

Out of the 873 glossinæ which I dissected and examined from this point of view, I found 139, almost 16 per cent, harbouring these long bacilli. Of these 139, three only harboured intestinal flagellates at the same time. In these three flies, the flagellates were numerous and the bacilli very scarce, or vice versa, so that even the exception which they represented would appear to confirm the general rule of incompatibility.

I have already noted, in my article on the culture of pathogenic trypanosomes, the manifest incompatibility between trypanosome culture forms and the bacteria which grow accidentally in the inoculated media. I decided to study the action of these bacilli on the actual blood forms of trypanosomes.

I made several cultures of these bacteria, and, with the kind assistance of Mr. GIBBINS, Bacteriological Assistant at the Entebbe Laboratory, isolated different strains of these bacilliform organisms and studied their characteristics. To avoid prolonging this account unduly, I will confine myself to indicating below the characteristics of one of the strains most frequently isolated :

Gram-negative bacillus, rounded ends, feebly motile. Grows readily on gelose and on broth, on the surface of which it forms an ash-coloured film. Reactions in media containing sugar : does not affect media containing salicin or lactose, but produces acid and gas in glucose, maltose, saccharose and mannite.

I inactivated old cultures of these bacilli with phenol and inoculated them into monkeys infected with trypanosomes.

One of the monkeys heavily infected with *T. rhodesiense* died fourteen hours after inoculation, the number of trypanosomes in the peripheral blood having noticeably decreased before death.

A second monkey, which exhibited *T. gambiense* in the peripheral blood, in the proportion of about one trypanosome per 60 microscopic fields (No. 6 Zeiss eye-piece and No. 40 Zeiss objective) after inoculation with these bacilli (?), revealed no trypanosomes in the 300 fields examined on the first and second days, but on the following days the flagellates reappeared, the proportions being about the same as before.

\* \* \*

This experiment in bacteriotherapy (or proteinotherapy) is mentioned here as a subsidiary incident in my study of the precipitin test as a means of identifying the blood found in the alimentary canal of glossinæ. It might perhaps be worth while carrying out tests on a larger scale, to study the action of hæmoclastic shock on latent trypanosomiasis, with a view to determining whether, independently or not of the doses inoculated, a parasitocidal effect is obtainable, or whether, on the contrary, the trypanosomes can be reactivated ; this would also be of some value in cases in which microscopic diagnosis is difficult.

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## ORGANISATION OF EXPERIMENTAL WORK ON GLOSSINÆ AT THE ENTEBBE LABORATORY.

### INTRODUCTION.

Dr. DUKE, following on Sir David BRUCE, has carried out in Uganda a long and uninterrupted series of investigations on wild and laboratory-bred glossinæ. His years of experience have enabled him to develop this particular line of investigation and to specialise in it, rendering his work practical and accessible to any careful investigator.

There are still countless experiments to be carried out with glossinæ in the different regions and it seemed to me desirable, therefore, to explain the practical organisation of Dr. DUKE'S work on these flies, through which he has obtained most valuable results never surpassed by other African institutes.

A highly specialised expert engaged in his own particular task finds everything easy and cannot always realise the difficulties—non-existent for him—which may be encountered by the novice.

A visitor in search of information may sometimes ask questions and thus indicate points in the experts' technique which require more detailed explanation in order to be properly understood by the uninitiated.

Dr. DUKE entrusted me with his experimental work on glossinæ when he was away from Entebbe for some weeks, and I had to ask him for most detailed directions before being able to undertake it. Thinking that the knowledge I thus acquired might be of use to Portuguese colonial doctors and perhaps to doctors in the other African colonies, where trypanosomiasis are transmitted by tsetse-flies, I decided to draw up a short report, which I have illustrated as best I could, endeavouring to make up by drawings and photographs for the inadequacy of my own powers of description.

My article, being originally written in Portuguese, could not be revised by Dr. DUKE before being translated. I have simply noted my own impressions, which may not be entirely in accordance with the actual facts. I am alone responsible, therefore, for any mistakes and omissions that may be found.

\* \* \*

Most of the experiments carried out with tsetse-flies can be divided into two main groups, according to whether wild glossinæ or laboratory-bred glossinæ are used.

I. *Wild glossinæ* are used to study the infectivity of a region from the point of view of trypanosomiasis, either by dissecting the glossinæ and classifying the trypanosomes which they harbour, or by feeding the flies on laboratory animals susceptible to infection by such trypanosomes, and in which the biological characteristics of the flagellates may be studied.

For this group of experiments it is necessary to catch wild glossinæ in the bush, some of which may be infected and to dissect them either before or after being fed on laboratory animals, so as to transmit the trypanosomes of which they may be the carriers.

II. With *clean laboratory-bred glossinæ*, it is possible to determine whether any given animal is infected with trypanosomes, by applying the glossinæ to it

(xenodiagnosis) or, if we know in advance that it is infected with trypanosomes, we can study their transmissibility and hence the epidemiological infectivity of the strain.

For this group of experiments, it is necessary to collect pupæ and breed clean glossinæ; the flies which hatch out are placed in special little boxes the top and bottom of which are made of netting, so that when the boxes are applied to the animals' skin the insects can bite between the meshes.

It may be of interest to study in detail how Dr. DUKE has organised his service at the Entebbe Laboratory for the purposes, *inter alia*, of his tsetse experiments.

#### *Collection of Pupæ.*

It is necessary to go to the fly belts in order to find the pupæ in the places where the glossinæ deposit their larvæ. Their preference in this matter is known to vary according to each particular species. The habitat of the different glossinæ in each region is so well known that there is no need to dwell on it. Anyone undertaking research on glossinæ must, of course, employ natives who are experts at collecting the pupæ and catching the flies (*vide* photographs 14, 15, 16, 17 and 24).

In Uganda, fly-catching has become a regular profession which is very popular with the natives; the work is attended by danger (cases of infection are by no means rare), but their careless ignorance and inveterate idleness make them look only on immediate advantages, *i.e.*, the possibility of earning first as much as in any other trade (or even more) and of being engaged by the month for easy work which need not be done every day.

The pupæ found in the breeding-grounds are taken in little boxes to the Laboratory, where they are kept under suitable conditions, so that adult insects may hatch out.

#### *Method of catching Wild Glossinæ.*

Wild glossinæ are caught in the fly belts with a net like a butterfly-net, on the end of a cane (*vide* photograph 15). Some of the fly-boys who are particularly expert never let a single fly within their reach escape them. It is chiefly a matter of practice. I tried more than once to catch these flies. One's first efforts are not very successful, until one has acquired the knack of twisting the net so as to roll it round the cane, imprisoning the fly in its folds.

The glossinæ, directly they are caught, are put into Bruce boxes, which I shall describe later.

Some authorities recommend that for long journeys earth and damp leaves should be put in the glossinæ boxes. The wild flies used at the Entebbe Laboratory are caught from one to three hours' distance away and reach the Laboratory in good condition, without its being necessary to keep the boxes damp.

These flies can be at once used for laboratory experiments.

#### *Clean Laboratory-bred Glossinæ.*

The pupæ, on arrival at the Entebbe Laboratory, are placed in a long net bag (*vide* photograph 23). The lower part of the bag is made of thicker material and stretched over two hoops. The bag is hung in a passage where the light is not very strong, a bucket of water being placed under it to ensure the necessary humidity.

Quite soon, during the course of the next few days, the glossinæ begin to hatch out. Every morning the head fly-boy transfers the adult flies to the Bruce boxes, where they are kept all through the experiments. When doing this, the fly-boy plunges his right hand into the bag and with his left hand presses the folds of the net against his right arm to prevent the flies from escaping.



He gets hold of from four to six glossinæ at a time, taking them between the thumb and first finger; it is quite easy then to put them one by one, through the little opening, into the Bruce box.

Skilled as the head fly-boy has become in the course of his long experience (he was doing this work under Sir David BRUCE), the tsetse-flies often get out into the laboratory and neighbouring premises, having escaped as a rule during the process of transfer from the breeding-bag to the experimental box. The newly hatched glossinæ are, of course, not infected, but it is quite possible—though not very probable—that infected tsetse-flies may escape from the box when the dead flies are being taken out each day, so that it is advisable, whenever one is bitten, to try to catch the fly at once and dissect it to ascertain whether it is infected and infectious (trypanosomes in the salivary glands), in case preventive treatment should be necessary.

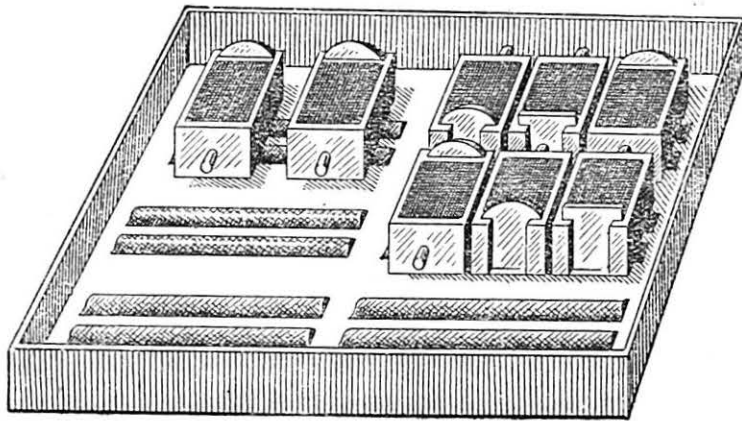


Fig. 1

The fly-boy collects about fifty flies in each box and hands them over to Dr. DUKE. A label is put on the box, showing the number of the experiment, the number of the animal to which the box is to be applied and the date of the first application; the boxes are then placed in large, shallow zinc trays (*vide* Fig. 1 and photograph 25); the trays are filled with water to a depth of from 3 to 5 cm. and cross-

pieces are arranged with the ends clear of the sides of the tray, the boxes being placed on them so as to rest just above the surface of the water.

This arrangement gives the flies the necessary humidity and at the same time keeps them out of reach of ants. The boxes are arranged in the tray in groups isolated from one another by water, so that even if ants should manage to find a way in, all the experiments would not be ruined.

Bruce boxes are better suited to these experiments than the wide-necked bottles with net covers recommended by some investigators. They are quite easy to make, as may be seen from my sketch (*vide* Fig. 2). They are of ordinary wood, rectangular about 15 cm. by 10 cm. and 7½ cm. high. At one end of the box, there is a sliding door, A, and at the other an opening closed with a wooden plug, B. The sliding door enables the box to be cleaned out, and the opening at the opposite end is used to put the live flies in and to take out those which die in the course of the experiment.

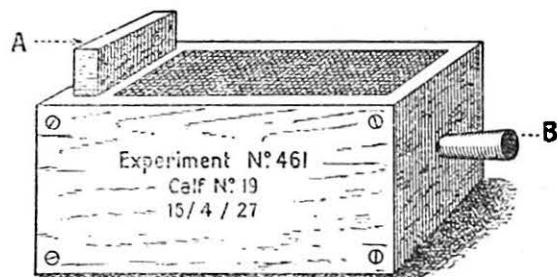


Fig. 2

The top and bottom of the box are made of the usual mosquito-proof wire netting employed for covering windows. The netting at the bottom, at all events, should be flush with the sides of the box, so that when the box is applied it should be in contact with the animals' skin and the glossinæ should have no difficulty in biting between the meshes. Ordinary cotton mosquito netting can also be employed. The



objection to the use of this material is that it tears very easily, but it does present certain advantages : the experimental glossinæ in boxes covered with wire netting sometimes refuse to bite animals (crocodiles, monitors, etc.) on which they are in the habit of feeding naturally, but can generally be induced to do so through cotton netting.

*Method of applying Glossinæ to Animals.*

Laboratory-bred glossinæ do not harbour trypanosomes when they emerge from the pupæ and before they have bitten infected animals. Dr. DUKE and Captain FRASER have proved by experiments that hereditary transmission of tsetse trypanosomes does not take place.

In experiments with laboratory-bred flies, the first step is to infect the flies on trypanosome-infected animals. Certain precautions are necessary to make the glossinæ suck their blood.

If the skin of the animal to which the box of clean glossinæ is to be applied is protected by a thick coat of hair or feathers, these must be got rid of over a sufficient area.

The fly-boy takes the boxes from Dr. DUKE and fixes them on the animal, being careful to cover the net covered top of the box with a damp cloth which will give the glossinæ sufficient moisture and protect the boy's hands, which the flies prefer to the skin of any animal (*vide* photographs 26 and 28).

Young glossinæ are not voracious. They have very little desire for food the first day after leaving the pupæ. At first, they have to be made to bite, and even so the death rate is much higher than on the following days, either because they are not fed or because of some malformation.

To avoid errors arising from trypanolytic crises, during which the trypanosomes disappear from the peripheral blood of infected animals, Dr. DUKE makes a point of applying four or five boxes of clean glossinæ on ten consecutive days, so that there may be more chance of obtaining flies which have absorbed blood during the intervals between these negative crises. When there is only a limited number of flies, he applies them as they come out of the pupæ. When the supply is adequate, he adopts the method which long experience has shown to be most satisfactory: this is shown in the following table.

PLAN OF GLOSSINA EXPERIMENTS  
(Extract from Daybook)

No. of fly-box	DAYS OF EXPERIMENTS : .....										
	1	2	3	4	5	6	7	8	9	10	11
421	cock	starved 1 ♂	cock 1 ♀	starved	C. 2 ♂ 1 ♀	s. 2 ♂	starved	Dissection { 12 ♂ 10 ♀	nihil		
422	starved	calf 25	starved 1 ♀	calf 25	s.	calf 25	s.	Dissection { 14 ♂ 10 ♀	2 ♂	gut and gl. +++	
423	goat 398	starved 5 ♀	goat 398	goat 398	s. 2 ♂ 1 ♀	C.	s. 1 ♀	C. 1	s. 1 ♀	C. 1 ♀	s. 1 ♀
424	monkey 620	starved 5 ♀	monkey 620	monkey 620	s. 2 ♀	C. 1 ♂ 2 ♀	s.	C. 1 ♂	s. 2 ♀ 1 ♀	C. 2 ♂ 1 ♀	s.
425		goat 398	s. 4 ♀	goat 398	goat 398	s. 1 ♂ 3 ♀	C. 1 ♀	s. 1 ♀	C.	s. 1 ♂	C.
426		monkey 620	s. 5 ♂ 2 ♀	monkey 620	monkey 620	s. 2 ♀	baboon	s. 1 ♂ 3 ♀	B. 1 ♂	s.	B. 1 ♂
427			patient X	patient X	patient X	patient X	s. 1 ♂ 3 ♀	C. 2 ♀	s. 1 ♂ 1 ♀	C.	s. 2 ♀
428				patient X	s. 2 ♀	patient X	patient X	s. 1 ♀	C.	s.	C. 1 ♂ 1 ♀
429						goat 398	goat 398	goat 398	goat 98	s. 1 ♂ 2 ♀	C. 1 ♀
430						monkey 620	monkey 620	monkey 620	monkey 620	s. 2 ♀	C. 1 ♀
431						goat 398	goat 398	goat 398	goat 398	s. 1 ♂ 3 ♀	s. 1 ♂ 3 ♀
432						monkey 620	monkey 620	monkey 620	monkey 620	s. 4 ♀	s. 1 ♂ 3 ♀
433						patient X	patient X	patient X	patient X	s. 3 ♀	s. 2 ♀
434							patient X	patient X	s. 1 ♂ 5 ♀	patient X	patient X

(Continuation)

No. of fly-box	DAYS OF EXPERIMENTS : .....											
	12	13	14	15	16	17	18	19	20	21	22	
421												
422	Applied on 10th day.											
423	C. 3 ♂ 2 ♀	s. 1 ♂	C. 2	s.	C. 2 ♂ 2 ♀	s.	C. 2 ♂ 1 ♀	s.	C. 1 ♂	s.	C. 2	
424	C. 4 ♂ 1 ♀	s. 1 ♂	C. 2 ♂	C.	C. 1 ♂ 1 ♀	s. 2 ♀	C.	s. 6 ♂ 1 ♀	C. 1 ♀	s.	C. 1 ♂ 1 ♀	
425	s. 1 ♂	C. 1 ♀	s. 1 ♂ 1 ♀	B. 1	s.	C. 2 ♂	s.	C. 1 ♂ 1 ♀	s.	C. 3 ♂	s.	
426	s. 2 ♂	B.	s. 1 ♂ 1 ♀	s. 1 ♂	s.	B. 1 ♂ 1 ♀	s.	B. 2 ♂	s. 1 ♀	B. 1 ♂	s.	
427	C.	s. 1 ♂	C. 1 ♂	C. 1 ♂	C. 1 ♀	s.	C. 2 ♀	s.	C. 1 ♂ 1 ♀	s.	C. 1 ♂ 1 ♀	
428	s.	C. 1 ♂	s. 1 ♂	C. 1 ♂	s. 1 ♂	C.	s.	C. 1 ♂ 1 ♀	s.	C.	s. 1 ♂	
429	s.	C. 1 ♂ 1 ♀	s. 1 ♂	C. 1 ♂	s. 1 ♂	C.	s.	C. 1 ♂	s.	C. 1 ♂ 1 ♀	s.	
430	s. 1 ♂ 1 ♀	C.	s. 1 ♂	C.	s. 1 ♂	C.	s.	C.	s. 1 ♂ 1 ♀	C.	s.	
431	C.	s.	C.	s.	C.	s.	C.	s.	C.	s.	C.	
432	C. 2	s. 1 ♀ 1 ♀	C.	s. 1 ♂	C.	s. 1 ♀	C.	s. 1 ♀	C. 1 ♀	s.	C. 1 ♂	
433	C. 1 ♂ 1 ♀	s.	C. 2 ♂ 1 ♀	s.	C. 1 ♂	s.	C. 2 ♂	s.	C. 1 ♂	s. 1 ♀	C.	
434	s. 2 ♀	C.	s. 1 ♂ 1 ♀	C.	s.	C. 1 ♀	s.	C. 2 ♂	s.	C.	s. 1 ♀	

PLAN OF GLOSSINA EXPERIMENTS (continued)

No. of fly-box	DAYS OF EXPERIMENTS :										
	23	24	25	26	27	28	29	30	31	32	33
425	s. 1 ♀ gut +++ C. 1 ♂ 1 ♀	C. 1 ♀ C. 1 ♀ s.	s. C. 1 ♂	sheep 480. C. 1 ♂ s. 1 ♀ gut +++	s. 1 ♂ 1 ♀ sheep 480	sheep 480 C. 1 ♂ s.	s. sheep 480	sheep 480 C. s.	s. 1 ♂ s. 1 ♀ sheep 430	sheep 430 C. 1 ♂ 1 ♀ s. 1 ♂ gut gl. +++	s. 1 ♂ s. sheep 430
426	B. 3 ♀ 1 ♀	s. 1 ♂	B.	s. 2 ♂ 1 ♀	B.	s.	B. 1 ♀	s. 1 ♂	B.	s. 2 ♂ 1 ♀	B.
427	s.	C.	s. 2 ♂	C.	s. 1 ♀	s.	s.	s. 1 ♂	s. 1 ♂	s. 2 ♂ 1 ♀	s. 1 ♂ 1 ♀
428	C.	s.	C. 1 ♂	s.	C.	s.	monkey 648	monkey 648	monkey 648	monkey 648	monkey 648
429	C.	s.	C. 1 ♂	s.	C.	s.	C. 1 ♂ 1 ♀	C. 1 ♂ 1 ♀	sheep 430	s. 1 ♂ 1 ♀	sheep 430
430	C. 1 ♀	s.	C.	s. 1 ♂	C. 1 ♂	s.	C.	C.	s. 2 ♂	s. 1 ♂	C. 1 ♀
431	s.	C.	s.	C.	s.	C.	s.	s.	C.	s.	s.
432	s.	C.	s.	C.	s.	C. 1 ♀	s.	s.	s. 1 ♂ 2 ♀	sheep 430	s.
433	s. 3 ♀	C.	s. 2 ♀	C.	s.	C.	s.	C. 3	s. 1 ♂	C.	s.
434	C.	s. 1 ♂ 1 ♀	C.	s.	C.	s. 1 ♂	C. 1 ♀ gut and gl. +++	s.	C.	monkey 643 s. 1 ♂ 2 ♀	s. 1 ♂ monkey 643

(Continuation)

No. of fly-box	DAYS OF EXPERIMENTS :											
	34	35	36	37	38	39	40	41	42	43	44	
421												
422												
423	sheep 430	s.	s.									
424	s. 2 ♂ 1 ♀	Diss. 9 ♂	nihil									
425	s.	sheep 430	s.									
426	s. 1 ♂	s. 1 ♀	Diss. 9 ♂ 8 ♀	s.								
427	monkey 643	s.	monkey 643	nihil								
428	s. 1 ♀	monkey 643	s.	s. 1 ♀	s. 1 ♂ 1 ♀							
429	s.	sheep 430	s. 1 ♂ 1 ♀	sheep 430	s. 1 ♂ gut +++	Diss. 10 ♂ 12 ♀ ; 1 ♀ gut +++						
430	s.	C.	s.	C.	s.	s.	sheep 430	s.	Diss. 13 ♂ 19 ♀ ; 2 ♀ gut and gl. +++			
431	sheep 430	s. 2 ♂	sheep 430	s.	sheep 430	sheep 430	sheep 430	sheep 430	s.	Diss. 12 ♂ 19 ♀ ; 2 ♂ gut and gl. +++		
432	C. 2 ♂	s. 1 ♂ 1 ♀	C.	s.	C. 1 ♂	s.	C. 1 ♂	s.	s. 2 ♂	Diss. 12 ♂ 16 ♀ nihil		
433	monkey 643	s. 1 ♀	monkey 643	s. 7 ♀	monkey 643	s.	monkey 643	s.	s. 2 ♂ 1 ♀	Diss. 19 ♂ 7 ♀ ; 1 ♂ gut and gl. +++		
434	s.	monkey 643	s. 2 ♀	monkey 643	s.	monkey 643	s. 1 ♂	monkey 643	s. 1 ♂	Diss. 17 ♂ 13 ♀ nihil Diss. 19 ♂ 10 ♀ nihil.		
										Diss. 18 ♂ 8 ♀ ; 1 ♀ gut +++		

Note: s. = starved; C. = cock; B. = baboon; ♂ and ♀ = glossinae, male and female, dead; gut. +++ = trypanosomes present in glossina gut; gl. +++ = trypanosomes present in salivary gland of glossina; nihil. = no trypanosomes in the gut or salivary glands of glossinae dissected; Diss. = dissection of glossinae remaining in box.

EXPLANATION OF TABLE.

The table shows only 14 glossina experimental boxes, but Dr. DUKE actually works with an average of from 40 to 60 boxes, each containing about 50 flies.

As regards boxes 421 and 422, only the final but not the early stages of the experiments are indicated in the table.

The glossinæ in box 423 were applied to goat 398 (which was thought to be infected) on the 1st, 3rd and 4th days. They were then fed on alternate days on hens (refractory to human trypanosomes) until the 25th day. From then until the 35th day, they were applied to sheep 430 and after being starved for two days were chloroformed and dissected. The effect of starving before dissection makes it possible to eliminate the blood absorbed by the fly, thus facilitating microscopic examination.

Several glossinæ died during experiment 423, but dissection did not reveal trypanosomes, except on the 23rd day, when flagellates were found in the gut of a female fly. Their presence shows that goat 398, to which the box was applied, did have trypanosomes in its blood.

On the 37th day, when the remaining flies—16 males and 7 females—were killed and dissected, two males were found to have trypanosomes in the gut and salivary glands, while one female had flagellates in the gut only. The presence of trypanosomes in the salivary glands indicates that the goat trypanosomes were transmissible by the glossinæ used in the experiment, and it is quite probable that sheep 430, to which the flies were applied during the last few days before dissection, when their salivary glands were already infective, would itself prove to be infected.

In experiment 424, the glossina box was applied to monkey 620, thought to be infected. After three applications, the flies were fed on animals refractory to human trypanosomes, until the 35th day, when they were killed, dissected and examined.

No trypanosomes were found in the gut or salivary glands of the glossinæ which died during the experiment or were killed, and this is equivalent to saying that monkey 620 was not infected or that if it was, the trypanosomes were not transmitted to the glossinæ.

\* \* \*

The table records the case of an infected animal bitten on 10 consecutive days by four boxes of glossinæ, each applied only three times; from this it may be seen approximately how long the trypanosomes take to develop in the glossinæ. Goat 398, for example, was bitten from the 1st to the 10th day; on the 1st by glossinæ box 423, on the 2nd by box 425, on the 3rd by box 423, on the 4th by boxes 423 and 425, on the 5th by box 425, on the 6th by box 429, on the 7th by box 431, on the 8th by box 429, on the 9th by boxes 429 and 431, and on the 10th by box 431.

Monkey 620 and patient A were bitten on 10 consecutive days by glossina boxes 424, 426, 430 and 432 (monkey) and by boxes 427, 428, 433, and 434 (patient) respectively.

\* \* \*

A fair idea of the work involved may be obtained from the programme, for example, for the 9th day, which was as follows :

1. Examine each box, take out dead flies and place them in dishes, noting on each in pencil the number of the box; enter in the register the number of dead males and females for the day.

2. Replace in the incubator the boxes of glossinæ to be starved and apply the others to the animal, susceptible or refractory (hens, baboons); for example, boxes 423, 424, 427 and 434 are starved on the 9th day, while boxes 425 and 428 are fed on hens (C. = cock), box 426 on a *Cynocephalus* (baboon), boxes 429 and 431 on goat 398, boxes 430 and 432 on monkey 620 and box 433 on patient X.

3. Dissect and examine all flies that appear to be dead in the different boxes (12 on the 9th day) and note the number of salivary glands or intestines containing trypanosomes, according to boxes.

4. Chloroform the flies in box 422, which was 35 days old on the 9th day. Count the remaining flies (14 males : 10 females), dissect and examine them (2 males show flagellates in the gut and salivary glands); reject positive salivary glands or inoculate into a new animal.

\* \* \*

Each box is applied to the infective animal on the 1st, 3rd and 4th days. The flies are starved on the 2nd, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th, 21st, 23rd, 25th, 27th, 29th, 31st, 33rd and 35th days. On the even days, the box is applied to animals refractory to the trypanosomes in question (hens, *Cynocephalus* (baboons), *Cercocebus*). After the 35th day, the flies can be killed and dissected and a search made for trypanosomes. If any are found, this proves that the animal bitten by the glossinæ still has trypanosomes in its blood and that they are transmissible by the species used in the experiment. With the object of determining whether trypanosomes are cyclically transmissible to another animal by the particular species of glossina, from the 25th day until dissection, the infected glossinæ are fed on a new animal, as in the case of boxes 423 and 425 shown in the accompanying table.

Each glossina box is applied three times to the infective animal, the latter being bitten on 10 consecutive days; there is thus more likelihood of some glossina sucking the blood in the intervals between trypanolytic crises.

For the purpose of infecting glossinæ by feeding them on animals infected with trypanosomes and again when infecting animals with infective glossinæ, the species susceptible to the particular trypanosomes are of course employed. Photographs are reproduced below of various animals susceptible to the trypanosomes studied by the League Commission on Sleeping-Sickness (*vide* photographs 26, 27, 28, 29, 30, 31, 32 and 33).

Clean laboratory-bred glossinæ after being applied three times to an animal infected with trypanosomes are then fed, on alternate days till the end of the experiment, on animals refractory to these particular trypanosomes.

The animals immune to human trypanosomes generally employed at the Entebbe Laboratory are hens and occasionally primates of the genus *Papio* (baboons) and *Cercocebus* (mangaby) (*vide* photograph 35). The latter are more difficult to obtain and handle than hens (*vide* photograph 37), which have, it is true, one slight drawback by comparison: they are refractory to human trypanosomes but sometimes are infected with *T. gallinarum*.<sup>1</sup> In blood preparations, however, these flagellates manifest such typical characteristics that they are easily recognisable, when once seen.

Sleeping-sickness trypanosomes are only feebly motile: their movements are serpentine, due to constant vibration of the undulating membrane. *T. gallinarum* is much more motile, with only slight undulations, passing rapidly across the field of observation like a great rigid bacillus.

<sup>1</sup> *Vide* Figure 1, Report No. II, Appendix E, page 113.

We reproduce hereinafter photographs of animals employed in the Entebbe Laboratory which are immune to polymorphic trypanosomes of African mammals (*T. brucei*, *T. gambiense*, *T. rhodesiense*, etc.) (*vide* photographs 34, 35, 36, 37 and 38).

The method of employing clean laboratory-bred flies may be summed up as follows : they are infected by being fed three times on an infected animal ; they are next fed on alternate days on animals resistant to the trypanosomes in question, this being continued until the 25th day, when the trypanosomes absorbed have completed their cycle of evolution. From the 25th to the 35th days, the glossinæ, which are now infective, are fed on alternate days on new animals, to which they may transmit their metacyclic trypanosomes. The flies are then dissected. The dissection and examination of flies which have died during the experiment are carried out each day.

#### *Dissection of Glossinæ.*

The purpose of dissection is to determine whether the flies are infected and infective (with metacyclic trypanosomes).

L. LLOYD AND W. B. JOHNSON (1924) decided that dissection was in itself a sufficient means of identifying most of the trypanosomes found in glossinæ. The various species that develop in the tsetse-fly are known to manifest a special trophism for specific organs of the fly and this has recently been taken as a basis for classification, the criteria being the morphological characteristics exhibited in the vertebrate host and the method of evolution in the non-vertebrate host : the glossina.

As shown below, a typical morphology for each stage of evolution in the glossina is characteristic of the trypanosomes in question :

Trypanosomes	Morphology in the vertebrate host	Evolutionary cycle in glossina	
		Trophism of non-infective forms	Trophism of infective metacyclic forms :
		First phase	Last phase
<i>T. brucei, gambiense, rhodesiense, pecaudi, togolense, ugandense nigeriense.</i>	Polymorphic (long, medium, short) flagella, free or otherwise.	Gut	Salivary glands
<i>Congolense, nanum, dimorphon, pecorum, montgomeryi, confusum, simiæ</i>	Monomorphic flagella, not free	Gut	Proboscis
<i>Vivax, capræ, uniforme, cazalbouï</i>	Monomorphic flagella, free	Proboscis	Proboscis
<i>Grayi</i>	?	Mid gut	Rectal extremity

A number of investigators, foremost among them Dr. DUKE, maintain that all these groups form a single zoological species, which includes several varieties without typical characteristics and not sufficiently differentiated to be of specific zoological importance. The table shows the practical utility, from the point of view of trypanosomiases, of dissecting wild and laboratory-bred glossinæ.

L. LLOYD'S classic technique for dissection is undoubtedly excellent as regards both the search for trypanosomes and the study of tsetse anatomy. At the same time, the technique employed by Dr. DUKE at the Entebbe Laboratory seems simpler and quicker and appears to us entirely satisfactory for the diagnosis of trypanosomes.

It is as follows : Using the scissors, cut the wings, legs and abdominal extremity of the tsetse-fly ; place on a slide one drop of normal saline, with the fly thus prepared beside it ; with the left hand, take hold of the thorax, keeping the fly on its back. Lay the severed abdominal extremity in the drop of normal saline. With a dissecting needle in the right hand exert slight pressure on the glossina from the middle towards the posterior extremity. The first effect will be to force out the internal reproductive organs, the intestine, the Malpighian tubes and a mass of fat bodies. The pressure should be repeated, keeping the abdominal extremity in the normal saline, and the crop and salivary glands will follow. Let go of the fly with the left hand, take a second needle, and with the two needles tear open the gut, releasing the trypanosomes, if present. Place a cover-slip over the drop of normal saline containing the viscera.

The various organs of the glossina can easily be distinguished with a dissecting microscope or a low-power objective : the *gut* is the biggest tube, containing blood with the red cells still intact or converted into a dark green substance (*vide* microphotographs 1, 2 and 3) ; the *four Malpighian tubes*, branching out from two tubes connected with the posterior extremity of the mid gut are thinner and longer, with sinuous contours, and very like the small intestine of some vertebrate species (*vide* microphotographs 2, 4, 5, 6 and 7) ; the *fat bodies* are elliptical in shape and of areolar structure (*vide* microphotographs 4, 6, 7, 8) ; the *tracheæ* are dark ramified tubes, with the tracheal structure very clearly marked (*vide* microphotographs 7, 8, 9, 10) ; the sucking stomach is a kind of stomach-like pouch, with thin walls, containing gas-bubbles, visible to the naked eye. The *salivary glands* consist of two long, transparent tubes, with the distal part wider than the proximal part, the general appearance being like that of a bluish-grey *Trichocephalus* on a dark background ; they are distinguishable by their longitudinal muscular fibres, producing double striation, longitudinal and transverse (microphotographs 11, 12, 13). Lastly, there are the *tubes of the reproductive organs*, which are transparent, like the salivary glands, but without striated longitudinal muscular fibres, and end in a rounded body ; this is somewhat the colour of a chestnut in the male and bright yellow in the female (*spermatheca*) (*vide* microphotographs 14, 15, 16, 18).

When dissecting any of these abdominal viscera, it is important to get rid of the many ramifications of the tracheal canals and the adipose tissue which lines the abdominal cavity.

Search should first be made in the gut for trypanosomes pathogenic for man. After tearing the gut if the fly is infected, the microscopic field will show a swarm of flagellates, characterised by their active movements and distinguishable with a moderately high objective, not necessarily an immersion lens when the intestine has not been completely opened up, an examination should be made of the cut extremities, from which the trypanosomes will issue, if present. If the intestines are full of blood, it will be difficult to see the trypanosomes, but if they are empty, the flagellates can be seen through the walls.

Flagellates are never found in the salivary glands if absent from the gut. They begin by multiplying rapidly in the intestinal canal and only the last phase of their evolutionary cycle (when they do complete it) takes place in the salivary glands. For the purposes of a study of polymorphic trypanosomes in African mammals (*brucei*, *rhodesiense* and *gambiense*), it is useless to look for these intestinal forms in the salivary glands if they have not been found in the gut of the glossina. If, however, they are present in the gut, the contents of the salivary glands should be carefully examined under the microscope *through the walls*.

This examination requires special care, as only a few trypanosomes may be present, and even when plentiful, they may be overlooked in a superficial examination. They

are shorter than the intestinal forms, vacuolated, and so faintly motile that it requires most careful examination to detect them among the granular contents of the gland. It is essential to adjust the microscope accurately to see the lumen of the gland tube; by first focussing the upper wall and then the lower wall, the canal midway can be readily examined.

The small metacyclic trypanosomes in the glands appear swimming in the salivary secretion or attached by one extremity to the inner wall.

They can be recognised by their small dimensions, their granular or empty appearance and their slight undulations, like those of a water-plant gently stirred by the motion of the water. There is no difficulty in distinguishing them from the long free intestinal forms whose rapid movements stir the whole microscopic field.

Anyone who has once seen metacyclic trypanosomes in the salivary glands will never confuse them with the intestinal forms, even when the latter lie over the salivary glands and fill the microscopic field. If the instrument is properly focussed on the gland cavity it is almost impossible to make a mistake. More frequently, however, the metacyclic forms may be overlooked in a superficial examination, as they do not stand out sufficiently against the gland contents.

The metacyclic forms in salivary glands inoculated by the bite of a tsetse-fly or with the syringe are known to be the only ones that are infective. The intestinal forms are quite inoffensive, when inoculated. A glossina with intestinal forms is said to be infected, but only when it exhibits metacyclic forms in the salivary glands is it infective; the trypanosomes under consideration are those which cause sleeping-sickness in man and "nagana" in domestic animals.

A search for other trypanosomes in the glossina necessitates examining the parts of the body in which they complete their evolutionary cycle, that is, in the rectum (*T. grayi*) or the proboscis (*congolense* and *vivax* groups).

When dissecting the rectal extremity of the gut, care must be taken not to injure the posterior part of the abdomen; only a very small segment should be cut, or the viscera should be extracted intact.

The proboscis should first be detached by pressing with the point of a needle on the bulb of the labium. It is then placed in a drop of normal saline, on a slide, which must not be too thin or it will not resist the slight pressure necessary to separate the various parts of the proboscis and release the trypanosomes.

In conclusion, when searching for trypanosomes in the digestive canal or the salivary glands of glossina the legs and wings should be cut off, that they may not get in the way, and the posterior extremity cut, to facilitate the evacuation of the abdominal viscera; this rapid evisceration makes it possible to examine the trypanosomes in the rectum and the middle part of the digestive canal, as well as in the salivary glands.

The process employed for studying trypanosomes located in the biting parts is equally simple: the point of a dissecting needle is placed upon the bulb of the labium, to detach the proboscis from the head, and the proboscis is then crushed between the slide and cover-slip. This rapid technique, as employed at the Entebbe Laboratory, is most satisfactory for the diagnosis of trypanosomes in glossina. If there were any reason to preserve the glossina organs intact, it would, of course, be necessary to dissect carefully with an appropriate microscope.

The present account is far from complete. It represents a series of notes on the organisation of experimental work on glossina at the Entebbe Laboratory, and is designed to give colonial doctors some idea of the methods and technique employed and the general trend of these experiments. I trust that the illustrations may make up for the shortcomings of my text.

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MICROPHOTOGRAPHS.

1. Fragment of glossina gut (large tube), and two Malpighian tubes (small tubes).
  2. Junction of Malpighian tubes with intestine.
  3. The four glands of the rectal end of the intestine.
  4. Showing the relative sizes of the intestine (large tubes), Malpighian tubes (small tubes) and fat bodies strung out like beads or in confluent masses (dark portion of the microphotograph).
  5. Four Malpighian tubes.
  6. Three Malpighian tubes, and five fat bodies.
  7. Centre, Malpighian tube; below, fat bodies full of little droplets of fat, and a small black tracheal tube; above, a fragment of salivary gland, standing out faintly against the lighter half of the microphotographic field (the glands are transparent).
  8. Fat bodies and, in the centre, tracheal ramifications.
  9. Branch of trachea, showing its striated structure.
  10. Tracheal ramifications.
  11. Fragment of salivary gland, showing its longitudinal fibrils, transversely striated. (Slightly enlarged.)
  12. The two parts, thick and thin, of a salivary gland, giving the latter the general form of a *Trichocephalus*. (Slightly enlarged.)
  13. Showing the relative thickness of a salivary gland and a Malpighian tube.
  14. 15 and 16. Three views of the internal reproductive organs of the male glossina; black spheres, light tubes, *vas deferens*, and seminal vesicles. A number of black bodies, the fat bodies, are also visible.
  - 17 and 18. Two views of the tubes of the internal reproductive organs of the female glossina.
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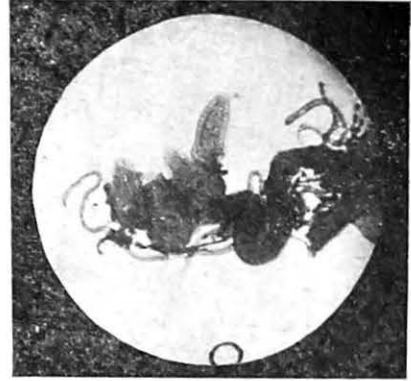
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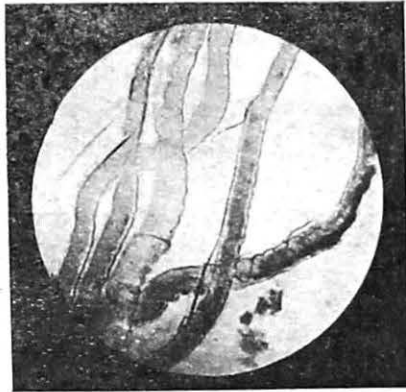
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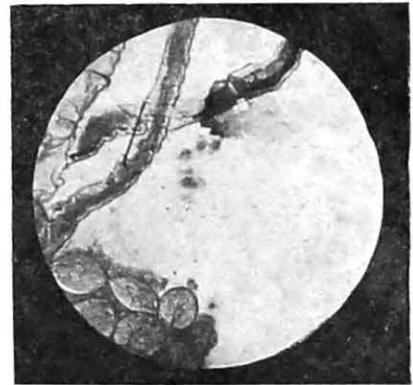
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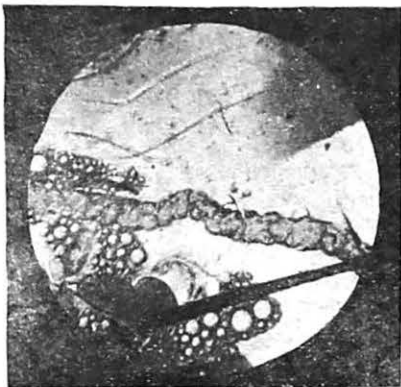
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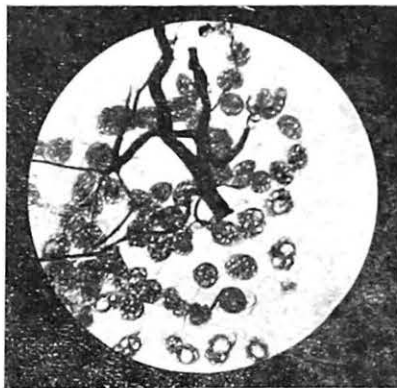
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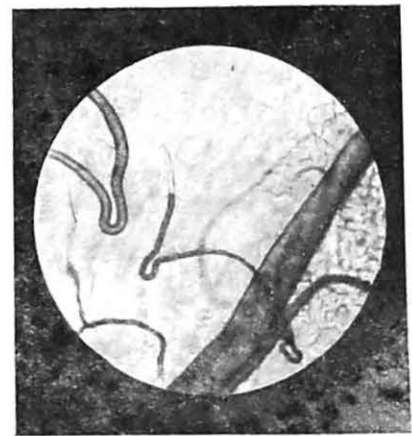
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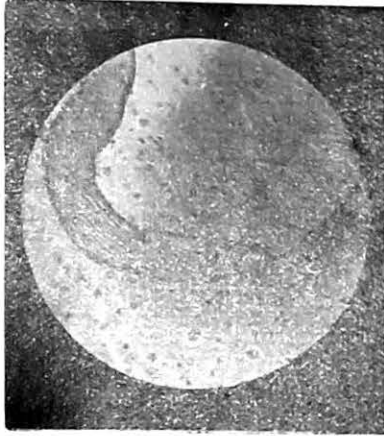
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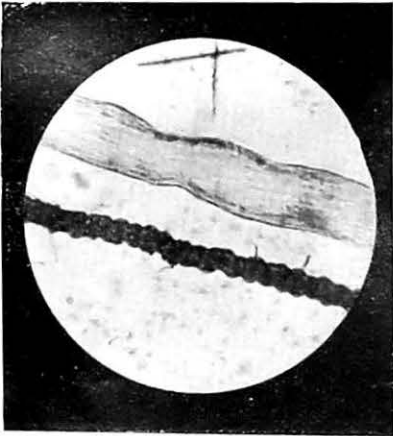
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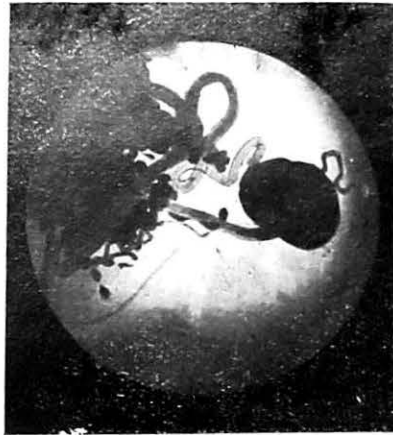
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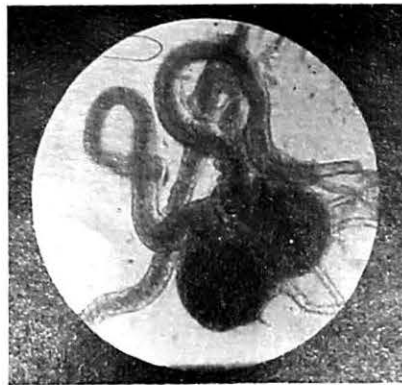
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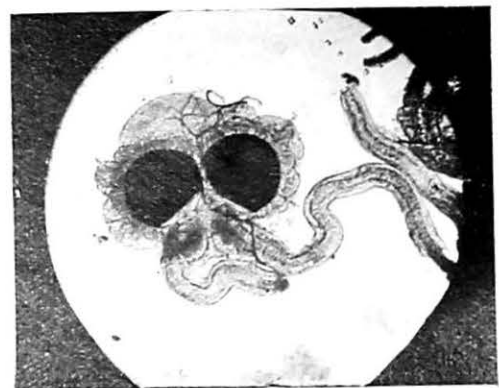
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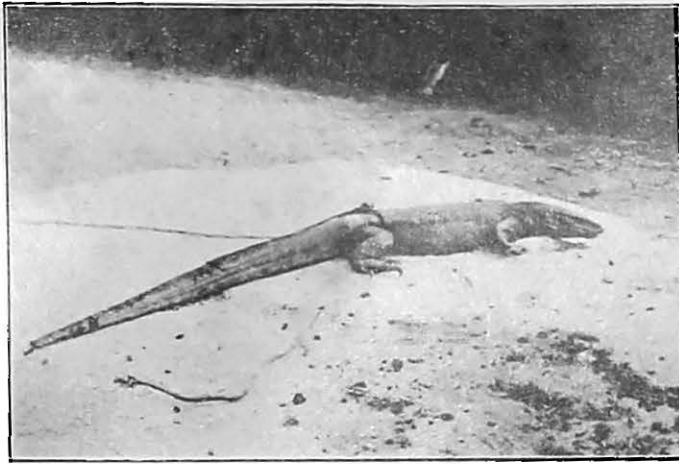


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PHOTOGRAPHS.

1



*Photograph No. 1: Monitor (Varanus), a big African lizard on which Glossina palpalis feeds.*

2



*Photograph No. 2: Bleeding a monitor, the blood to be used as an antigen.*



*Photograph No. 3:* Bleeding a large crocodile, the blood to be used as an antigen.



*Photograph No. 4:* Mr. GIBBINS, Assistant at the Entebbe Laboratory, drawing off the blood of a large crocodile which had died a natural death.

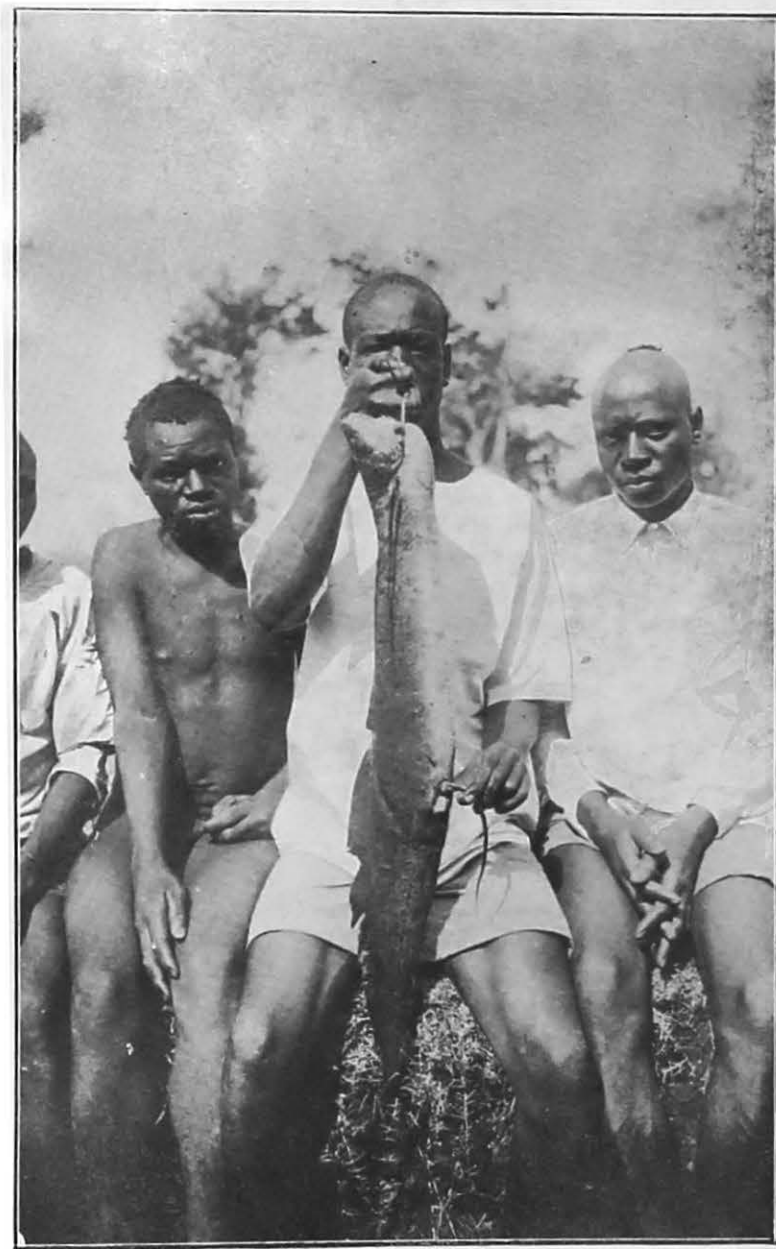




*Photograph No. 5 :* Rearing crocodiles hatched from eggs in the Laboratory incubator. On the right is seen a box containing eggs.



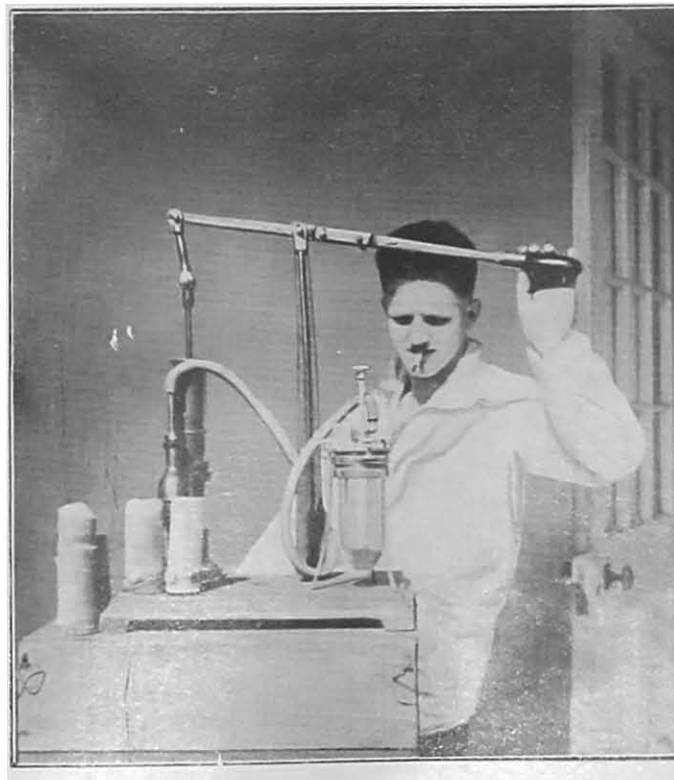
*Photograph No. 6:* Bleeding a crocodile brought to the Laboratory alive.



*Photograph No. 7:* Sleeping-sickness patients showing the lung-fish (*Proptopterus*), which comes up to the surface of the water to breathe close to the haunts of tsetse flies.

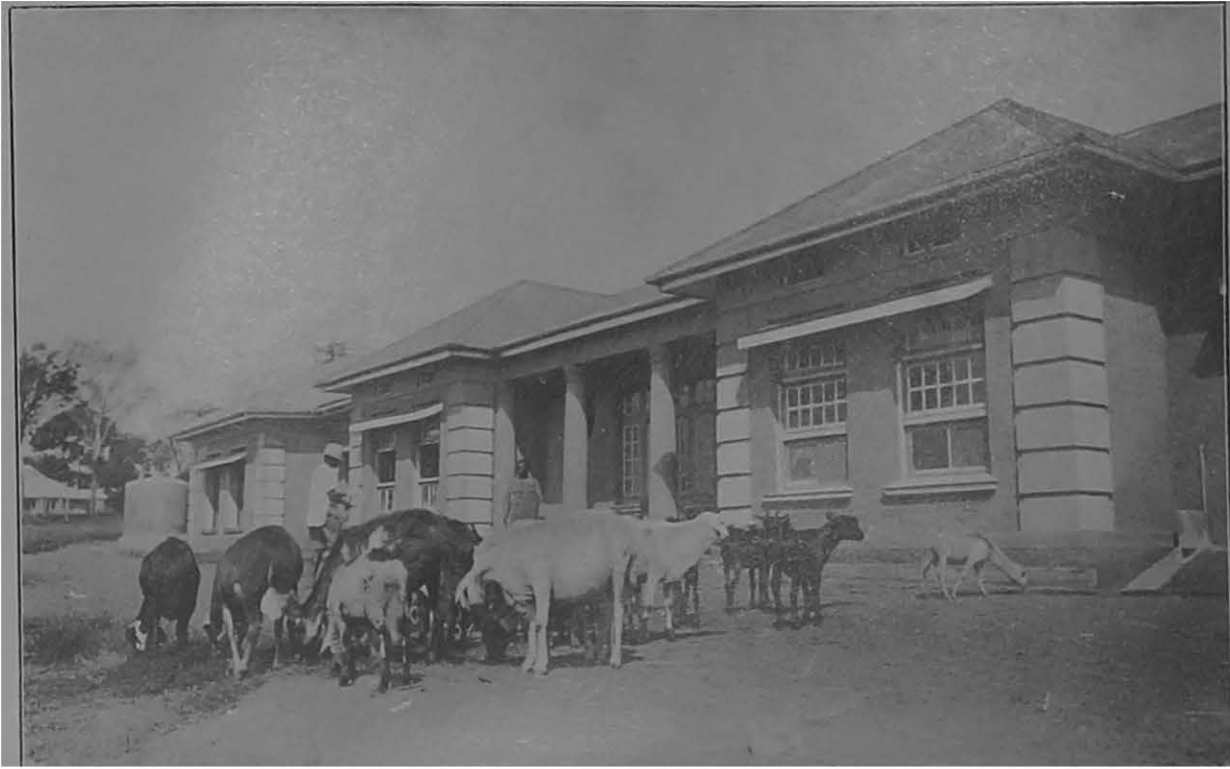


*Photograph No. 8:* Material and apparatus for the precipitin test; the native is holding the book in which the antigen papers are kept; behind are seen the ordinary tubes containing the frothy antigen solution, and in front the smaller tubes for the precipitin test.



*Photograph No. 9:* Apparatus for filling ampoules with anti-serum by the vacuum process. Shows the central rod used to raise the ampoules above the surface of the serum.



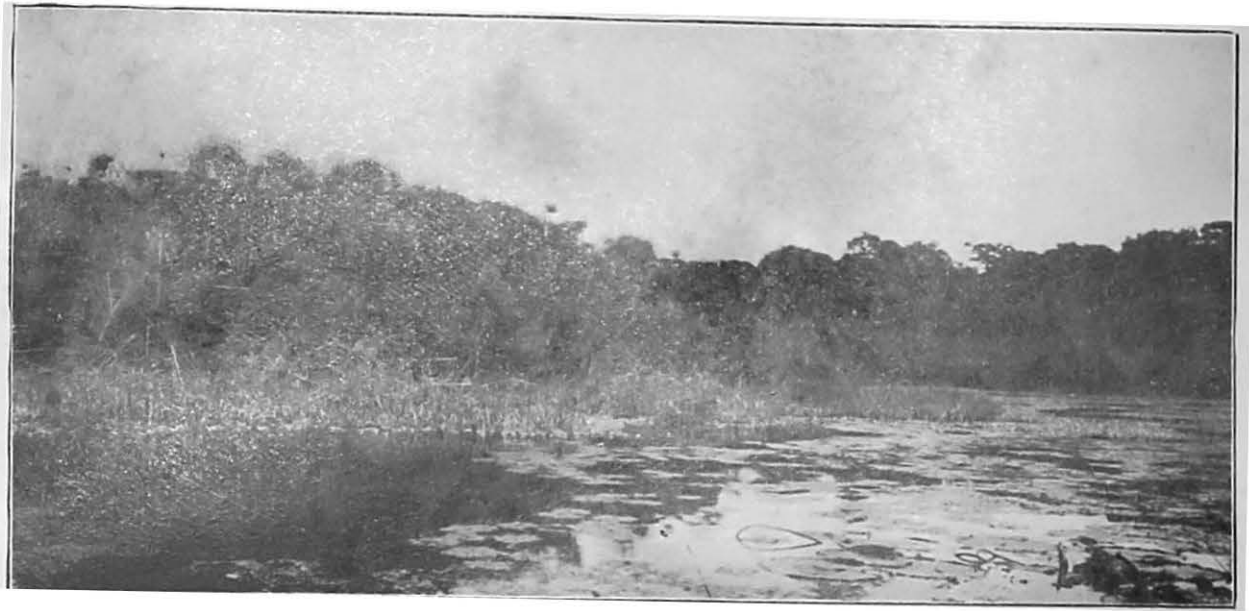


*Photograph No. 10:* Bacteriological Section of the Entebbe Laboratory.



*Photograph No. 11:* Entebbe Laboratory and Lake Victoria.

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*Photographs Nos. 12 and 13: Two views of Glossina palpalis breeding-grounds on the shores of Lake Victoria ; thick bush coming down to the water's edge or separated from the Lake by a bank of papyrus which is seen between the trees and the water,*



*Photograph No. 14:* Fly-boys catching tsetse flies in a clearing on the shores of Lake Victoria Nyanza.



*Photograph No. 15:* The head fly-boy (who worked with Sir David BRUCE) catching flies in the bush near the Entebbe Laboratory.



*Photographs No. 16 and 17:* Fly-boys looking for glossina pupæ in the sand, under the vegetation on the shores of the Lake.





*Photograph No. 18:* Saucer containing about twenty thousand pupæ from which the laboratory-bred flies were hatched which the League of Nations Commission used in its researches.



*Photograph No. 19:* View of bush inhabited by the *Glossina palpalis*, bordering on Uganda rivers.



*Photograph No. 20:* Road through the forest.

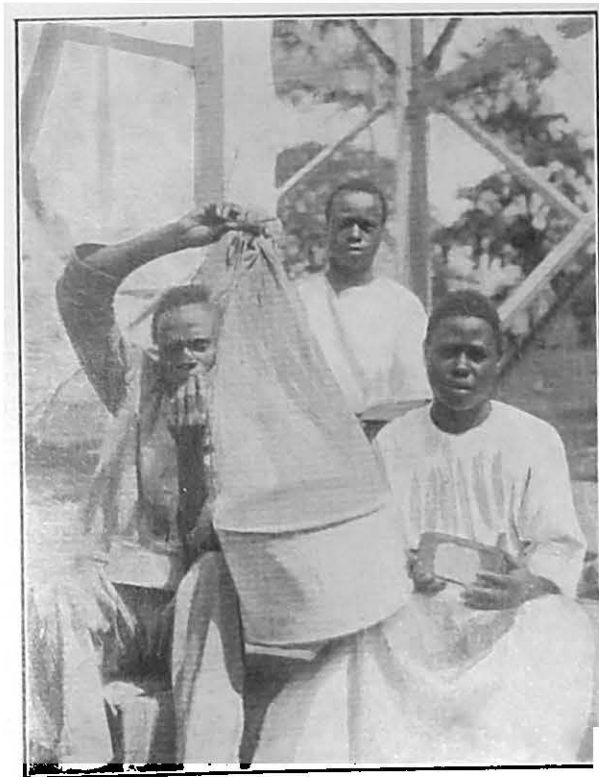
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*Photograph No. 21:* Panorama of a papyrus-covered river. "Raffia" palms are seen and, behind, the forest on the opposite bank.



*Photograph No. 22:* One of the little bridges on a road which crosses the papyrus-covered river. Behind are seen "raffia" palms and forest.

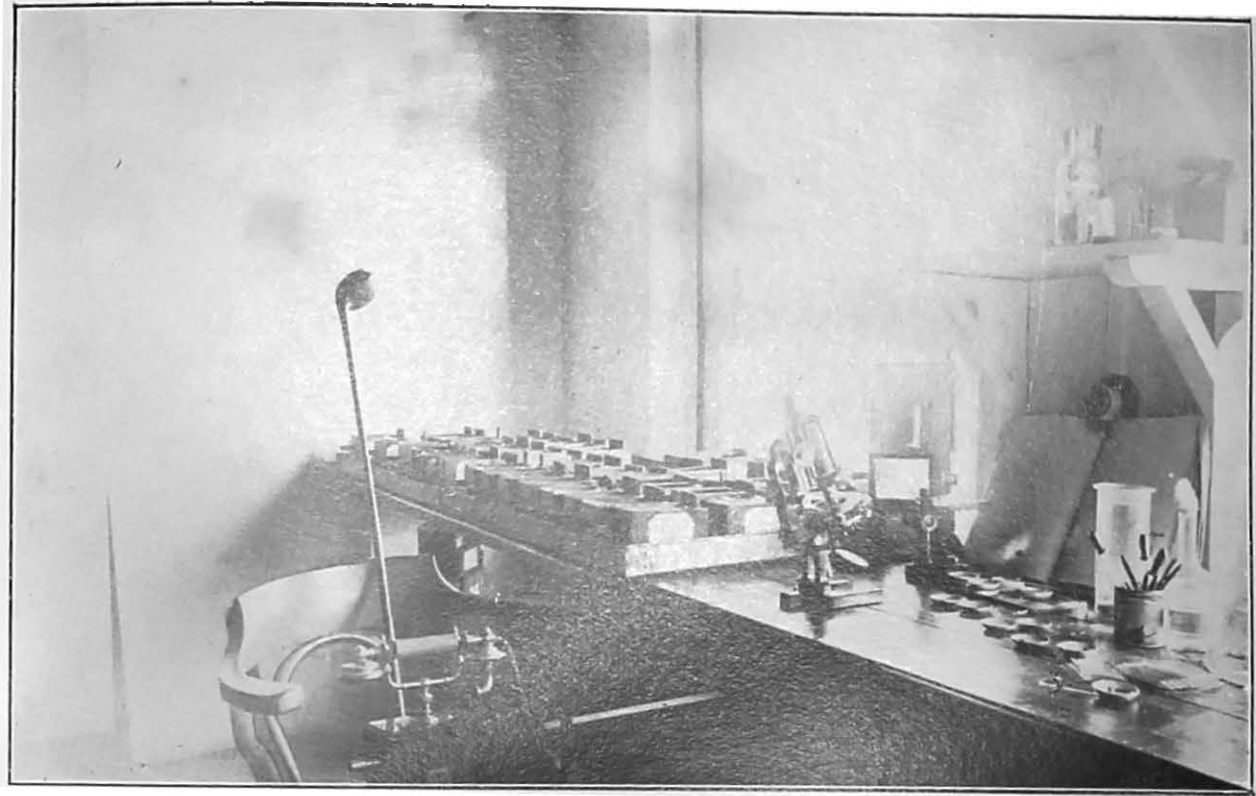


*Photograph No. 23:* Net bag in which the glossinæ hatch out from their pupæ. When hatched, they are placed in the box which the fly-boy on the right is holding.



*Photograph No. 24:* Fly-boy catching glossinæ on one of the few rivers not covered with papyrus.

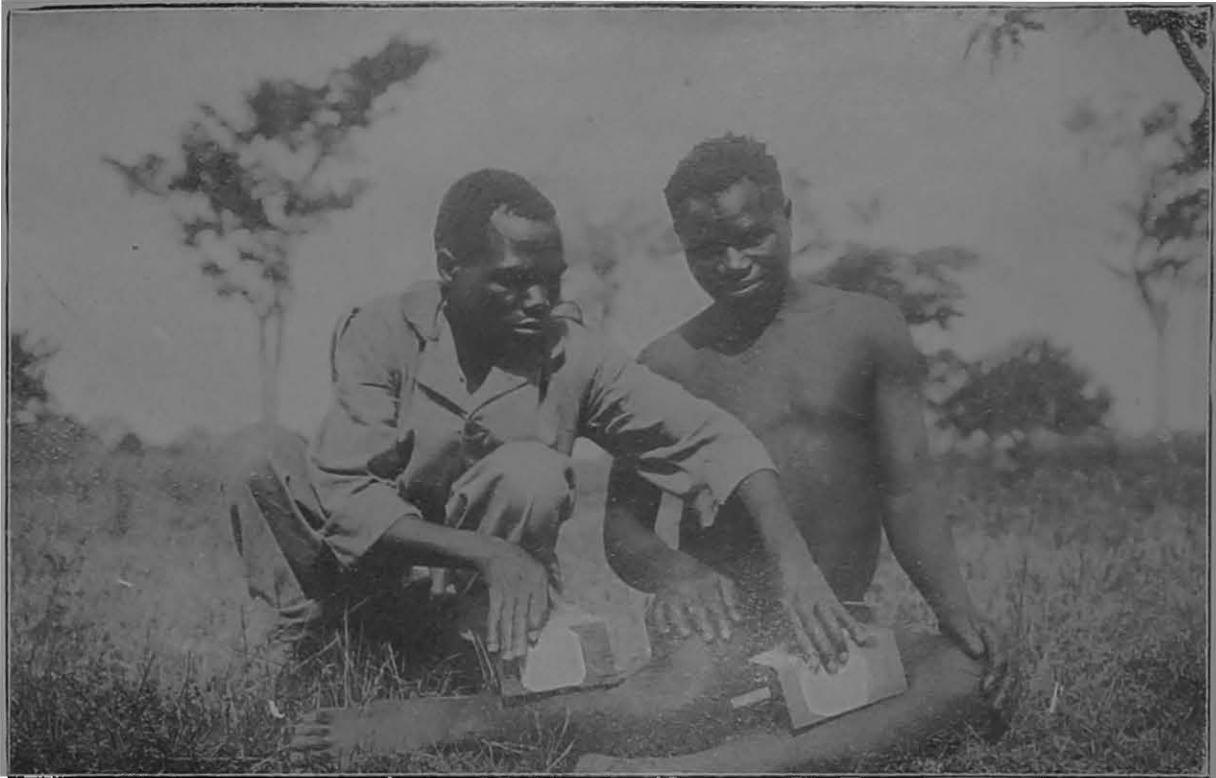




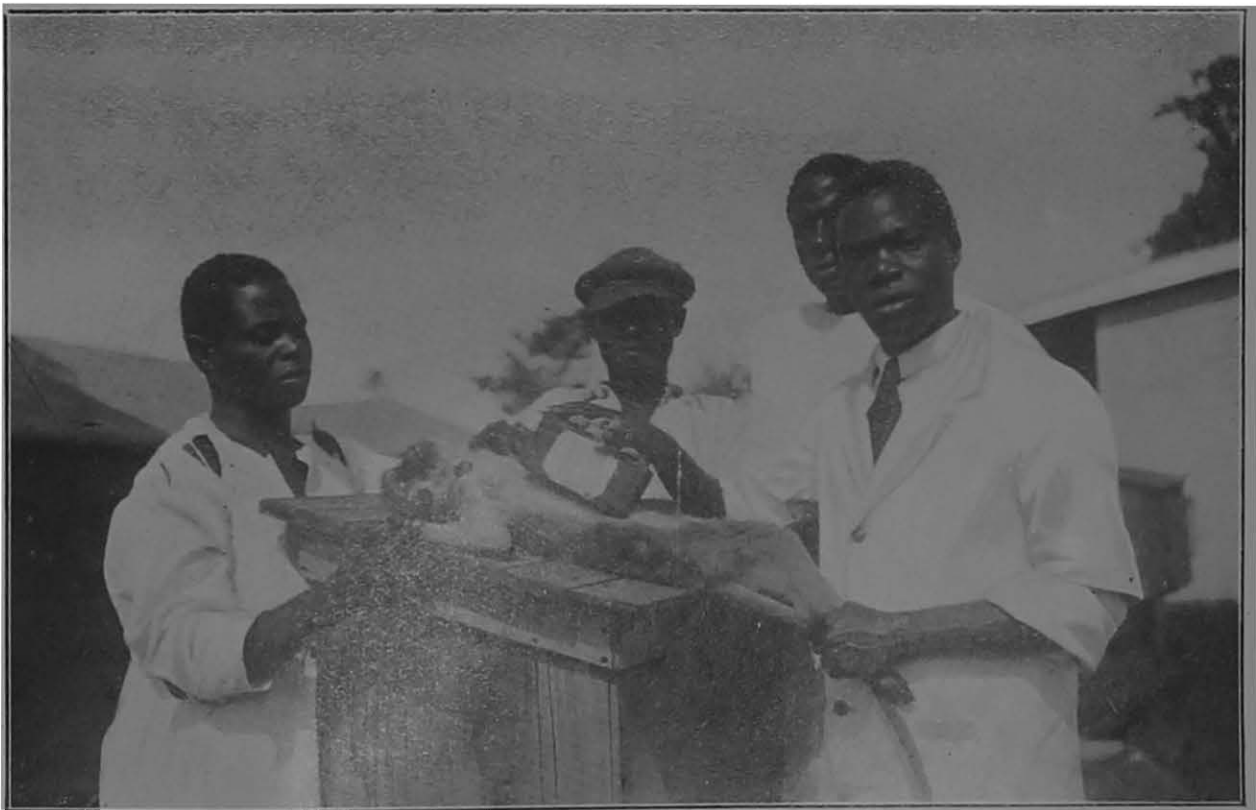
*Photograph No. 25:* Dr. DUKE's work-table. On the left, glossinæ boxes in trays containing water. On the right, dishes with dead flies awaiting examination.



*Photograph No. 26:* Laboratory-bred flies being placed on Congo sleeping-sickness patients, with the object of infecting the glossinæ.



*Photograph No. 27 :* Laboratory-bred flies being placed on Congo sleeping-sickness patients, with the object of infecting the glossinæ.



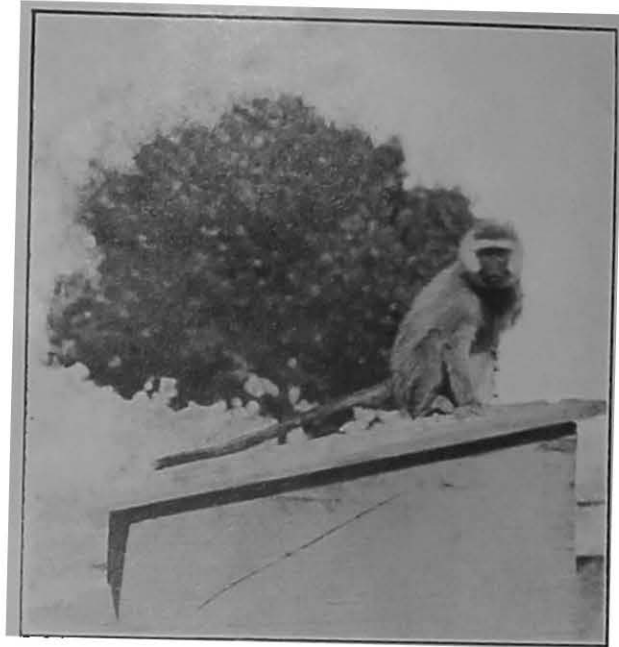
*Photographs Nos. 28, 29 and 30 :* Glossinæ infected by the trypanosomes of sleeping-sickness patients being placed on a monkey (Photograph No. 28), a sheep (Photograph No. 29) and a calf (Photograph No. 30).



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Two monkeys susceptible to human trypanosomes, employed at the Entebbe Laboratory: large monkey (*vide* Photograph No. 31) and small monkey (*vide* Photograph No. 32). General view of the monkey village at the Entebbe Laboratory (*vide* Photograph No. 33).

*Animals immune to human trypanosomes, employed  
at the Laboratory for feeding glossinæ.*

34



*Photograph No. 34: Big baboon with young.*

35



*Photograph No. 35: Mangaby.*

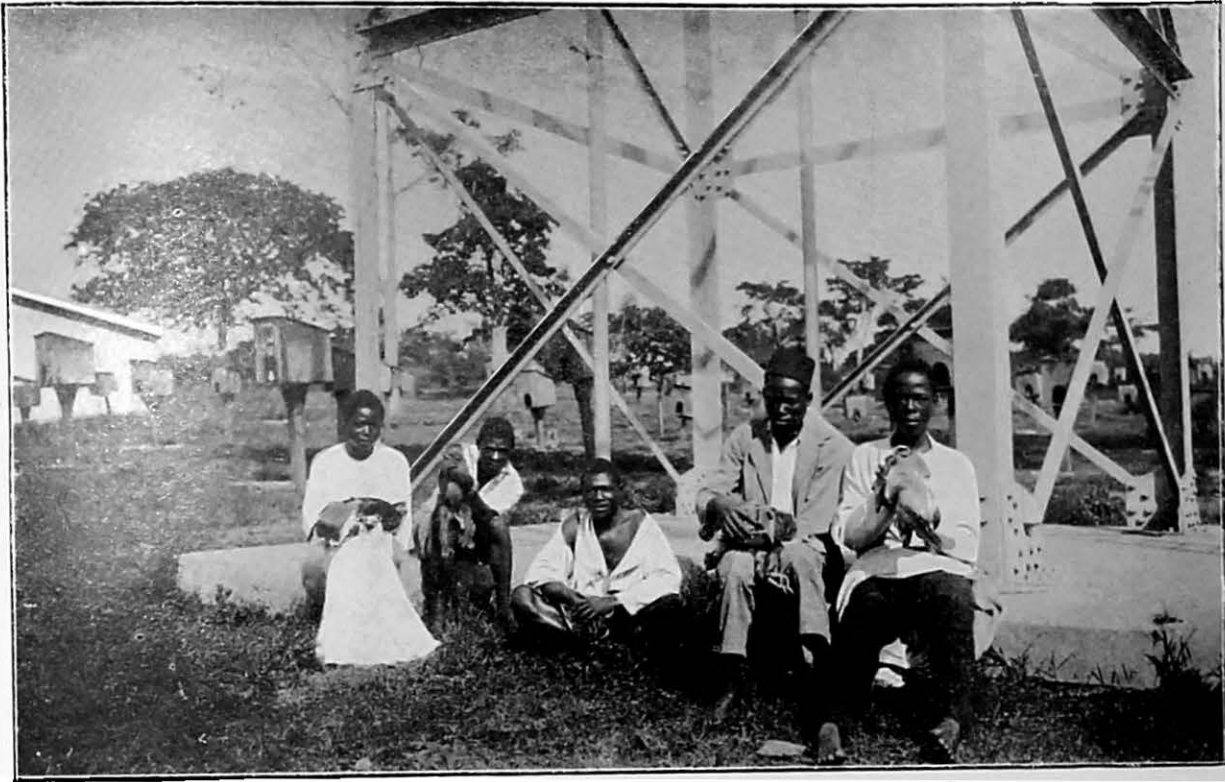




*Photograph No. 36 :* Baboon houses.



*Photograph No. 37 :* Chicken-run with hens partially plucked, ready for the application of glossina boxes.



*Photograph No. 38:* Natives feeding glossinæ on animals immune to human trypanosomes (hens and baboons).

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V.

**PATHOLOGICO-ANATOMICAL AND SEROLOGICAL OBSERVATIONS  
ON THE TRYPANOSOMIASES**

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1.

## THE PATHOGENIC POTENCY OF SOME TRYPANOSOMES

(POST-MORTEM INVESTIGATION ON FIFTY-THREE MONKEYS)

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The animals I examined numbered seventy, of which fifty-three were monkeys (*Cercopithecus sp.*). I shall give here the results obtained in the latter because they form a homogeneous and relatively important group, and because the lesions found strongly resemble those known in man from the few rare autopsies completed with histological examination. This group also has the advantage of furnishing conditions very favourable for the comparative study of the strains used. For this purpose, all the experiments were made on a uniform plan : microscopic examination was made of all the organs even when they showed no appreciable change macroscopically or only common lesions. It must also be mentioned that very often it was not possible to determine the cause of death at the autopsy. If I had not resorted to other more precise, sometimes very laborious, methods, the interpretation of the facts would have been unsafe, and some unexpected and extremely interesting lesions would even have been passed over unobserved. Of the fifty-three monkeys examined post mortem, fifty were inoculated and forty-three showed trypanosomes in their blood ; the others, inoculated without a result, or dead at the outset of their captivity, were examined so as to have controls and particulars of the common complications observed in the monkeys of the first group (malaria, filariasis, porocephalasis, etc.).

The experimental animals were infected either by cyclical transmission (*Glossina palpalis*) or by direct inoculation with trypanosomes isolated in man or animals from various sources (Uganda, Kenya, Damba Island, Tanganyika, Congo) having different carriers (*G. palpalis*, *G. morsitans*, *G. swynnertoni*).

The number of animals simultaneously under experiment was too large to allow the symptoms to be followed attentively. Blood examination was repeated daily until the appearance of the trypanosomes, and from time to time in animals which had been inoculated for a long time or whose general condition was obviously bad.

The very frequent association of the trypanosomiasis with malaria and the restlessness of the recently captured monkeys rendered any study of the pulse and temperature in vain.

In rare cases, it was possible to ascertain a distinct and persistent state of somnolence in monkeys that, post mortem, showed well-characterised cerebral lesions. Lastly, a true cachexia characterised the end of the infections that ran a slow course. When, on the other hand, the development had been rapid, death was preceded by very serious symptoms. In some cases, it was possible to observe an almost sudden death occurring in animals that had shown evident signs of cardiac insufficiency.

In the following table, I summarise the general results :

TABLE OF MONKEYS EXAMINED.

A. *Trypanosomes of Human Origin from G. palpalis (gambiense Type).*

Monkey No.	Date			Strain	Transm.	Duration: days	Anatomical changes
	inoc.	+	died				
354	12 V 27	17 V 27	9 VI 27	G (?) Risiki II pass.	direct	28	Hæmorrhagic hypersplenia; incipient myocarditis; pericarditis; meningo-encephalitis; infiltrative lesions in liver, suprarenals, kidney.
353	12 V 27	17 V 27	10 VI 27	G (?) Risiki II pass.	direct	29(?)	Hypersplenia; incipient epicarditis and myocarditis; cellular infiltrations in liver, suprarenals, salivary glands.
260	27 XII 26	19 I 27	28 II 27	G Bochi II pass.	direct	61	Hæmorrhagic hypersplenia; incipient meningo-encephalitis; cellular infiltrations in liver, salivary glands. Complication: terminal pasteurellosis.
258	27 XII 26	25 I 27	4 IV 27	G Acholi I pass.	direct	98	Hypersplenia with incipient sclerosis; pericarditis; incipient meningitis; cellular infiltrations in liver. Complications: skin wound, terminal streptococcal septicæmia, malaria.
222	28 X 26	30 XI 26	14 II 27	G Dabiro I pass.	cycl.	107	Hypersplenia, perisplenitis; incipient meningo-encephalitis; cellular infiltrations in liver. Complications: terminal septic peritonitis; filariasis.
287	10 II 27	24 II 27	9 VI 27	G Kakowa I pass.	cycl.	120	Hypersplenia; incipient pericarditis.
252	17 XI 26	20 XII 26	1 IV 27	G Kavirondo (KL.) I pass.	cycl.	135	Fibrous perisplenitis, splenitis and lymphadenitis; myocarditis; pericarditis; incipient meningo-encephalitis. Complications: terminal staphylococæmia, malaria, filariasis.
276	17 I 27	—	10 VI 27 killed	G Awasi II pass.		173	Hypersplenic with incipient cirrhosis; advanced pericarditis and myocarditis; cellular infiltrations in liver, choroid plexuses, salivary glands. Complication: malaria.
237	7 XI 26	5 XII 26	22 V 27	G Awunu III pass. atoxyl	cycl.	197	Cirrhosis of spleen and ganglia; advanced pericarditis and myocarditis; thickening of choroid plexuses; sub-ependyma cellular infiltrations. Complication: cerebral filariasis.
112	10 VIII 26	24 VIII 26	20 IV 27	G Tekisoka I pass. atoxyl.	cycl.	240	Cirrhosis of spleen and ganglia; fibrous epicarditis and myocarditis; meningo-encephalitis; cellular infiltrations of liver and salivary glands; gelatiniform bone-marrow. Complication: cerebral filariasis.
96	21 V 26	11 VI 26	26 II 27	G Blasio I pass.	direct	245	Cirrhosis of the spleen; hyperplasia of ganglia and bone-marrow; infiltrative and fibrous lesions of the myocardium, liver, kidney. Complications: filariasis, malaria, erratic helminthiasis and peritonitis.
80	11 V 26	28 VI 26	22 II 27	G Walubani III pass.	direct	281	Subcapsular cirrhosis and hæmorrhages of the spleen; cellular infiltrations of the liver; incipient meningo-encephalitis. Complication: malaria.

A. *Trypanosomes of Human Origin from G. palpalis (gambiense Type) (continuation).*

Monkey No.	Date			Strain	Transm.	Duration: days	Anatomical changes
	inoc.	+	died				
126	30 VI 26	17 VII 26	1 V 27	G Adjambo II pass.	cycl.	300	Cirrhosis of spleen, myocardium, kidney; fibrous vaginalitis; thickening of epiploön; colloid degeneration of bone-marrow; incipient meningo-encephalitis. Complications: malaria, porocephaliasis.
41	3 III 26	10 V 23	24 VI 27 killed	G Walubani II pass.	cycl.	478	Hypersplenia with fibrosis; exudative pericarditis; meningo-encephalitis. Complications: subcutaneous and cerebral filariasis.

B. *Trypanosomes of Human Origin from G. morsitans and G. swynn. (rhodesiense Type).*

Monkey No.	Date			Strain	Transm.	Duration: days	Anatomical changes
	inoc.	+	died				
338	28 IV 27	3 V 27	8 V 27	R Boga V pass.	direct	10	Hæmorrhagic hypersplenia; hæmatopericardium due to recent cardiac puncture; pericarditis; infiltrative lesions of the choroid plexuses.
307	21 III 27	28 III 27	1 IV 27	R Boga IV pass.	direct	11	Hæmorrhagic hypersplenia; incipient pericarditis; interstitial myocarditis in foci; cellular infiltrations of liver and suprarenals; incipient meningitis.
308	21 III 27	28 III 27	1 IV 27	R Boga IV pass.	direct	11	Hæmorrhagic hypersplenia; incipient myocarditis and pericarditis; cellular infiltrations of liver and suprarenals.
312	25 III 27	30 III 27	7 IV 27	R Boga V pass.	direct	13	Hæmorrhagic hypersplenia; œdema of myocardium; epididymitis; cellular infiltrations and degeneration of liver. Complication: malaria.
281	27 I 27	1 II 27	10 II 27	R Kimori III pass.	direct	14	Hæmorrhagic hypersplenia; exudative myocarditis and pericarditis; exudative epididymitis and vaginalitis; incipient meningo-encephalitis. Complication: malaria.
351	8 V 27	13 V 27	22 V 27	R Boga VI pass.	direct	14	Hypersplenia; hæmorrhages of spleen, liver, gastric mucosa; œdema of myocardium; exudative pericarditis.
282	29 I 27	5 II 27	19 II 27	R Boga III pass.	direct	20	Hypersplenia; myocarditis; pericarditis; cellular infiltrations of liver and suprarenals; incipient meningitis. Complication: malaria.
309	21 III 27	28 III 27	14 IV 27	R Boga IV pass.	direct	24	Hæmorrhagic hypersplenia; exudative pericarditis; incipient myocarditis; vaginalitis; cellular infiltrations of the liver and choroid plexuses.
314	26 III 27	7 IV 27	22 IV 27	R Boga V pass.	cycl.	28	Hæmorrhagic hypersplenia; exudative pericarditis; myocarditis; cellular infiltrations of liver, kidney, suprarenals, choroid plexuses. Complication: terminal bacteriæmia.
313	25 III 27	9 IV 27	26 IV 27	R Boga V pass.	direct	31	Hæmorrhagic hypersplenia; myocarditis; exudative pericarditis; nephritis with large white kidney; incipient meningo-encephalitis; cellular infiltrations of liver, suprarenals, salivary glands, genital organs, skin.
311	25 III 27	5 IV 27	29 IV 27	R Boga V. pass.	direct	34	Hæmorrhagic hypersplenia; exudative polyserositis; myocarditis; nephritis; incipient meningo-encephalitis; cellular infiltrations of liver, salivary glands, suprarenals, skin.

C. *Trypanosomes of Animal Origin, from G. palpalis*  
(*T. of Damba Island Antelope*).

Monkey No.	Date			Strain	Transm.	Duration: days	Anatomical changes
	inoc.	+	died				
365	23 V 27	28 V 27	31 V 27	D V pass.	direct	8	Hæmorrhagic hypersplenia; exudative pericarditis; incipient myocarditis and infiltrations of choroid plexuses.
360	19 V 27	27 V 27	9 VI 27	D VI pass.	direct	22	Hæmorrhagic hypersplenia; hæmorrhages of the mucous membranes; exudative pericarditis; incipient myocarditis; cellular infiltrations of the suprarenal capsules and choroid plexuses. Complication: porocephaliasis.
263	12 I	—	15 II	D II pass.	direct	33	Hypersplenia; hæmorrhages of the skin and of the mucous membranes of the liver; exudative pericarditis; cellular infiltrations of the liver. Complications: erratic helminthiasis and localised peritonitis.
265	20 I	4 II	24 III	D III pass.	direct	64	Hypersplenia; pericarditis and myocarditis; nephritis; vaginalitis; incipient meningo-encephalitis; cellular infiltrations of the salivary glands and suprarenals. Complication: malaria.
292	16 II 27	23 II 27	22 IV 27	D XXIII pass. cycl. transm.	direct	66	Hypersplenia; pericarditis; myocarditis; cellular infiltrations of kidneys, suprarenals and salivary glands. Complication: terminal peritonitis.
199	4 X 26	12 X 26	18 II 27	D pass.	cycl.	136	Hypersplenia; myocarditis; polyserositis; incipient meningitis and nephritis; cellular infiltrations of liver and suprarenals.
208	23 X 26	16 XI 26	17 V 27	D pass.	direct	206	Hypersplenia with incipient cirrhosis; exudative pericarditis; myocarditis with incipient fibrosis; meningo-encephalitis; cellular infiltrations of salivary glands and genital organs.
180	31 VIII 26	7 IX 26	28 IV 27	D XI pass.	direct	208	Hypersplenia with incipient cirrhosis; exudative pericarditis; myocarditis; meningo-encephalitis; cellular infiltrations of liver, suprarenals, salivary glands. Complication: streptococcal septicæmia.
122	29 VI 26	12 VIII 26	18 III 27	D pass. atoxyl	cycl.	232	Cirrhosis of spleen, myocardium, kidney and liver; incipient meningo-encephalitis; colloid degeneration of bone-marrow. Complication: malaria
121	29 VI 26	10 VII 26	29 III 27	D pass.	cycl.	275	Cirrhosis of the spleen, exudative pericarditis; incipient myocarditis; vaginalitis and epididymitis; cellular infiltrations of the suprarenal capsules.
111	12 VI 26	1 VII 26	26 III 27	D pass. atoxyl trypars.	cycl.	289	Hæmorrhagic hypersplenia; exudative myocarditis, pericarditis and vaginalitis; incipient meningo-encephalitis; cellular infiltrations of the liver; suprarenals, salivary glands.
3	21 XII 25	11 I 26	15 II 27	D XIX pass.	cycl.	426	Hypersplenia with cirrhosis; exudative pericarditis; myocarditis; meningo-encephalitis; cellular infiltrations of the liver. Complication: porocephaliasis.

D. *Trypanosomes of Animal Origin from G. morsitans (brucei Type).*

Monkey No.	Date			Strain	Transm.	Duration: days	Anatomical changes
	inoc.	+	died				
286	9 II 27	15 II 27	17 II 27	B Tanganyika II pass.	direct	8	Hæmorrhagic hypersplenia; hæmorrhages of the gastric mucosa; cellular infiltrations of liver. Complications: malaria, filariasis.
327	10 IV 27	15 IV 27	18 IV 27	B Uganda II pass.	direct	8	Œdema and incipient cellular infiltrations of the myocardium; cellular infiltrations of epiploön; long-standing cirrhosis. Complications: malaria, filariasis, œsophagostomatosis.
336	27 IV 27	2 V 27	21 V 27	B Uganda pass.	direct	24	Hæmorrhagic hypersplenia; incipient myocarditis; incipient exudative pericarditis; nephritis; infiltrative and degenerative lesions of liver.
337	27 IV	2 V	28 V	B Uganda pass.	direct	31	Hypersplenia; incipient myocarditis; cellular infiltrations of the choroid plexuses; degenerative lesions of liver.
335	27 IV 27	29 V 27	2 V 27	B Tanganyika IV pass.	direct	32	Hæmorrhagic hypersplenia; exudative polyserositis; incipient myocarditis; cellular infiltrations of the choroid plexuses. Complication: cerebral filariasis.
294	17 II 27	24 II 27	28 III 27	B Tanganyika III pass.	direct	40	Hæmorrhagic hypersplenia; exudative pericarditis; incipient myocarditis; cellular infiltrations of the choroid plexuses.

*Negative or Uninoculated Monkeys.*

Monkey No.		Died.	Anatomical changes
210	Negative	27 III 27	Splenic and hepatic cirrhosis; malaria.
232	Negative	16 II 27	Pulmonary abscess and peritonitis from erratic nematodes; filariasis.
261	Negative	14 II 27	Peritonitis; porocephaliasis; filariasis.
269	Negative	8 III 27	Streptococcal fibrinous pleuro-pericarditis.
271	Negative	29 III 27	Ulcerous wound of the abdominal wall; septic peritonitis.
279	Negative	10 VI 27	Splenic and incipient hepatic cirrhosis; chronic peritonitis.
985	Negative	31 V 27	Staphylococcal septicæmia; skin wound.
Autop. XV	Not inoc.	24 II 27	Diplococcal meningo-encephalitis.
Autop. XXVII	Not inoc.	26 III 27	Traumatic lesions (fracture of skull, subdural hæmorrhage); filariasis.
Autop. XXVIII	Not inoc.	26 III	Traumatic lesions.

## STATISTICAL AND CRITICAL STUDY OF THE MORBID ANATOMICAL CHANGES.

As far as standard treatises are concerned, the morbid anatomy of sleeping-sickness and the trypanosomiasis will be summed up in changes of the blood, lymphatic organs and nervous system. In some animals, special symptoms have been noted in the skin, genital organs and eye, but numerous experiments have shown that these lesions are not absolutely characteristic of different species of trypanosomes, nor even of different species of susceptible animals, and the latest works have brought to light very common histological lesions observable in nearly all the organs.

The researches on this subject by WOLBACH AND BINGER have shown the proliferative nature and interstitial site of these very scattered though limited lesions due to the ubiquity of the parasites and their habitual presence in the lymphatic spaces.

These observations have perceptibly modified the pathogenic conception of the trypanosomiasis and suggested analogies with other protozoal diseases, particularly syphilis. Nevertheless, numerous uncertainties still obscure the mechanism of the pathogenesis, notably as regards the determining cause of death from trypanosomiasis.

The disease is frequently complicated by other affections, either concurrent or terminal, sometimes to the extent of rendering impossible the interpretation of the morbid anatomical picture, and experimental pathology has hitherto yielded only indifferent results, considering the nearly always unfavourable conditions as to quality of the viruses, means of transmission, species of animals ordinarily used, and the difficulty encountered in demonstrating parasites in the lesions found.

In this respect, the morbid anatomical material I have been able to draw upon was very valuable; instead of finding very monotonous or slightly marked lesions, such as one generally obtains in laboratory animals by means of virus increased in potency by very numerous passages, I was able to find in the monkeys well-characterised specific lesions, very interesting from their close connection with human pathology.

### A. *Changes in the Serous Membranes.*

In the literature of trypanosomiasis, one finds numerous observations of serous effusions in the pleuræ, pericardium and peritoneum considered as transudates and regarded in the same light as the œdema that very often accompanies them.

The presence of trypanosomes has also been noted in these fluids, where they were sometimes very numerous in comparison with those that could be found in the blood. CLAPIER found them in large numbers in the fluid of hydroceles, and NEWHAM in the peritoneal fluid.

The morbid anatomical observations I have made on this subject have led me to consider the existence of true trypanosomal serositis and polyserositis.

Indeed, from the very first cases examined and nearly always afterwards, I was easily able to demonstrate the typical character of the inflammatory exudates in the fluids of the pericardium, peritoneum and tunica vaginalis.

The precautions taken at the time of collecting the fluids sufficiently eliminate possible errors; when cytological examination of the fluids revealed blood elements, it could be said with certainty that they had not been introduced artificially. Moreover,

I have often had the opportunity of examining the effusions of the tunica vaginalis in sections of preparations fixed without the sac having been opened, and of judging the morphological composition.

The quantity of fluid drawn off was nearly always enough for the Rivalta test, for examination between slide and coverslip and for the preparation of several smears. Very often I was even able to make researches *in vitro* and to observe either the formation of fibrinous networks resembling cobwebs or else massive coagulation.

I refrained from centrifuging in order always to obtain comparable results and not in any way to disturb the cytological formula of the exudate.

When the quantity of fluid was insufficient to allow these researches, I confined myself to a few smears, applying the slides to the carefully exposed organs.

Apart from easily discernible post-mortem changes, these exudates showed the following characteristics : They were yellowish liquids, sometimes tinged with red, turbid, of definitely positive Rivalta reaction, and within a few hours showed *in vitro* cobweb-like fibrinous precipitates or massive coagulation.

In cases examined immediately after death, microscopic examination between slide and coverslip showed living trypanosomes, sometimes extremely numerous and always much more so than in the blood. In cases showing incipient putrefaction, the trypanosomes were non-motile, changed, or not demonstrable.

The examination of the Giemsa-stained smears confirmed the presence of the parasites if they were sufficiently well preserved, and sometimes yielded information on the existence of other micro-organism that had caused septic complications or originated in post-mortem contaminations ; but this examination was chiefly useful in revealing the cytological character of the exudates. (See Fig. 4, Plate I.)

The most certain results given above were therefore obtained in cases examined immediately or less than three hours after death, and in the absence of other infectious complications. The effusions most noticeable by their frequency and quantity were those in the pericardium ; in the peritoneum, the quantities were very much less and rarely reached 0.5 c.c. ; nevertheless, in monkey *III*, which was infected by a Damba antelope strain and died after 284 days, there was a serosanguinolent effusion of 25 c.c., with a small capsular laceration of the spleen. In the tunica vaginalis I found considerable effusions more rarely. The pleura hardly ever showed any appreciable change.

Most frequently it was only in the pericardium that I found any collection of fluid ; sometimes it was accompanied by a noticeable increase of the peritoneal fluid. Effusions of the tunica vaginalis, which were rarer, were constantly associated with an abundant pericardial effusion.

The most constant cytological formula of the pericardial exudates is the following :

Globular cells of endothelial origin . . . . .	12
Small basophile mononuclears with nuclear displacement . . . . .	25
Mononuclears with horseshoe nucleus . . . . .	35
Lymphocytes . . . . .	23
Neutrophile polynuclears . . . . .	5
Erythrocytes . . . . .	10

In the exudates of the peritoneum and tunica vaginalis, the leucocytes and erythrocytes were sometimes more numerous.



The following table summarises the most characteristic cases :

Monkey No.	Strain and transmission	Duration of the infection	Quantity of exudates			Trypanosomes
			Peric.	Perit.	Tunica vagin.	
252	G c	135 days	3 c.c.	—	—	Very scarce
307	R d	11 days	0.25 c.c.	—	—	Few
312	R d	13 days	2 c.c.	—	traces	Very numerous
313	R d	19 days	3 c.c.	—	—	Very numerous
282	R d	20 days	4 c.c.	traces	—	Very numerous
311	R d	22 days	0.5 c.c.	—	—	Very numerous
281	R d	26 days	6.5 c.c.	—	traces	Numerous
292	D d	66 days	2 c.c.	—	—	Very numerous
199	D c	136 days	0.25 c.c.	traces	—	Very numerous
122	D c	232 days	4.5 c.c.	—	—	Scarce
121	D c	275 days	3 c.c.	—	traces	Very numerous
3	D c	426 days	4 c.c.	traces	—	Very numerous

This table shows the importance of pericardial effusion and its co-existence with exudates of the tunica vaginalis and peritoneum. These effusions are rarer in monkeys inoculated with trypanosomes of the *gambiense* type, which nevertheless produced infections that were both long and debilitating. Indeed, histological investigations on sections revealed in the thickness of the serous membranes a true inflammatory process due to the presence of trypanosomes. (See Figs. 1, 2, 3, Plate I.)

The most prominent changes were in the epicardium, epiploön and parietal tunica vaginalis; they were characterised by copious cellular infiltrations of histogenous and lymphatic origin, which very often began in the neighbourhood of vascular formations and invaded the interstitial spaces. They consisted of the same exudative elements as are found in the effusions in addition to some plasma cells, sometimes polynuclear macrophages and numerous leucocytes. The lymphatic vessels were tightly packed with these elements and a few small hæmorrhages by diapedesis revealed changes of the capillary walls. Trypanosomes were very numerous in these lesions, either free in the interstitial lacunæ or enclosed in the protoplasm of the phagocytic elements.

Investigations on sections of the mesentery, abdominal wall and parietal pericardium revealed only very limited phenomena comparable to those in the other tissues.

The same is true of the sections of visceral organs, which did not present any symptoms equal to those observed on the surface of the peritoneum. These are therefore true specific localisations and we shall see later on their continuity with deeper lesions, especially when we come to speak of the changes in the myocardium.

Although the question of trypanosomal serositis has not yet been considered in human pathology, it is probable that the same lesions are present in sleeping-sickness.

In the autopsies, effusions were very often observed in the pericardium and peritoneum. Otherwise it would have been impossible to discover trypanosomes in them because of post-mortem phenomena, but the cytological formula would doubtless have revealed their inflammatory nature.

It should be noted that hydrocele fluids rich in trypanosomes examined by CLAPIER contained numerous lymphocytes and small mononuclears. Here, then, are researches to be made which, even in a few patients, might prove useful from the point of view of diagnosis.

### B. Changes in the Myocardium.

The experimental lesions of the myocardium reported in the literature of the African trypanosomiasis are very limited. They deal particularly with regressive phenomena, such as granular degeneration, fatty infiltration, fibrillary fragmentation and small hæmorrhages (lesions frequently met with in the most diverse infectious diseases), or else with proliferation of the connective tissue. BATTAGLIA found lymphocytic infiltrations of basophile granulations in the tissue of the cardiac valves and large vessels as well as in the subendocardial and subpericardial tissue of dogs or rabbits inoculated with *T. vespertilionis* and *T. lewisi*.

A very severe specific myocarditic process, characterised at the beginning by regular massive deposits of the trypanosome, was one of the most interesting findings in my autopsies on monkeys. The extent and depth of the lesions were in some cases so great that no doubt was possible as to their importance as a direct cause of death.

The evident signs of pericarditis I was able to demonstrate right from the first autopsy on a monkey suggested to me, at the outset of these researches, the most careful study of the myocardium; nevertheless, it must be said that in general there were no important macroscopic changes in this quarter. The dilatation of the ventricles, the slightly greyish colour of the tissue and its diminished firmness might indeed be nothing but manifestations of common more or less advanced regressive changes. The œdema of the epicardium, however, which was very obvious at the base of the ventricles, and the cytological character of the pericardial effusion sometimes indicated an inflammatory condition.

In reality, it is only histological examination that could reveal the nature and importance of the lesions.

From the finding in the first cases of myocarditis accompanied by massive localisation of trypanosomes, I took care to prepare smears and stain them by Giemsa's method. These preparations, made with fresh sections of myocardium previously washed and dried, gave some interesting information.

The finding of numerous mononuclear cells denoted the presence of foreign elements of inflammatory infiltration in the muscle tissue, and the cases autopsied before the onset of post-mortem changes showed also remarkable quantities of free or phagocytosed trypanosomes morphologically typical or strangely modified.

The myocardium was often the seat of numerous infiltrations of small round cells, very limited and localised in the neighbourhood of vascular formations. These sometimes not very significant and very common changes were comparable to those found in other organs.

The trypanosomal myocarditis found in the monkeys, on the other hand, was an extremely serious process, always complicated by exudative pericarditis and capable of producing great destruction of the myocardium.

I found it in more or less advanced forms whose histological characteristics suggested a more or less chronic course. The specificity of the lesions was beyond doubt; there were enormous quantities of very characteristic trypanosomes, so that any confusion with other micro-organisms was impossible; moreover, smears of fresh myocardium, stained with Giemsa, showed as parasitic elements nothing but trypanosomes.

I will describe in more detail my histological observations of trypanosomal myocarditis later on (see pages 282-294); here I must confine myself to considerations of a general nature.

The histological manifestations at the outset consist of lesions of the capillaries and interstitial tissue, accompanied by marked œdema which strongly dissociates the muscle fibres and even their fibrils.

The trypanosomes multiply rapidly in the pericapillary spaces among a few lymphocytoid cells and macrophages ; they are specially numerous on the surface of the cardiac fibres and even penetrate into their interior.

The more advanced histological manifestations are a very extensive infiltration of round cells, in which the predominant elements are lymphocytoid cells with basophile protoplasm without granulations, lymphocytes, mononuclears with bilobate nucleus, very often charged with inclusions of clumped trypanosomes. Plasma cells, polynuclear leucocytes and a few erythrocytes are added in greater or less numbers to this multitude of elements which proliferate *in situ* and are strongly reminiscent of the proliferative infiltrations of granulomatous inflammations.

The muscle fibres are affected by dystrophic and regressive changes ; they lose their transverse striation and become greatly reduced by atrophic degeneration, but without reaching the stage of necrosis.

The trypanosomes are easily demonstrable among the infiltrative elements, inside the phagocytes, but as a general rule they are not very numerous in advanced manifestations ; very often they have a globular form reminiscent of *leishmania*.

Between the incipient phase and the advanced forms of the inflammatory process, I observed intermediary forms, which were the most numerous. The same changes were present, varying in intensity without presenting any essential difference. It is therefore only a single pathological process, a true specific inflammation of granulomatoid type. The different histological appearances depend on variable factors, notably the properties of the trypanosome in question.

In the following table, I summarise the most characteristic cases, particularly from the point of view of the possible determination of the parasites in the lesions :

Monkey No.	Strain and transmission	Duration of the infection	Pericardial effusion	Lesions in the myocardium
252	G c	135 days	3 c.c.	Not very advanced
237	G d	197 days	traces	Very advanced
312	R d	13 days	2 c.c.	Initial stage
351	R d	14 days	3 c.c.	Initial stage
281	R d	26 days	6.5 c.c.	Not very advanced
282	R d	20 days	4 c.c.	Advanced
313	R d	19 days	3 c.c.	Initial stage
311	R d	22 days	0.5 c.c.	Initial stage
265	D d	64 days	—	Very advanced
121	D c	275 days	3 c.c.	Advanced
111	D c	289 days	7.5 c.c.	Very advanced
335	B d	32 days	0.5 c.c.	Initial stage
336	B d	24 days	0.5 c.c.	Initial stage

As one can easily see from this table, the lesions do not seem to depend directly upon the duration of the infection ; I can, moreover, state that they had no relation even to the number of trypanosomes in the blood. They were therefore due to an occasional localisation, which probably may occur at various stages of the disease.

Since these lesions have all the characteristics of proliferative inflammation, I expected to find some cases with fibrous lesions, and, sure enough, I found them several times in the form of thickening of the subepicardial and subendocardial tissue and in the form of cirrhosis of the myocardium in confluent foci or extensive callosities.

The cirrhotic forms were well characterised in the following cases :

Monkey No.	Strain and transmission	Duration of the infection	Pericardial effusion	Lesions of the myocardium
208	D d	206 days	4.5 c.c.	Cirrhosis beginning
126	G c	300 days	traces	Cirrhosis advanced
80	G d	281 days	traces	Cirrhosis very advanced.

Up till now I have always found very limited changes in other animals ; perhaps the monkey has a particular predisposition to these localisations ; be that as it may, they are of paramount importance as links with human pathology. Very similar changes are not rare in sleeping-sickness, and, although not much importance has been attributed to them in comparison with other more obvious lesions of the lymphatic organs and nervous system, they have nevertheless been reported by MOTT, CASTELLANI, LOW and BREINL, from whose descriptions it can be asserted that they are manifestations of the same process ; and since, in the monkey, infections with the *rhodesiense* type of trypanosome gave very frequent and very severe lesions, it is extremely probable that in human infections of this type myocardial affections play an important part.

### C. Changes in the Nervous System.

Numerous observations have shown that nervous changes are not rare in the animal trypanosomiasis. They often assume special forms, such as hæmorrhagic myelitis in equidæ infected with dourine, but sometimes one sees cerebral lesions resembling those described by MOTT in man, though less pronounced. BREINL and HARVEY found them in monkeys infected with *T. gambiense* ; GRAY in a dog infected naturally ; BRUMPT in the rat inoculated with the same type of trypanosome. VAN HOOFF recently demonstrated that there were cytological changes of the cerebro-spinal fluid in monkeys infected by strains of *gambiense* and Damba types, which drew his attention to their close analogy to the manifestations observed in man that were first described by CASTELLANI.

I have, in fact, very often found cerebral changes in monkeys. In some cases, the lesions were obvious even on macroscopic examination, and the histological sections showed a picture identical with that which in man characterises the extreme phases of sleeping-sickness. Some of the monkeys that gave these results had shown serious symptoms, with a well-marked lethargic condition during the last ten to fifteen days ; but most frequently there was no appreciable nervous manifestation, and macroscopically I found only congestive changes.

Only examination of the cerebro-spinal fluid and histological researches enabled the characteristic changes to be determined.

In other cases, the examinations were negative.

In all cases, I examined the cerebro-spinal fluid as well as several pieces taken from nerve centres. I collected the cerebro-spinal fluid by suboccipital puncture after dissection of this region.

The quantity of fluid I was able to recover varied from 0.5 to 1.5 c.c. ; it was nearly always sufficient for examination between slide and coverslip, for some smears and for Rivalta's test, which I made as for effusions of the serosæ ; sometimes I had enough to observe also the possible formation of MYA's cobweb-like fibrinous network. This and Rivalta's tests gave definitely positive results when the microscopical examination revealed numerous cellular elements.

Giemsa-stained smears sometimes showed the presence of trypanosomes ; but most often they brought to light only lymphocytoid cells and mononuclears.

I took from different places some pieces for histological examination (cortical substance, basilar nuclei, cerebellum, protuberance, bulb, spinal marrow) and generally fixed them with sublimate in alcoholic solution, rarely with formol. The lesions found were of various degrees :

I. Severe changes, shown at autopsy by thickening of the pia mater, and cytological and chemical modifications of the cerebro-spinal fluid.

The meningeal lesions were appreciable with the naked eye, especially at the level of the protuberance and bulb.

Histological examinations showed more or less advanced phases of a perfectly characteristic meningo-encephalitic process. The pia mater, the perivascular lymphatic spaces and the nerve tissue were the seat of very pronounced cellular infiltrations. In the pia mater, which was visibly thickened, the cellular infiltrations were diffuse, though more marked around the vessels. The infiltrative elements were small round or polymorphous cells, rare plasma cells, leucocytes, lymphocytes, sometimes erythrocytes ; and occasionally rare morular cells or globular cells, with small nucleus, originating on the surface of the choroid plexus and ventricles.

Some of the round cells had all the characteristics of small blood lymphocytes ; the most numerous ones were provided with more abundant protoplasm, definitely basophile and with a subcentral bean- or horseshoe-shaped nucleus ; their diameter varied from 6 to 12  $\mu$ . The morular cells were very rare, even in the most advanced cases ; they were always situated on the surface or in the subpial spaces ; they had all the well-known characteristics and were in no way distinguishable from hyaline globe cells ; they showed a marked affinity for fuchsin.

In the nerve substance, the infiltrations were more localised and, as a general rule, closely grouped in thick perivascular cuffs, which sometimes completely filled the lymphatic space. These perivascular infiltrative elements were almost the same as in the meningeal infiltrations, accompanied by neuroglial cells and more frequently still by morular cells ; the leucocytes and plasma cells, on the contrary, were much rarer. Even round the small vessels these infiltrations were very pronounced ; and at the level of the basilar nuclei, the protuberance and the medulla oblongata they were most numerous and most evident. Diffuse infiltrations, sometimes complicated by small hæmorrhages, were generally associated with cellular infiltrations of small vessels and very often were still more pronounced round perivascular lymphatic spaces. The most easily identifiable elements in these infiltrations were small globular cells very like plasma cells or else like lymphocytes, and ramified cells of neuroglial nature. The morular cells were sometimes present in large numbers in the white matter and in the gray matter, where they were seen in close connection with the nerve cells.

Figures 1, 2, 3, 4 of Plate II show the depth of these lesions. I found such lesions in the following cases :

Monkey No.	Strain and transmission	Duration of the infection	Cerebro-spinal fluid	Lesions
112	G d	240 days	Lymphocytes, mononuclears	Advanced
126	G d	300 days	Lymphocytes, mononuclears	Advanced
180	D d	208 days	Trypanosomes	Very advanced
3	D c	426 days	Lymphocytes, mononuclears, Mott's cells, trypanosomes	Very advanced

The extent and depth of the nervous changes were evidently proportional to the duration of the infection.

In monkeys 112 and 126, the lesions were less pronounced, although very evident in small vessels, nerve tissue, choroid plexuses and meninges. It was in monkeys 3 and 180 that the lesions reached the highest degree. The meninges, the vessels and the surrounding nerve tissue were the seat of very strong infiltrations with numerous morular cells.

The Damba Island strain showed in this respect a pathogenic power superior to that of the *gambiense* type of strains. It must be mentioned that monkey 3 had already, four months previously, given a very changed cerebro-spinal fluid on suboccipital puncture (VAN HOOFF).

The very interesting histological preparations from these cases were the object of special studies on the origin and relationships of the infiltrative cells, particularly the morular cells.

I shall describe the results of these studies separately (see pages 302-308).

II. Slight changes, not shown at autopsy unless by cytological and chemical modifications of the cerebro-spinal fluid. Macroscopically, no change was appreciable on the meninges except a very pronounced hyperæmia, to which only a relative significance could be attributed.

Histological examination revealed very slight though extensive meningeal and vascular lesions.

The pia mater was not thickened but strewn with lymphocytes and mononuclears, which were specially numerous in the neighbourhood of vascular formations. In the choroid plexuses, a very marked infiltration of round cells and leucocytes surrounded the vessels with a continuous layer, and numerous trypanosomes were visible among the infiltrative elements, sometimes in very considerable quantities, revealing their way of access to the ventricular fluid.

The lymphatic spaces of the nerve tissue showed an incipient perivascular infiltration. Numerous very pale round nuclei were ranged against the wall of the lymphatic space ; more rarely they were ranged against vascular walls and provided with a little protoplasm radiating outwards in slender prolongations. The same nuclei were numerous and regularly arranged all along the capillaries ; they differed in no way from the satellite nuclei of nerve cells. Clearly, therefore, we had to deal with neuroglial elements proliferating *in situ* around vascular formations. All the same, these elements were not the only ones that marked the beginning of the changes.

In the walls of the vessels indeed there could be noticed an endothelial and adventitial proliferation, much more marked, too, when the lesions were more advanced ;

they represented the beginning of the formation of perivascular cuffs. In the subpial strata of the protuberance and bulb, round cells could be seen with small nucleus and abundant acidophile protoplasm, very similar to the free cells originating in the epithelium of the ventricular walls and choroid plexuses. The epithelium of the ependyma was sometimes in proliferation and obstructed the cavity with a syncitial mass.

I found this histological picture in the following cases :

Monkey No.	Strain and transmission	Duration of the infection	Cerebro-spinal fluid	Lesions
260	G d	61 days	Negative	Incipient
222	G c	107 days	Lymphocytes, mononuclears	Incipient
80	G d	281 days	Negative	Incipient
351	R d	14 days	Negative	(Tryp. in the chor. pl.)
282	R d	20 days	Lymphocytes, mononuclears, trypanosomes	Incipient
281	R d	26 days	Lymphocytes, mononuclears, trypanosomes	Incipient
313	R d	31 days	Lymphocytes, mononuclears, trypanosomes	Incipient
199	D c	136 days	Lymphocytes, mononuclears	Incipient
121	D c	275 days	Lymphocytes, mononuclears	Incipient
337	B d	31 days	Lymphocytes, trypanosomes	Incipient
335	B d	32 days	Lymphocytes, mononuclears trypanosomes	(Tryp. in the chor. pl. beginning)

This table, then, shows incipient lesions ; note the precocity of the changes in the cerebro-spinal fluid, the localisation of trypanosomes in the choroid plexuses and their entry into the cerebro-spinal fluid in infections of rapid course provoked by trypanosomes of the *rhodesiense* and *brucei* types, though the nervous lesions are not at all pronounced. That is another proof of the tendency to extravascular invasion shown by strains of these types also in other tissues. It follows from all the preceding that each type of trypanosome studied is capable of penetrating into the nerve centres, that the first action is manifested in the extreme vascular ramifications and that the cerebro-spinal fluid is affected by way of the choroid plexuses.

The Damba strain of trypanosomes and those of the *gambiense* type gave, in infections of slower course, the most profound lesions, analogous to those seen in the last stage of sleeping-sickness ; trypanosomes of *rhodesiense* and *brucei* types gave incipient histological lesions, in spite of their presence in the cerebro-spinal fluid and in the perivascular spaces of the choroid plexuses, which confirms the connection between the histological changes and the duration of the infection that one sees in the most serious manifestations.

#### D. Changes in the Hæmatopoietic Organs.

The literature on the experimental trypanosomiasis contains a great deal of information on lesions of the spleen and lymphatic glands. These organs more than any others have attracted the attention of experimenters.

They are, in fact, the morbid anatomical manifestations that most constantly and most obviously characterise, from the very beginning, trypanosomal infections, except in some animals, like the ruminants, in which the parasites are always very rare in the blood. There is, then, a certain connection between the quantity of trypanosomes and splenomegaly. Changes of the spleen are in reality never very pronounced in man, and the very frequent co-existence of malaria very often makes it difficult to determine the precise value of these changes.

These reservations apply also to the lymphatic glands and bone-marrow.

The classical researches of MOTT, BREINL and a great many other authors (LAVERAN AND MESNIL, BATTAGLIA, BALDWIN) have shown that, in infections running a rapid course, the hæmatopoietic organs are the seat of regressive and necrotic, but above all of hyperplastic and circulatory changes, whereas, in infections of slow course, fibrous changes are the most frequent.

The lesions of these organs have even been credited with a very important rôle as direct cause of death, either by the profound alteration of the hæmatopoietic tissues not allowing a sufficient regeneration of the destroyed elements (BREINL), or by the hæmorrhagic lacerations of the spleen that are very frequent in small animals.

Mention must also be made of the researches of BRADFORD AND PLIMMER, SAUERBECK, RODET AND VALLET, and LANFRANCHI on the value of the spleen as a protective organ. According to these authors, trypanosomal infections are most serious in splenectomied animals; the parasites are destroyed post mortem more rapidly in the spleen than in other organs, and intrasplenic inoculations give rise to slower infections.

I examined, in smears and sections, the spleen, the glands and the bone-marrow.

*Spleen.* — The fact that trypanosomiasis in the monkeys was nearly always associated with malaria imposes certain reservations on the interpretation of the lesions found, and, according to some investigations made on uninoculated monkeys, these reservations concern above all the intense hæmatocathæresis and the cirrhosis. In some cases where death had been caused by bacterial infections of septicæmic type, the same reservations had to be made for the hæmorrhagic changes. The spleen of the infected monkeys showed three well-marked types of lesions.

In very rapid infections or those interrupted by extraneous complications, the spleen was enlarged, of almost normal consistency, with more or less marked signs of hyperæmia. The most interesting characteristic of these spleens was the hypertrophy of Malpighi's follicles (hyperplastic spleens).

In more serious and prolonged infections without extraneous complications, the spleen was very much larger, of firm consistency, swollen with blood, with prominent hæmorrhagic macula on the capsule, which showed notable thickenings; Malpighi's follicles were not visible (hæmorrhagic spleens).

In infections of slower course terminating in eight to fourteen months, the spleen was less voluminous, with very pronounced thickenings of the capsule, of fibrous consistency, blackish in colour, and rich in connective tissue trabeculæ (cirrhotic spleens).

The histological picture of these three types was equally characteristic.

*Hyperplastic Spleens.* — In smears the lymphocytes were very numerous, associated with round or polymorphous mononuclears pigmented or charged with inclusions of cellular detritus sometimes enclosing trypanosomes. The polynuclear leucocytes were very numerous when there were bacterial complications. The trypanosomes were



always few in number, changed or reduced to their nucleus only; very often they were not demonstrable here, although they were so in other organs.

On examination of the sections, one noticed: follicles of a diameter of 90 to 100  $\mu$ , very compact and regularly rounded, with a few macrophages in the central zone and numerous karyokinetic figures in the small lymphocytes of the peripheral zones; lymphatic reticulum largely infiltrated with small mononuclear cells and leucocytoids with bilobate nucleus; lacunar system covered with numerous and prominent endothelial cells and containing macrophages, lymphocytes and a few erythrocytes; capsulo-trabecular system lined with numerous plasma cells and small mononuclears; vessels swollen with blood.

This histological picture was very pronounced in the following cases:

Monkey No.	Strain and transmission	Duration of the infection	Complications
263	D d	33 days	Filariasis
265	D d	64 days	Malaria
199	D c	135 days	Malaria, filariasis
282	R d	20 days	Malaria
338	R d	10 days	—
309	R d	24 days	—
313	R d	31 days	—
314	R c	28 days	Terminal septicæmia
337	B d	31 days	Helminthiasis

*Hæmorrhagic Spleens.* — In smears the blood elements were extremely abundant. The macrophages and mononuclears were very altered; the pigment was not at all abundant. The trypanosomes were more or less numerous according to the quantity of blood contained in the smear. Examination of sections revealed the following picture: follicles very reduced (sometimes absent over a large extent of surface) or represented by a few small clusters of lymphocytes round the arterioles; lymphatic reticulum completely infiltrated or dissociated by erythrocytes; lacunar system compact, of alveolar appearance, containing almost exclusively erythrocytes; plasma cells (in the neighbourhood of the capsule, vessels and connective trabeculæ); capsule irregularly thickened; hæmorrhagic and necrotic zones in the subcapsular strata.

This histological picture was very marked in the following cases:

Monkey No.	Strain and transmission	Duration of the infection	Complications
260	G d	61 days	Septicæmic infection with pasteurella
307	R d	11 days	Malaria
311	R d	34 days	Terminal septicæmia
365	D d	8 days	—
360	D d	22 days	—
121	D c	275 days	—
111	D c	426 days	—
286	B d	8 days	Malaria, filariasis
327	B d	8 days	Malaria, filariasis, helminthiasis
336	B d	24 days	—
335	B d	32 days	Filariasis

In monkey No. III, there was a capsular laceration and sero-sanguineous effusion in the peritoneum.

*Cirrhotic Spleens.* — The smears contained a great quantity of lymphocytes, macrophages, epithelial and plasma cells. The pigment was very abundant; the trypanosomes were extremely rare or not demonstrable; very often there were plasmodia. On examination of sections one found a noticeable generalised cirrhosis; the follicles, very numerous but not very scattered, and of very irregular form, presented a large central zone of macrophages and sometimes a hyaline thickening of the arterioles. The lymphatic plexus was thickened and sprinkled with plasma cells; the lacunar system being contracted contained macrophages, lymphocytes and erythrocytes. The thickened capsule was sometimes covered with papillary growth; the trabeculæ and the vessels, very rich in fibrous tissue, were surrounded or infiltrated by plasma cells or small fibroblasts; the pigmentation was very marked.

This histological picture was evident in the following cases :

Monkey No.	Strain and Transmission	Duration of infection	Complications
258	G d	98 days	Malaria
222	G c	107 days	Filariasis
252	G c	135 days	Malaria, filariasis
237	G c	197 days	Helminthiasis
80	G d	281 days	Malaria
126	G d	300 days	Malaria
122	D c	232 days	Malaria
112	G c	240 days	Filariasis

In other cases, I found combined lesions where the hæmorrhagic changes were accompanied by hyperplastic or fibrous degenerations. These pictures showed that a true lymphatic hyperplasia had been produced in the infections with rapid course produced by various human or animal strains of *gambiense* type or by the Damba antelope strain; this hyperplasia is not very pronounced or completely checked by a cirrhotic process.

*Lymphatic Glands.* — The ganglionic changes were characterised by very different phenomena, even in the same animal. The inguinal-crural and axillary glands often presented hyperplastic changes, congestions, and sometimes hæmorrhages which even extended into the surrounding tissue.

Occasionally in this connection one found thickened and sometimes sclerosed ganglia, which mostly had no relation to the rapid course of the infection.

The frequent association with filariasis, the existence of cutaneous lesions, and the unknown antecedents with regard to regional infections made understanding of the fibrous changes extremely difficult.

Better worth consideration was the information gained from the cervical mesenteric and retro-peritoneal glands, which, however, seldom presented marked differences; they were almost always the seat of a marked hyperplasia of the lymphatic elements and a moderate hyperæmia, phenomena which had no special characteristics.

All the same, in some cases, the lymphatic ganglia presented the same modifications as the spleen, especially in the cases of hæmorrhagic changes (monkeys 260,

III, 121), and cirrhotic changes (monkeys 126, 258, 222, 80, 122, 3). The histological changes were not different from those which MOTT, BREINL, LAVERAN and others have carefully described : hyperplasia of the follicles, increase of macrophages in the sinuses, perivascular infiltrations of round and plasma cells, and sometimes the presence of globular hyaline cells ; thickening of the lymphatic plexus, capsular and trabecular fibroses in the most advanced forms.

The demonstration of trypanosomes was generally much easier than in the spleen, but their number was very different from the number of trypanosomes contained in the blood. I have never found in the glands quantities of trypanosomes comparable with those of the localisations in the myocardium, the serous membranes and the choroid plexuses. The trypanosomes were either free and of typical form, or frequently clumped or even phagocytosed by the globular cells with bilobate nuclei.

*Bone-marrow.* — In my post-mortem on monkeys, I examined the bone-marrow of the femoral diaphysis in smears and sections. I always found a red marrow, even in uninoculated monkeys ; the macroscopic and histological characteristics were almost always interesting.

The commonest change and the one most easily discoverable on autopsy was the more or less gelatinous consistency of the marrow. Very rarely in incipient infections the bone-marrow presented a firm consistency which made it possible to remove the whole mass intact. More frequently it was like pap, which made the preparation of contact smears very difficult. These conditions were very often due to hyperæmic-hæmorrhagic phenomena. In some cases, on the other hand, the bone-marrow presented a singularly viscous consistency and had a greyish colour. One found in the smears of myelocytoid cells some lymphocytes and some very rare leucocytes.

On examining sections, one found three different well-characterised pictures, which corresponded to hyperplastic, hæmorrhagic or aplastic states.

The hyperplastic bone-marrow was characterised by the almost complete disappearance of adipose spaces, very abundant in the normal state ; this resulted in a very cellular marrow where the hæmatopoiesis and the leucopoiesis were very active, considering the presence of numerous erythroblasts and especially of numerous myelocytes ; one found besides large lymphocytic infiltrations, sometimes in clearly visible groups of follicular aspect.

This aspect of the bone-marrow was the most frequent and corresponded to a lymphatic hyperplasia in the spleen and the ganglia.

The hæmorrhagic bone-marrow was characterised by a cavernous dilatation of the lacunar spaces gorged with blood and by true hæmorrhagic effusions which produced a profound dissociation of the medullary tissue. This change was very marked in monkey III, which showed also splenic and ganglionic hæmorrhages.

The gelatinous bone-marrow was characterised by an extreme diminution of the hæmatopoietic tissue and by the presence of an abundant hyaline substance, feebly basophile, containing lymphocytes, mononuclears and erythrocytes in little groups.

This type of medullary change was very pronounced in the following three monkeys :

Monkey No.	Strain and transmission	Duration of infection	Complications
126	G d	300 days	Malaria
122	D c	232 days	Malaria
112	G c	240 days	Malaria, filariasis

The spleen of these three monkeys showed an intense cirrhosis (see the preceding table), and the degeneration of their hæmatopoietic organs was the most serious of the whole series.

I was unable to undertake hæmatologic examinations of the infected monkeys. I can only state that in monkeys long infected the visible mucous membranes were always pale and that hæmatoblasts in circulation must be very rare in these animals ; in fact, I have never found them in the heart blood removed at autopsy, although they were frequent in the blood of other animals.

The three monkeys which showed these changes in the hæmatopoietic organs were seriously attacked by a grave anæmia to which the accompanying malaria gave perhaps a special gravity.

In man, hyperplastic changes of the bone-marrow are also very common in sleeping-sickness ; colloid degeneration has been observed in four autopsies by BREINL, who considers the changes in the hæmatopoietic organs as the most important factor in causing death, supposing that the renewal of the blood elements, destroyed in great quantity, had become impossible.

#### E. *Changes in the Liver.*

Under the title of lymphoid transformation, PETTIT has described in experimental trypanosomiasis animals an hepatic change, characterised by cellular infiltration around the vessels and localised especially in the interlobular portal spaces.

The degenerative, hæmorrhagic and necrotic changes, as well as the cirrhotic manifestations described by other authors, have no specific character. LAVERAN AND MESNIL do not believe that the changes described by PETTIT can be characteristic, granting that analogous phenomena are frequently seen in other protozoal maladies. However, these infiltrations are certainly produced by the trypanosomes ; in fact, STEVENSON has shown that the parasites may be very numerous in proximity to the bile ducts.

The same lesions are demonstrable in man (BREINL). In my post-mortems on monkeys, I found them almost constantly in different degrees.

The macroscopic characters of the liver are of little interest. I noticed a moderate hyperæmia, a more or less soft consistency and sometimes little hæmorrhages which corresponded to the hæmorrhages of other organs. In uncomplicated cases, I have found the parenchyma almost normal.

The manifest changes were in the perivascular tissue ; they were generally very limited.

The smears stained with Giemsa showed some well-preserved hepatic cells, much blood and very few infiltrative elements.

Examination of sections gave the following picture : hepatic cells generally little altered ; more or less extensive cellular infiltrations round the vessels and the bile ducts in the portal spaces, sometimes even round the centrolobular veins and round the roots of the subhepatic system ; Kupffer's cells in normal condition or slightly projecting and remote from the epithelial trabeculæ without evident inclusions. The most interesting phenomenon was, then, the cellular infiltration in the portal spaces and round the venules. These infiltrations had the same characteristics as those seen in other organs. The name chosen by PETTIT does not entirely correspond with their characters, since the infiltrative elements are generally only small, round or polymorphic histiocytes accompanied by mononuclears with bilobate nuclei, plasma cells, rarely true lymphocytes and polynuclear leucocytes. (See Fig. 2, Plate III.)

In some cases where these changes were more manifest and more extensive, I have found very pronounced perivascular cellular cuffs with numerous hyaline globe cells. These infiltrative changes were always limited and the trypanosomes in them were not numerous. The following cases are chosen from among those which present the most evident manifestations of this nature :

Monkey No.	Strain and transmission	Duration of infection	Complications
80	G d	281 days	Malaria
222	G c	107 days	Filariasis
258	G d	98 days	Malaria, staphylococcic septicæmia
3	D c	426 days	—
180	D d	208 days	—
121	D c	275 days	—
111	D c	289 days	—

As will be found in this table, the most marked hepatic changes occur in the slowly developing cases ; the comparison of this table with those of other lesions also shows that these cases were among the most serious.

On the other hand, the hepatic changes were very slight or negative in the monkeys infected with strains of *rhodesiense* and *brucei* type.

A hyperplasia of the connective tissue at the same site was frequent in other monkeys, especially as the result of very slowly developing infections, but I do not know that I would attribute a specific rôle to these lesions, which one equally meets with in some cases of very rapid infection, even in some monkeys that have not been inoculated. In the same way, I do not venture to attribute much importance to degenerative and necrotic lesions, which I observed very often in cases running a rapid course, because of the bacterial infections by which they were frequently accompanied. To sum up, one can say that the liver is the frequent but not constant seat of very limited lesions which reach neither the hepatic nor Kupffer's cells.

#### F. Renal Changes.

Renal lesions have very seldom been reported. The descriptions of them mention circulatory changes (MASSAGLIA, YAKIMOFF), fibrotic phenomena with thickening of the walls of the vessels and tubules and cellular infiltrations (BATTAGLIA).

I examined the kidneys in all my post-mortems ; in some cases I completed the macroscopical and histological examination by the examination of smears stained with Giemsa.

In general, I only found very faint circulatory and degenerative changes in the uncomplicated cases, which do not merit special consideration. In the three following cases, however, I found a true trypanosomal nephritis.

*Monkey 313.* — Inoculated by direct transmission with a strain of *rhodesiense* type obtained by KLEINE, in January 1927, from the patient *Boga* (*G. swynnertoni* region of Lake Tanganyika) ; 3rd passage ; duration of infection, 31 days. Serious infection without extraneous complications, but with very abundant localisations of parasites in the myocardium and the pericardium.

The kidneys were large, with tumefaction of the cortical substance, which was very pale and of a soft consistency. The capsule was not adherent, and the macroscopic aspect was that of a large white kidney.

As there were no noticeable lesions found other than those which most characterise this form of trypanosomiasis, I made smears, which, stained with Giemsa, showed numerous altered renal cells and an enormous quantity of infiltrative cells among which small mononuclears with displaced bean-shaped nuclei predominated, accompanied by larger mononuclears with bilobate or horseshoe nuclei, lymphocytes, plasma cells and polynuclears.

The protoplasm of the mononuclears contained clumped trypanosomes; the typical and free forms were not numerous, but a great number of free nuclei were found among the infiltrative cells. The histological picture was as follows:

The glomeruli without special change, or presenting a slight proliferation of endothelial nuclei in the vascular loops; abundant granular deposit and degenerated epithelial cells in Bowman's capsules and in the uriniferous tubules. Very deep lesions of the tubular epithelium, with vacuoles and hyaline globules. Massive interstitial and diffuse infiltration of the elements observed in the smears. (See Fig. 1, Plate III.)

The changes extended to the whole cortical substance; there were no fibrous alterations or thickening of the vascular walls.

The trypanosomes were not in massive quantities, but it was, however, very easy to find them among the elements of infiltration and in the interior of the mononuclears. Some inclusions of *leishmania* form were frequently found in the epithelial cells of the convoluted tubules, sometimes accompanied by elongated forms having all the morphological characters of trypanosomes.

The medullary substance was oedematous and hyperæmic; the collecting tubules were more or less obstructed by pathological products from the cortical substance.

These characters enabled the morbid anatomical picture to be classed as a subacute nephritis of large white kidney, particularly in the rarest forms that can be found in man and which have never been reproduced in the experimental pathology of the kidney.

*Monkey 311.* — Inoculated with the same strain by direct transmission at the 4th passage; duration of the infection, 34 days. Grave infection without extraneous complications. General changes analogous to those of the preceding case.

Kidney not much increased in volume, with a thickened, very pale, cortical substance; hyperæmia of the medullary substance.

On histological examination, the same form of interstitial nephritis, but with more pronounced characteristics. In the infiltrations the plasma cells predominated, and there were very numerous small polymorphous cells. The trypanosomes were rare.

*Monkey 265.* — Inoculated with a Sititunga antelope strain from Damba Island; direct transmission at 3rd passage; duration of the infection, 64 days; death following a cardiac puncture. Very serious infection with advanced pericarditis and myocarditis, complicated with malaria. On post-mortem and histological examination, the same findings as with monkey 313.

*Monkey 336.* — Inoculated with a strain of *brucei* type obtained in April 1927 from a dog not artificially infected (*G. morsitans* region of Mbarara, Uganda); direct transmission at 2nd passage; duration of the infection, 24 days. Serious infection with massive localisations of trypanosomes in the myocardium; incipient myocarditis.

Kidneys large, with the characteristics of a large mottled kidney.

On examining histologically, alterations were found analogous to those found in the kidneys of monkey 313, complicated with an extreme hyperæmia of the subcapsular veins and the base of the pyramids.

I found analogous changes in other monkeys, but very limited and in a stage of development which approached sometimes to a fibrosis with small foci.

In monkey 162, which was inoculated with a strain of *gambiense* type, and died after 300 days, there was a true cirrhosis.

Although I never found in the kidneys massive localisations of trypanosomes to be compared with those of the myocardium, the pathological process had the same characteristics, and, in the most pronounced cases, the kidney lesions were accompanied by lesions of the myocardium.

### G. Changes in Other Organs.

Besides the alterations already described and which deserve the name of major lesions of the trypanosomiasis, I have found minor lesions in some other organs, among which must be mentioned the suprarenal capsules, the genital organs, the salivary glands and the skin.

*Suprarenal Capsules.* — While other organs of internal secretion, such as the thyroid and parathyroid or the islands of Langerhans, showed no appreciable alterations, the suprarenal capsules were often affected; the lesions were either in the elements of the parenchyma or in the interstitial tissue and the vessels. Severe hyperæmia with moderate degeneration of the cortical cells was often present, accompanied sometimes by small cellular infiltrations around the vessels and the medullary substance. These changes were almost exactly like those which ELMASSIAN has described in rats inoculated with *T. brucei*.

More rarely I found well-characterised lesions, some of which were very extensive.

Abundant cellular infiltrations surrounded the vessels of the medullary substance as well as the elements of the parenchyma, to the point of disintegrating them and taking their place. (See Fig. 3, Plate III.) In the cortical substance, the endothelial cells of the capillaries were much degenerated and numerous round cells were found scattered among the dissociated and vacuolised epithelial cells. In cases where the post-mortem phenomena were not very far advanced, one easily found the trypanosomes by the side of the epithelial cells, sometimes inside their vacuoles in the pericapillary spaces and among the elements of infiltration.

The most marked alterations were hardly visible macroscopically; at post-mortem, the suprarenal capsules were yellowish or much injected and succulent; in smears, the elements of infiltration were numerous, and the cells of the parenchyma very much modified, without enabling a connection with the parasites to be established.

The most pronounced lesions were always accompanied by very grave changes in other organs, notably the heart.

Figure 3 of Plate III shows lesions of medium severity in monkey No. 180.

I found these lesions in the following cases :

Monkey No.	Strain and transmission	Duration of infection	Complications
307	R d	11 days	Malaria
282	R d	20 days	Malaria, helminthiasis
309	R d	24 days	—
265	D d	64 days	Malaria
292	D d	66 days	Malaria
199	D c	136 days	—
180	D d	208 days	Streptococcal septicæmia
111	D	284 days	—
121	D c	289 days	—

The suprarenal lesions were insignificant or lacking in monkeys inoculated with *gambiense* and *brucei* types.

*Genital Organs.* — The histological researches in the genital organs had for their principal object the demonstration of the possible presence of trypanosomes in the seminal ducts. This demonstration has not been made up till now; however, the genital organs are very seldom affected in trypanosomised animals; V. DURME has reported it in rabbits inoculated with *T. brucei*.

I fixed the testicles with the tunica vaginalis and the seminal vesicles. Under these conditions I was easily able to observe specific vaginalitis with its exudate containing trypanosomes. In spite of these serous localisations, I found only very moderate infiltrations in the epididymis and very limited ones in the testicle, except in monkey No. 121, which presented small noticeable interstitial infiltrations.

In Monkey No. 265, where the lesions were the most marked in the epididymis, I found a trypanosome of typical form inside a seminiferous tubule. The canals contained an almost homogeneous substance, without a trace of spermatozoa, with some round lymphocytoid cells. The testicle showed no special changes, but spermatogenesis had been arrested.

Spermatogenesis was absent in other monkeys, even with very limited genital lesions, which would explain the progressive diminution of genital activity in man during the course of sleeping-sickness; nevertheless, I do not venture to attach too great a value to these findings, knowing neither the influence of captivity nor the primitive state of development of these organs in the inoculated monkeys.

It is none the less true that trypanosomes can enter by the seminal ducts, and provoke specific lesions in the epididymis characterised by perivascular and pericanalicular infiltrations, as well as by the presence of the parasites.

The change in the genital organs was always accompanied by an exudative vaginalitis. I noticed this in monkeys 312, 281, 313, 265, 121, 111. These were therefore severe infections, affecting the heart and sometimes the kidney and the suprarenal.

*Salivary Glands.* — Examination of these organs was made to seek the existence of a sialo-adenitis, recently reported by BATTAGLIA.

The lesions I was able to observe in monkeys consisted of cellular cuffs around the interlobular venules, either in the submaxillary gland or in the parotid.

The cellular infiltrations were localised, and rarely extended very slightly into the surrounding tissue. The glandular elements and the interstitial tissue showed no other noticeable modifications.

Fig. 4, Plate III, shows these changes, well marked in monkey No. 260. I verified these lesions in the following cases:

Monkey No.	Strain and transmission	Duration of infection	Complications
309	R d	24 days;	—
260	G d	61 days	Pasteurellosis
265	D d	64 days	Malaria
111	D c	289 days	—
3	D c	426 days	—

*Skin and Muscles.* — I never found in monkeys cutaneous changes comparable to those which are so severe and common in the course of trypanosomiasis of Equidæ, and of those which WOLBACH AND BINGER have described in small laboratory animals.



I often noted the existence of hæmorrhagic spots in the subcutaneous tissue, which agree with the analogous manifestations in the internal organs and with genital œdema when there was inflammation of the tunica vaginalis.

In two monkeys inoculated with strains of *rhodesiense* type (Nos. 311 and 313), I observed papular and erythematous spots on the chest and abdomen. On histological examination, I found capillary congestion, small hæmorrhages from diapedesis into the papillary layer of the derma and perivascular infiltrations of small round cells the whole length of the deeper small vessels; there the trypanosomes were visible.

These symptoms in the monkey would correspond with the trypanids described by CASTELLANI in sleeping-sickness.

In the muscular system (abdominal, cervical and crural muscles), I only rarely found proliferations of the capillary endothelium, and very limited infiltrations of polymorphous cells. These phenomena were, however, of little importance and not pronounced enough to represent, even in an arrested form, a myositic process.

#### EXTRANEOUS COMPLICATIONS.

The question of extraneous complications, so important both from the clinical and morbid anatomical point of view, in human trypanosomiasis often arises in a very similar form in the interpretation of the lesions found in the monkey.

Indeed, the most common extraneous complications were malaria, filariasis, helminthic infections of intestinal origin and bacterial infections.

*Malaria.* — This is a very common complication in monkeys that have been in captivity a certain time.

On examining the blood between slide and coverslip, one frequently notices, after several minutes, the formation and division of microgametes animated with very lively movements; in the stained films, few annular and plasmodial trophozoites were found, but numerous globular gametocytes, rich in yellowish hæmozoin. In smears of the hæmatopoeitic organs, stained Giemsa, I often found these parasites, and their nuclear fragments sometimes made a difficulty in the interpretation of phagocytic inclusions.

The anatomical changes due to malaria never reached a high degree, but the pigmentation of the hæmatopoeitic organs appeared exaggerated with reference to the phenomena of hæmatocathæresis present.

In monkey No. 210, I found more serious symptoms, and it is probable that the malaria played a very important part in the cause of death.

This monkey had been inoculated, as control, with the blood of a non-infected she-goat; the blood, on examination, had in fact never shown the presence of trypanosomes, and the microscopic researches made after the post-mortem on the smears of organs and fluids gave negative results as regards exudative elements and bacteria. The spleen was a little enlarged, slightly succulent, hard and fibrous, of a blackish colour. The blood contained a great number of globular gametocytes, annular and plasmodial trophozoites. On examining sections and smears of the hæmatopoeitic organs, I found fibrotic phenomena and a very marked pigmentation; these phenomena, although less pronounced, were equally visible in the liver and kidneys; in the capillaries of the organs there were globular gametocytes and some pigment.

Morbid anatomical statistics have demonstrated no notable difference in the picture of the lesions, whether this complication was present or not; but, as it was not

possible to be sure of the duration, I was obliged to make reservations in the interpretation of accentuated fibrosis and very marked pigmentations of the spleen in the malarial monkeys.

*Filariasis.* — In smears of blood, pericardial liquid, cerebro-spinal fluid and several other organs, I often found microfilariae, sometimes very numerous. The adult filariae are found in the subcutaneous tissue and sometimes in the nervous system.

In no case was I able to attribute to this complication any influence on the histological lesions found.

I observed filariasis in some uninoculated monkeys. The monkey of post-mortem XXVII was a new arrival in the laboratory; it was found dead on the fourth day, having succumbed to its recent traumatic lesions, occasioned by the capture (ecchymoses, fracture of the skull, subdural hæmorrhage). The blood was rich in microfilariae; the pericardial fluid contained still more, but showed no alteration either with the Rivalta reaction or on cytological examination.

The finding of adult filariae in the central nervous system of five monkeys infected with trypanosomiasis merits note because of the possible relations between this localisation and the vascular lesions of the nervous system; I have stated this in a preliminary notice (see pages 309-313).

*Infections due to linguatulæ, strongyles and ascaridæ.* — The lesions I found were in connection with erratic parasites, localised in the mesenteric or retroperitoneal glands, and sometimes in the intestinal walls. These lesions had no characteristics that need mention here, but in some cases they were accompanied by localised or generalised peritonitis, which played an important part in the mechanism of death.

*Bacterial Infections.* — Infectious complications played a very important rôle.

In some cases, one might have attributed to the strain inoculated a virulence which did not exist, if one had not completed the post-mortem by bacterioscopic and minute histological researches. Indeed, a generalised bacterial infection very often prevented the trypanosomiasis from reaching its ultimate manifestations.

In monkey 180, there was an incipient pulmonary localisation, determinable on post-mortem, accompanied by streptococcal septicæmia. In monkey 312, I found miliary abscesses on histological examination of the liver; in some other monkeys (Nos. 258 and 260), I determined the presence of bacteria in the smears of organs, and I was able to diagnose general infections with pasteurella or staphylococci, by finding these bacteria at the sites of leucocytic exudations of the spleen, serous-membrane fluids and the interior of phagocytes.

More frequently, it was a case of terminal bacterial invasions, without any demonstrable histological manifestation.

A diplococcal meningo-encephalitic process was the cause of death in a monkey recently captured and not yet inoculated (post-mortem XXV). The examination of the cerebro-spinal fluid had shown numerous polynuclears and free or intra-cellular diplococci. The histological examination revealed an acute meningo-encephalitis, with rich infiltrations with polynuclears in the pia mater, in the perivascular spaces, and in the nerve substance. In short, the extraneous complications were of great importance, but relatively limited with regard to the interpretation of the lesions.

The slight morbidity of the uninoculated animals suggested that, after the first days of captivity, sufficient confidence could be placed in the hygienic conditions of

the installations, but the remarks I made above show the advantages of careful morbid anatomical study of each inoculated monkey.

Indeed, any evaluation of the virulence deduced only from the duration of the malady, even if based on the macroscopical post-mortem findings, would have been hazardous.

#### GENERAL CONCLUSIONS.

The post-mortem and histological study of fifty-three monkeys, of which forty-three were infected with recently isolated strains of trypanosomes of human and animal origin, has brought to light the susceptibility of these monkeys (*Cercopithecus sp.*) to present the most diverse manifestations of trypanosomiasis, and the relation between the specific lesions and the extraneous complications (malaria, filariasis, helminthiasis, cerebral porosis, bacterial infections).

The variety of the morbid anatomical lesions thus provided very favourable conditions for the comparative study of the strains used and their pathogenic connections. The results of this study enable us to distinguish three pathogenic types :

I. Chronic manifestations, with few trypanosomes, approaching in a few weeks (four to eight) sclerotic and infiltrative lesions of the hæmatopoietic organs, characteristic meningo-encephalitis, sclerosis of the myocardium and cachectic phenomena, predisposing the monkeys to extraneous complications.

This group characterises the pathogenic action of human trypanosomes of the *G. palpalis* regions.

II. Rapid manifestations, with numerous trypanosomes, approaching in a few weeks (two to four) terminal lesions, characterised by hyperplastic and hæmorrhagic phenomena in the hæmatopoietic organs, by true specific myocarditis, serositis and nephritis, by the early passage of the parasites into the cerebro-spinal fluid and lesions in the choroid plexuses.

This group characterises the pathogenic action of the human and animal trypanosomes of the *G. morsitans* and *G. swynnertoni* regions.

III. Irregular manifestations of an intermediary type, tending to reach in a few weeks (eight to ten), or even some months (eight to fourteen), lesions of the first or second type, and most frequently to chronic lesions associated with rapid terminal lesions.

This group characterises the pathogenic action of trypanosomes of animal origin, of *G. palpalis* regions (Damba Island).

Nevertheless, it must be noticed that there are some very great differences, and numerous important exceptions, due either to individual resistance, or to pathogenic potency, which is extremely variable in recently isolated strains ; these differences prevent any grouping of some border-line forms among the most rapid of the first group and the slowest of the second. Some cases, from reputed *G. palpalis* regions, suggest the existence of other glossinæ, or that the patients had contracted the disease elsewhere ; and the pathogenic manifestations in monkeys sometimes singularly agree with the results of morphological research.

The morbid anatomical process was always the same, with local manifestations more or less important, according to the number of parasites, which all showed their tendency to localise themselves in the interstitial spaces of the tissues and in the serous-membrane fluids.

The most considerable localisations determine, in the first stage, exudative infiltrations of polynuclears and lymphocytes ; afterwards, the mononuclears and macrophages are added, while the capillaries are the seat of serious trouble (œdema, hæmorrhage by diapedesis, endothelial proliferation). These elements, as well as the parasites, are gradually absorbed by the lymphatic ducts ; some plasma cells and some lymphocytoid and polymorphous histiocytes persist a long time in the form of abundant interstitial infiltrations, until the complete development of elements capable of fibroblastic activity.

This process has all the characteristics of granulomatous inflammations and it may attain to definite histological transformations in the form of cirrhoses.

The most remarkable manifestations affect the myocardium, the serosæ and the kidneys. More moderate infiltrative changes may affect the liver, the suprarenals, the meninges and the vascular terminations of the nerve centres, the genital organs, the salivary glands, the alimentary canal and present local symptoms that are sometimes of great importance.

Small perivascular infiltrations are also demonstrable in almost all the tissues of the organism, owing to the ubiquity of the trypanosomes in the interstitial spaces rather than in the blood.

Hyperplastic hæmorrhagic and sclerotic changes are manifested in the hæmatopoietic organs ; they are due to their cathæretic function, as well as to the direct action of the living parasites.

The results of these post-mortems enable one to explain the pathogenic power of the trypanosomes studied ; one can also say that they have brought to light the most faithful experimental reproduction of human lesions obtained till now and that they have called attention to new questions which must be carefully studied in man, such as serositis and cardiopathy.

Entebbe, June 1927.

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All the researches and the photographs were made in the laboratory of the Commission and in the bacteriological laboratory at Entebbe.

I wish here to thank Drs. L. DUKE and CORMACK for their kind reception and the facilities they accorded to me.

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2.

TRYPANOSOMES IN THE LESIONS, MASSIVE LOCALISATIONS  
AND CELLULAR INVASIONS

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The special methods that GIEMSA, SCHILLING, WOLBACH, STEVENSON and BATTAGLIA have proposed for staining trypanosomes in sections are not applicable to researches on a large scale. It would have been very difficult for me to have followed, by such methods, an extended histological and parasitological study; besides, they do not give the same differentiation in sections as in smears, they do not allow of mounting in balsam, and, in general, they require much manipulation, and are not very favourable for the study of histological changes.

Fortunately, since the first observations, I have ascertained that fixation with sublimate in alcoholic solution and a good staining with hæmatoxylin-eosin give very favourable results for research of trypanosomes in the tissues, and at the same time for the study of histological changes. I have therefore confined myself to this method, except for a few control experiments in the first cases.

When trypanosomes are present, one can see them easily. The microphotographs which I have been able to obtain, even with very limited means, show the advantages offered by this very simple method. The condition which is absolutely necessary in trypanosome research in tissues, whatever the method used, is good preservation of the pieces and good fixation. Post-mortem phenomena destroy these parasites very rapidly, especially after invasion by terminal bacteria.

Under the most favourable conditions (temperature, rapid death, absence of infectious complications, etc.), I have obtained good staining of trypanosomes up to about four to five hours after death. Later than this, the demonstration of trypanosomes was always doubtful.

There were then few opportunities of obtaining perfect results, and very often I had to keep careful account of these circumstances in order to explain negative or doubtful results. In fact, it often happened that I could not prove trypanosomes to be in the corpse, although they had been numerous before death, or else that I found them in a very much modified state.

There are different causes of the disappearance of trypanosomes. In the first place, one must consider bacterial infection of septicæmic type. When these complications were of sufficiently long standing to give histological manifestations (leucocyte infiltrations of the spleen, embolic abscesses, polynuclear serositis, etc.), the trypanosomes could not be demonstrated; and as the macroscopic changes due to these complications might give rise to error (splenomegaly, effusion, hæmorrhage), the diagnosis could only be considered on the basis of histological and bacteriological findings.

The very frequent terminal invasions of bacteria allow of easier demonstration of trypanosomes post mortem, in the blood or in the pericardial fluid. In the contrary case, it was also to histological examination that one had to turn in order to discuss the diagnosis (specific lesions, bacterial embolism without lesion or tissue reaction, etc.)

Post-mortem phenomena when very advanced equally prevented demonstration of trypanosomes, and, after their appearance, they determined the serious changes

of the parasites. These changes began in the peritoneal cavity by arrest of movement, loss of flagellar undulation, clumping, appearance of nucleus as a grain of coffee, vacuolisation and destruction of the protoplasm.

After these changes, nothing remained of trypanosomes except free nuclei, which might take on a coccoidal appearance, but generally stained with hæmatoxylin better than bacteria.

These first observations were necessary before considering the connection between the trypanosomes and the histological changes. It is a very important point, because the presence of parasites in the lesions can give rise to manifestations capable of eliminating all doubt as to their specific nature. Indeed, there is often need to doubt at this point, especially with regard to certain animals (dog, rabbit), which frequently have chronic unknown lesions in the liver, kidney, nervous system and hæmatopoietic organs; and one must also note that the demonstration of trypanosomes is sometimes difficult even at the site of specific changes.

For the study of trypanosomes in the tissues, I will confine myself, then, to portions from monkeys examined immediately after death, or before post-mortem phenomena were perceptible.

A great number of my smears and sections were very favourable for the study of trypanosomes in the different organs.

I will summarise here the most interesting facts that I have noticed up to the present.

#### *Trypanosomes in the Serous Membrane Fluids.*

I have given some information on this subject when treating of serous membrane lesions. Trypanosomes were numerous in these fluids, even when the latter presented the most pronounced changes. The number of the trypanosomes was generally in proportion to the exudative elements, which one can interpret as chemico-tactic action and, at the same time, a pronounced resistance to the defensive inflammation.

In some cases, the parasites were exceedingly numerous, especially in the liquid of the pericardium, the peritoneum and the tunica vaginalis. Most of them were free and very active in movement, but intra-cellular forms were not lacking and were found in some globular elements. These cells were most frequently mononuclear; the lymphocytes, both small and medium, had no phagocytic power whatever. The phagocytosed trypanosomes were almost always of globular form; and, as these forms were equally found among the free trypanosomes, it is probable that this phagocytosis is exerted principally on those forms that one considers dead, or manifestations of involution.

The normal fluids of the serous membrane and, above all, the exudates permit, therefore, active multiplication of the trypanosomes; indeed, forms in process of segmentation were found in great numbers, which leads us to consider these liquids as selective media, specially favourable when the inflammatory changes augment their albuminoid content.

The serous membrane fluids are, then, indicated for research as to diagnosis when ordinary means do not give results; here are some examples:

*Monkey 276.* — Inoculated January 17th, 1927, with a strain of *gambiense* type (patient *Awasi*), by cyclical transmission at 1st passage. The trypanosomes never appeared in the blood. The animal was killed with chloroform and examined on June 10th, 1927.

On autopsy, I found moderate splenomegaly; no other appreciable lesions. The blood contained no trypanosomes on examination between slide and coverslip. The pericardium contained  $\frac{1}{2}$  c.c. of cloudy liquid, with feeble Rivalta reaction, which,

by examination between slide and coverslip, presented living trypanosomes and globular cells.

*Monkey 41.* — Inoculated March 3rd, 1926, with a strain of *gambiense* type (patient *Walubani*), by cyclical transmission at 2nd passage. Trypanosomes had been seen in the blood from May 10th to June 2nd ; after that time, the search for trypanosomes in the blood was negative and the monkey was considered cured.

Autopsy was made (after chloroforming on June 24th, 1927), and demonstrated a cerebral filariasis, previously indicated by the presence of microfilariae in the cerebro-spinal fluid.

On autopsy, I found splenomegaly ; the pericardium contained 0.7 c.c. turbid liquid, which, on examination between slide and coverslip, showed living trypanosomes, globular cells and some microfilariae.

#### *Trypanosomes in the Cerebro-spinal Fluid.*

The cerebro-spinal fluid of monkeys collected on autopsy by suboccipital puncture frequently contained trypanosomes, much more frequently than I expected, especially in infection with trypanosomes of the *rhodesiense* and *brucei* types.

The trypanosomes in the cerebro-spinal fluid were always non-motile, squat, clumped or with stiff flagella, without perceptible undulation, or reduced to a nucleus. They were, then, abnormal forms, or forms in course of destruction.

Indeed, the trypanosomes reach the cerebro-spinal fluid by the choroid plexuses ; I have seen them very numerous in monkeys 311, 351, 354 and 337, among the elements which surround and link the vessels ; although in those cases the histological lesions were quite at their commencement or were not recognisable, and even the cerebro-spinal fluid did not always present important changes. It is just in these cases of rapid evolution, due to strains of *rhodesiense* or *brucei* types, that I most easily found trypanosomes in the cerebro-spinal fluid and the least lesions in the nerve tissues. So it is not only the presence of trypanosomes in the cerebro-spinal fluid which determines nerve lesions, but it is more likely the specific lesions (especially vascular) of the nervous system that determine the changes in the cerebro-spinal fluid and make it more favourable than the normal condition for the survival of parasites.

#### *Trypanosomes in the Hæmatopoietic Organs.*

One considers the hæmatopoietic organs to be the earliest and most constant seat of the trypanosomes, and diagnosis founded on puncture of ganglia shows the frequency and persistence of the parasites in these organs. Nevertheless, I have never found, during my researches on monkeys, in examining the lymphatic ganglia both in smears and in sections, such numbers of parasites as I found in the serous membrane fluid.

Perhaps the defensive phenomena are the more accentuated because of the activity of the macrophages, in the interior of which one can easily find clumped trypanosomes, at times in great numbers.

The distribution of the parasites in the ganglionic tissue is almost confined to the lacunar system.

The presence of more or less numerous trypanosomes in the spleen has been the subject of some discussion.

I have already cited, in Report No. 1, the works of several authors according to whom there is reason to consider the spleen as a protective organ and the seat of a trypanolytic action demonstrable especially after death. I must associate myself with this point of view ; in fact, in smears of the spleen, I have always found very few trypanosomes in comparison with those I found in other organs just as fully vascular,



such as bone-marrow and the liver. Generally, the free forms were vacuolised, clumped, or more frequently reduced to their single nuclei. The phagocytosed forms were very numerous, and sometimes of typical form.

These verifications prove that in the spleen the very energetic local defence is not only represented by phagocytic manifestations, but also by lytic action which may continue after death.

### *Massive Localisations and Cellular Invasions.*

In almost all the tissues, the demonstration of trypanosomes requires close and sometimes very long search; the habitual seat of the parasites is found in the lacunæ of the interstitial tissue, and their most common form is that found in the blood. Nevertheless, one finds in some organs important exceptions with regard to massive localisations and their intra-cellular position.

It is above all in the myocardium that I found massive localisations, and in the kidney and suprarenal capsules that I found undeniable manifestations of cellular invasion. These localisations cause serious disturbances in the organs, which strikingly remind one of granulomatous inflammation. What is more difficult to explain is the mechanism of these localisations and some morphological manifestations of the trypanosomes observed in them.

Trypanosomal myocarditis is always accompanied by exudative effusion in the pericardium and a much-accentuated epicarditis. The trypanosomes are very numerous in the exudate and sometimes also in the sub-epicardial connective tissue. These findings may perhaps explain the localisations in the myocardium as lymphatic propagation. In any case, it must be noticed that the sub-epicardial lymphatic vessels, as well as the deeper vessels, contain very few trypanosomes compared with the interstitial spaces. If it is lymphatic propagation, it must be propagation by way of the lacunæ.

The trypanosomes find in the myocardium conditions extremely favourable to their development; very often they adhere to the surface of muscular fibres, or else they penetrate to their interior where favoured by cleavage and fragmentation of fibre.

In morphological study of these masses one finds strange modifications with a tendency to short leishmanoid forms with large nuclei and a blepharoplast very evident or not demonstrable.

It is difficult to explain these morphological manifestations in an entirely satisfactory way; they, however, deserve close attention.

In the first place, one must notice that these manifestations are seen almost exclusively in the myocardium; they are, then, an expression of what takes place under these conditions of development. Whatever the cause, they present a very accentuated polymorphism, which may come from a too-rapid multiplication not attaining to regularly differentiated forms. In fact, the forms that one notices present signs of regression; is this a case of a less-differentiated state more favourable to rapid division or a true regression perhaps connected with the local histological reaction? The two hypotheses may be equally maintained.

With regard to other possible causes, it must be noticed that the post-mortem phenomena have no appreciable influence. These manifestations were visible even in the smears made directly after death. These phenomena may perhaps suggest other hypotheses with regard to invasion forms and their relation to the grave disturbance of general metabolism preceding death. These enormous quantities of parasites are certainly the product of a multiplication *in situ* so rapid that the process of division is necessarily modified.

Manifestations of cellular invasion are not rare in the cardiac fibres ; still more interesting are those that one may find in the epithelial cells of the kidney and suprarenal capsules. I have found them in some cases of very grave infection with trypanosomes of *rhodesiense* and Damba-antelope types. It was generally a case of leishmanial forms, which did not differ from those one sees in the interstices of the myocardium—and sometimes short forms. Besides the morphology of the parasite, the cellular changes deserve some notice.

It must be stated first that these observations were made on organs very much deteriorated. The cardiac fibres which contained trypanosomes showed signs of atrophic degeneration : pyknosis, œdematous imbibition, fibrillar dissociation, fragmentation ; the cells of the suprarenal and kidney were turbid and vacuolised.

It was just in the most degenerated elements that the trypanosomes appeared most frequently. One might wonder whether these cellular changes were not caused by the direct action of the parasites. I do not think so : the cells occupied by the trypanosomes were always rare in proportion to the number which showed these changes. The trypanosomes could easily have penetrated the interior of the cells where the protoplasm was dissociated or degenerated by these regressive processes, and it may be that these intra-cellular forms have no other signification. However, they sometimes take on an appearance very similar to that which is typical of *Trypanosoma cruzi*. I have long sought in these inclusions for characteristics which would show an intra-cellular developmental cycle ; the most striking fact was a variety of forms and arrangement which showed rather accidental relationships than a definite tropism and intra-cellular developmental forms.

The massive localisations as well as the intra-cellular forms are nothing but manifestations of malignity and invasive tendencies which very frequently were typical of Damba-antelope trypanosomes, and which nearly always characterised the trypanosomes of *rhodesiense* type.

#### CONCLUSIONS.

At the site of lesions, the trypanosomes have generally the same form as in blood drawn off from the periphery ; but in massive localisations they show a very accentuated polymorphism, which seems related to their power of invasion.

In certain tissues (myocardium, kidney, suprarenal), one can find typical trypanosomes, or leishmanoid forms. These manifestations of cellular invasion have characteristics which show accidental relationships with the protoplasm, changed or vacuolised by a regressive process, and do not show the phases of an intra-cellular cyclical evolution typical of other trypanosomes. It is a case of manifestations of malignity which almost always characterises the trypanosomes of *rhodesiense* type and very often those of the Damba-Island antelopes.

The most extensive localisations in the monkey were found in the myocardium, where they caused myocarditis ; in the choroid plexuses which favour early penetration into the cerebro-spinal fluid, and into the fluids of the serous membranes, where the trypanosomes were to be found even when their presence in the blood had not been proved. The trypanosomes are easily visible in tissues stained with hæmatoxylin-eosin, provided that the post-mortem phenomena are not advanced and that bacterial infections have not produced complications.

Entebbe, June 1927.

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3.

TRYPANOSOMAL MYOCARDITIS

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Cardiac lesions have frequently explained the mode of death in monkeys who died, so to speak, suddenly and without appreciable lesions in other organs.

A very characteristic specific myocarditis caused by massive deposits of trypanosomes explained, in these cases, the very rapid and fatal course of the infection.

I indicated these forms of myocarditis in the first report when pointing out that the gravest forms are frequent among monkeys infected with strains of the *rhodesiense* or Damba-Island type. I am going to present here the most salient histological characters of this affection, which played an extremely important rôle in experimental trypanosomiasis in monkeys, and which, moreover, presents striking analogies with observations in human pathology.

A. EARLY APPEARANCES.

The onset of trypanosomal myocarditis is determined by a massive deposit of parasites in the myocardium. This deposit does not present the features of an embolic process — the trypanosomes multiply in the interstitial spaces, along the muscle fibres external to the blood capillaries.

At autopsy, the myocardium is almost always pale and œdematous, an exudative pericarditis is equally the rule, and trypanosomes are extremely numerous in the pericardial fluid. According to these observations, one might postulate a propagation via the lymphatics, and occasionally, indeed, the more peripheral portion of the myocardium does show the severest degree of infection. But very frequently one finds large numbers of trypanosomes equally distributed throughout the myocardium, even in the deepest portion. The spread, therefore, does not necessarily occur by continuity, although this may be possible. The deposits of trypanosomes are probably also carried by the bloodstream.

The most striking point is the enormous number of parasites found in the myocardium; it is undoubtedly a question of a progressive invasion due to a very rapid multiplication *in situ* favoured by the special conditions of the myocardium. Indeed, no other organ is the seat of a similar phenomenon.

Nor are these deposits terminal manifestations determined by the enormous number of trypanosomes present in the blood. They may be produced at any time during the course of the disease, even when the trypanosomes are not numerous in the peripheral circulation. It is a striking contrast to see, in the midst of tissue richly infiltrated with parasites, blood-vessels which contain few or none. It therefore appears very probable that the localisation of these deposits is fortuitous.

The morphology of the deposited parasites presents some striking features. In smear preparations or sections of the myocardium, they show extreme pleomorphism: side by side with short and truncated or even rounded forms, there are others which are long and flagellate, these being the most typical; there are also very small as well as extremely large forms.

Most frequently leishmanial forms are grouped in nests, or they may be arranged in rows within the muscle bundles, following the longitudinal axes of the muscle fibrils.

These special forms may perhaps result from an atypical or premature multiplication related to their mode of invasion. In any case, they have no apparent relationship to post-mortem phenomena. I have found them to be very numerous in some monkeys autopsied immediately after death, and they were no more numerous in some others in which post-mortem changes were manifest. It is possible that these atypical forms are related to the phenomena described by VIANNA, which recall in a striking degree the cyclical life-history of *Trypanosoma cruzi* in the tissues and particularly in the myocardium. This is a further question which merits careful study.

In Fig. 2 of Plate IV, I have reproduced these morphological manifestations, and some microphotographs in Plate VII reproduce others.

At the site of these massive deposits of trypanosomes, one nearly always observes the first signs of an inflammatory reaction. A very marked œdema separates the cardiac fibres and an imbibition of their protoplasm shows up the individual fibrils. The muscular protoplasm is turgid, the transverse striation is poorly marked or lost altogether — in the interstitial tissue there is a commencing inflammatory proliferation which gives rise to infiltrating elements, viz., macrophages, small and large mononuclear cells with basophile protoplasm, lymphocytes and some leucocytes; erythrocytes and some polynuclear cells are frequently associated with these. The macrophages appear first; then follow the small and large mononuclears with basophile protoplasm and bilobate nuclei in increasing numbers.

These mononuclears probably arise from the cells in the lymphatic spaces. Their most obvious rôle is an active phagocytosis; many of them contain inclusions of clumped trypanosomes.

The earliest manifestations were seen when an external agency (cardiac puncture) interrupted the course of a severe infection; but in some cases there was no associated external factor; the lesions were very severe: very extensive invasion of parasites, well-marked œdema, separation and rupture of cardiac fibres and grave anæmia due to compression of the capillaries by œdema and to endotherial lesions.

These lesions were so pronounced that no doubt was possible as to their rôle in the causation of death. Some examples follow:

#### *Monkey 312.*

Strain from patient *Boga* (region of *G. swynnertoni*, Tanganyika); 5th passage by direct transmission. Inoculation on March 25th, 1927; trypanosomes present in the blood on March 30th; death on April 7th, 1927.

At autopsy, I found 2 c.c. of turbid fluid in the pericardium; on examination between slide and coverslip, this fluid contained many still living trypanosomes and many spherical cells. The heart was dilated, pale and flabby. In the ventricular blood I found numerous living trypanosomes.

#### *Smear Preparations:*

*Pericardial fluid.* — Numerous trypanosomes typical in shape; some circular forms very rare. Small and large mononuclears with peripheral bean- or horseshoe-shaped nuclei; lymphocytic cells and some leucocytes. Contained in the mononuclears, numerous inclusions of clumped trypanosomes. Microfilaria.

*Myocardium.* — A few small and large mononuclears, large numbers of clumped trypanosomes, sometimes very short and sometimes very big.

*Sections:*

*Parietal pericardium.* — Nothing specific.

*Visceral pericardium.* — Some very limited infiltration into the sub-endothelial tissue, of macrophages and small mononuclears in keeping with the deposits of large numbers of trypanosomes of spherical shape.

*Myocardium.* — Well-marked œdema, muscle fibres showing granular degeneration with transverse striation lost; capillaries widely separated from the fibres and poorly marked. In some of the interstitial spaces, large numbers of short or spherical trypanosomes.

*Monkey 309.*

The same strain, 4th passage by direct transmission. Inoculation on March 21st, 1927, trypanosomes in the blood on March 28th; death on April 14th, 1927.

At autopsy, the pericardium contained some traces of sero-sanguineous fluid related to a cardiac puncture performed four days previously. The heart was very pale, flabby and friable.

*Smear Preparations:*

*Heart blood.* — Numerous typical well-preserved trypanosomes.

*Myocardium.* — Small and large mononuclears with bilobate or horseshoe-shaped nuclei; very numerous macrophages with two to four nuclei. Large numbers of trypanosomes of different shapes and sizes, free or contained in the macrophages or mononuclears. (See Fig. 4, Plate VII.)

*Sections:*

*Parietal pericardium.* — Rare small cellular infiltrations round the vessels.

*Epicardium.* — œdema, abundant infiltration of macrophages and mononuclears. Trypanosomes very numerous.

*Myocardium.* — Very marked œdema; muscle fibres dull, atrophic, generally preserving their striation, widely separated. In the interstitial spaces, massive deposits of polymorphous trypanosomes, mostly short spherical forms or very large leishmanoids; long, slender forms also numerous. Numerous accumulations of spherical or long forms. Numerous isolated trypanosomes on the surface or in the interior of the muscle fibres. Incipient infiltration of macrophages and mononuclears; some free red-blood corpuscles; poorly marked capillaries with altered endothelium. (See Fig. 1, Plate IV.)

*Monkey 311.*

The same strain, 4th passage by direct transmission. Inoculation on March 25th, 1927; trypanosomes in the blood on April 5th; death on April 29th, 1927.

At autopsy, the blood contains  $\frac{1}{2}$  c.c. of turbid fluid; Rivalta reaction positive; on examination between slide and coverslip, many spherical cells are seen, as well as immobile trypanosomes in large numbers. Heart pale, with œdema at the base; visceral pericardium studded with small white patches in the vicinity of the blood-vessels.

*Smear preparations :*

*Pericardial fluid.* — Very numerous large and small mononuclears, some leucocytes and erythrocytes, numerous free trypanosomes.

*Myocardium.* — Some mononuclears and macrophages, lympho- and leucocytes, polymorphic cells.

*Sections :*

*Parietal pericardium.* — Well-marked perivascular infiltrations of mononuclears and lymphocytes.

*Visceral pericardium.* — Infiltrations very rich in mononuclears, macrophages, lymphocytes and some leucocytes. Well-marked œdema in the vicinity of the vessels, small hæmorrhages by diapedesis ; lymphatic vessels engorged with cells similar to those in the interstitial spaces. Large numbers of typical trypanosomes.

*Myocardium.* — Well-marked œdema, turgid muscular fibres, showing atrophy, dissociation and rupture but conserving their striation. In the spaces, massive deposits of pleomorphic trypanosomes, the majority being typical in shape and size. Infiltrating cells few in number, represented by macrophages, mononuclears and leucocytes. Blood capillaries poorly marked and containing very little blood ; endothelium much altered, sometimes separated and sometimes proliferating.

*Monkey 337.*

Strain taken from a dog from Mbarara (region of *G. morsitans*) ; natural infection ; 3rd passage by direct transmission. Inoculation on April 27th, 1927 ; trypanosomes in the blood on May 2nd ; death on May 28th, 1927.

At autopsy, the heart did not present any appreciable changes and the quantity of pericardial fluid did not appear to be increased.

*Smear Preparations :*

*Pericardial fluid.* — Exudate of mononuclears and lymphocytes, numerous trypanosomes.

*Myocardium.* — Polymorphic cells leuco- and lymphocytes ; trypanosomes.

*Sections :*

*Parietal pericardium.* — Nothing specific.

*Visceral pericardium.* — Œdema and infiltration of mononuclears and leucocytes.

*Myocardium.* — Abundant infiltration of macrophages, mono- and polynuclears, leuco- and lymphocytes. Numerous trypanosomes. Turgid muscle fibres without striation. In the interstitial spaces, groups of pleomorphic trypanosomes. (See Fig. 1, Plate V).

The first of these cases shows the earliest stages, the course of the illness having been interrupted by a bacterial infection of the septicæmic type. In fact, the liver contained some incipient miliary abscesses showing cocci.

The second case showed more pronounced phenomena ; perhaps the cardiac puncture played some part in the rapid termination ; in any case the cardiac changes were sufficiently pronounced to account for death.

The third and fourth presented no trace of any intercurrent complication.

#### B. ADVANCED APPEARANCES.

I had many opportunities of observing the ultimate phases of this form of myocarditis, which is characterised by a histological reaction more or less diffused according to the distribution of parasites.

In general, the inflammatory reaction does not bring the multiplication of trypanosomes to a sudden standstill. I several times found massive numbers of parasites when the histological action was advanced. In any case, the pericardial fluid can contain enormous quantities of trypanosomes, while presenting the most marked features of an exudate, with numerous cellular elements and large amounts of albumen and fibrinogen. The phagocytic activity of the macrophages and of the other infiltrating elements in the myocardium is clearly manifest, except in the case of the true lymphocytes, which show no aptitude for phagocytosis. The smallest mononuclears, closely resembling lymphocytes but with displaced and horseshoe-shaped nuclei, show active phagocytosis, as do also the larger mononuclears, with bilobate nuclei. In fact, in smear preparations of fresh myocardium stained with Giemsa, I found certain of these cells containing in their protoplasm four to five clumped trypanosomes — the presence of numerous Karyokinetic phases proves that they multiply *in situ*.

In sections, the histological distribution of these infiltrations corresponded perfectly to the deposits of parasites in the early stages — that is to say, they were in patches or in more or less extensive layers, attaining their maximum where the deposits of trypanosomes were most considerable. Their distribution in the ventricles was almost uniform, the auricles were sometimes very gravely infected, so too the right ventricle and musculi papillares. The valves presented no noteworthy changes.

The most salient feature of these manifestations was the extent and severity of the interstitial lesions compared with the primarily slight infection of the cardiac fibres. One may say that the latter are more seriously affected by the formation of the exudate than by the direct action of the trypanosomes. Indeed, the cellular infiltration is sometimes so pronounced as almost completely to replace the muscle fibres over a large area ; the result is frequently a dissociation and profound modification of the myocardium ; the separated, atrophic or ruptured fibres, showing granular degeneration, lose their transverse striation.

The capillaries are the site of extreme alterations, which greatly embarrass the cardiac circulation. The inflow of blood is very much diminished. The sections show, over large areas, nothing but œdematous spaces studded with trypanosomes, infiltrated with polymorphic cells without trace of capillaries or of blood, save for a few red corpuscles escaped from their vessels by diapedesis.

It is this failure of circulation that causes the extreme pallor, the soft and friable consistence of the myocardium and perhaps also the arrest of its function.

Nevertheless, it is not uncommon to find cases in which even this phase is passed ; the infiltration is increased and at the same time an evolution of connective tissue begins to show. The polymorphic cells are more abundant ; plasma cells make their appearance. This will result in a new tissue formation presenting characters which recall certain granulomata, produced in other tissues by, *e.g.*, leishmaniosis or fram-bœsia ; at the same time, there is no trace of nodular new formations or of true giant cells or even of necrotic foci.

These granulomatoid forms of myocarditis have been found more frequently in prolonged and severe infections with strains of the *rhodesiense* type. They were accompanied by other lesions at the level of the kidneys or suprarenal capsules, but the lesions noted in the myocardium were of such an intensity that they could without doubt be regarded as the direct cause of death.

Some examples follow :

*Monkey 281.*

Strain from the patient, *Kimori* (region of *G. swynnertoni* Tanganyika) ; 3rd passage by direct transmission. Inoculation on January 27th, 1927 ; trypanosomes in the blood on February 1st ; death on February 10th, 1927.

At autopsy, the pericardium contained 6.5 c.c. of turbid fluid ; Rivalta reaction positive ; formation of a spider's-web clot after seven hours *in vitro*. On examination between coverslips : numerous spherical cells and some erythrocytes, numerous immobile trypanosomes. Heart dilated, pale, soft ; visceral pericardium turgid, showing a few small fibrinous deposits ; myocardium showing no appreciable change.

*Smear preparations :*

*Pericardial exudate.* — Numerous lymphocytes, small and large mononuclears with bilobate nuclei, pericardial cells of endothelial origin, leuco- and erythrocytes. Numerous well-preserved trypanosomes.

*Sections :*

*Parietal pericardium.* — Moderate hyperæmia ; some perivascular infiltrations with small round cells or with polymorphs.

*Visceral pericardium.* — Well-marked œdema ; rich infiltration of mononuclears and macrophages in the sub-endothelial tissue ; small superficial papillary vegetations. A few trypanosomes, spherical in shape, in the interstitial tissue and among the infiltrating elements.

*Myocardium.* — Some œdema ; interstitial and perivascular cellular infiltrations ; muscle fibres turgid and without transverse striation. Very numerous trypanosomes between the capillaries and muscle fibres, typical in shape, very rarely clumped.

*Monkey 282.*

Strain from the patient *Boga* (region of *G. swynnertoni*, Tanganyika) ; 3rd passage by direct transmission. Inoculation on January 29th, 1927 ; trypanosomes in the blood on February 5th ; death on February 18th, 1927.

At autopsy, the pericardium contained 4 c.c. of turbid fluid and a little flake of fibrin. The Rivalta reaction was positive. On examination between coverslips, numerous spherical cells and erythrocytes ; no mobile trypanosomes. Heart showing no noteworthy external changes ; myocardium slightly pale and flabby.

*Smear Preparations :*

*Pericardial exudate.* — Globular endothelial cells, lymphocytes, small and large mononuclears, leucocytes, numerous erythrocytes. Numerous much-altered trypanosomes.

*Myocardium.* — Many polymorphic cells and lymphocytes ; numerous trypanosomes, for the most part much altered or reduced to the nucleus only.



*Sections :*

*Parietal pericardium.* — Moderate hyperæmia ; some perivascular infiltrations of polymorphic cells and lymphocytes.

*Visceral pericardium.* — Pronounced œdema and very extensive cellular infiltration of macrophages, mononuclears, lymphocytes and erythrocytes. Delicate fibrinous clot. Trypanosomes few in number ; in shape, spherical or typical.

*Myocardium.* — Pronounced œdema, muscle fibres separated, atrophic and dissociated and ruptured in the peripheral layers ; capillaries poorly defined ; massive quantities of typical or globular trypanosomes in the interstitial spaces of all the layers and on the surface of the muscle fibres. Diffuse cellular infiltrations very rich in polymorphic cells, mononuclears and lymphocytes ; leucocytes and erythrocytes very rare.

*Monkey 265.*

Strain from the Damba Island antelope Situtunga from Lake Victoria (*G. palpalis*); 3rd passage by direct transmission. Inoculation on January 20th, 1927 ; trypanosomes in the blood on February 4th ; death on March 24th, 1927, after cardiac puncture.

At autopsy, coagulated blood and 2.5 c.c. of sero-sanguineous fluid found in the pericardium. Heart pale and flabby, with traces of the recent puncture of the left ventricle ; myocardium greyish.

*Smear Preparations :*

*Myocardium.* — Numerous polymorphic cells, mononuclears and lymphocytes ; leucocytes and plasma cells very rare. Free, pleomorphic trypanosomes not very numerous, leishmanoid inclusions in some of the mononuclears. (See Fig. 5, Plate IV.)

*Sections :*

*Parietal pericardium.* — Small perivascular infiltrations of lymphocytic and polymorphic cells.

*Visceral pericardium.* — Richly cellular infiltration round the vessels in the sub-endothelial tissue and in the adipose tissue. Lymphatic vessels charged with small mononuclear cells with bilobate nuclei, lymphocytes and macrophages. A few trypanosomes in the perivascular connective tissue.

*Myocardium.* — Infiltration extreme, rich in polymorphic and lymphocytic cells in all the layers. Some polynuclears ; cells with three to five nuclei, erythrocytes, plasma cells all mixed up without special topographical distribution. Atrophic, dissociated, ruptured turgid muscle fibres, sometimes without clear transverse striation. Capillaries poorly defined. Fairly numerous pleomorphic trypanosomes, free or engulfed, on the surface of the muscle fibres and among the infiltrating elements. (See Fig. 2, Plate V, and Fig. 4, Plate IV.)

Trypanosomal myocarditis presents during this phase all the features of a subacute inflammatory process. The course of the illness was only from two to four weeks. That is just about the time taken by other specific inflammations to develop their histological appearances, which present some analogous features.

C. CHRONIC FORMS.

Side by side with these severe and rapid forms of myocarditis, I have observed more moderate types characterised histologically by a slower progress.

I have found these chronic forms in monkeys inoculated some months previously with strains of the *gambiense* or Damba Island antelope type, and, in some cases, after treatment with arsenical preparations.

The histological picture, sometimes very similar to the forms previously described, presents well-marked differences as regards the distribution of the lesions, the numbers of parasites, the alterations of the muscular fibres and of the blood-vessels, and the character of the infiltrating elements.

In some cases, the infiltrations and parasitic deposits are topographically limited, infecting the more peripheral layers close to the visceral pericardium. In the mesial layers, the lesions are less evident and more localised to certain spots. A connective tissue proliferation of the sub-endothelial tissue of the visceral pericardium proves that the alterations in the serous membrane have existed for some time. The visceral pericardium is œdematous, but the fibrous tissue is visibly increased and causing a real thickening. In this tissue, which is studded with numerous fibroblasts and new-formed capillaries, an infiltration of polymorphic and round cells extends more or less uniformly, showing occasionally pseudo-nodular groupings of lymphocytic and plasma cells and mononuclear cells. The trypanosomes are found among the infiltrating elements and in the lymphatic spaces.

The myocardium presents atrophic appearances and an endothelial proliferation in the capillaries. Trypanosomes are few in number or rare. The cellular infiltrations and parasitic deposits sometimes only infect the peripheral layers underlying the visceral pericardium and endocardium and the muscoli papillares. Some muscle fibres show regressive changes; others, on the contrary, are manifestly hypertrophied. Cellular infiltrations are more or less extensive, but small polymorphic cells with basophile protoplasm and plasma cells predominate instead of small mononuclears and macrophages. A delicate reticulum of connective tissue is always visible between these elements, proving the fibroblastic activity of some of them.

These, then, are the chronic forms in which propagation has probably taken place from the primarily infected serous membrane.

This chronic myocarditis presents no macroscopic changes at autopsy, apart from the pericarditis which occasionally accompanies it, and it was sometimes impossible to discover the parasites in sections.

I have found these appearances particularly in monkeys inoculated with human or animal strains in regions of *G. palpalis*, *i.e.*, of the *gambiense* and Damba types. In some cases, they were accompanied by a meningo-encephalitis and their rôle in the causation of death was secondary.

The following cases have been chosen from among those in which parasites could be most definitely observed in the lesions.

#### *Monkey 121.*

Strain from the Damba Island antelope Situtunga (*G. palpalis*); 26th passage by cyclical transmission. Inoculation on June 29th, 1926; trypanosomes in the blood on July 10th, 1926; death on March 29th, 1927. (Treated.)

At autopsy, the pericardium contains 3 c.c. of turbid fluid; Rivalta reaction positive; examination between coverslips shows abundant spherical cells — extremely numerous living trypanosomes. When kept *in vitro*, the fluid coagulates completely in five hours. Heart pale and flabby with well-marked œdema under the pericardium at the base; a few small opaque patches on the visceral pericardium.

#### *Smear Preparations :*

*Pericardial fluid.* — Numerous small and large mononuclears, some lymphocytes; leucocytes rare. Trypanosomes extremely numerous.

*Myocardium.* — Cell nuclei much altered, coarse chromatic reticulum, small and medium-sized mononuclears with basophile protoplasm. Very numerous, well-preserved free trypanosomes of diverse shapes.

*Sections :*

*Parietal pericardium.* — Very limited little perivascular infiltrations.

*Visceral pericardium.* — Thickening of connective tissue and well-marked œdema ; infiltrations of round cells and lymphocytes in a network of fibroblasts scattered here and there in groups round the vessels and in pseudo-nodules. Very numerous trypanosomes among the infiltrating elements.

*Myocardium.* — Proliferation of connective tissue in the subpericardial layers, well-marked œdema and infiltration of polymorphic and round cells of plasmatic type. Endothelial proliferation in the capillaries. Cardiac fibres atrophic and vacuolated, turgid, without clear transverse striation, with hyperchromatic nuclei. Numerous trypanosomes among the infiltrating elements and in the connective tissue spaces. Deeper layers showing no noteworthy changes in the interstitial tissue.

(This monkey was treated with atoxyl from the 73rd day of his illness onward. Parasites disappeared from the blood for 12 days.)

*Monkey 180.*

Strain from the Damba Island antelope Situtunga (*G. palpalis*) ; 10th passage by direct transmission. Inoculation on August 31st, 1926 ; trypanosomes in the blood on September 7th, 1926 ; death on April 28th, 1927.

At autopsy, the pericardium contains 0.25 c.c. of turbid fluid. Rivalta reaction positive. Examination between coverslips shows many spherical cells ; no trypanosomes. Heart dilated, with punctiform subpericardial hæmorrhages. Myocardium the colour of a " dead leaf ".

*Smear Preparations :*

*Pericardial fluid.* — Round cells, mononuclears with basophile protoplasm, leucocytes, macrophages, erythrocytes ; streptococci ; no trypanosomes.

*Myocardium.* — No appreciable change.

*Sections :*

*Parietal pericardium.* — Some very limited perivascular infiltrations.

*Visceral pericardium.* — No appreciable change in the surface and in the subpericardial connective tissue, except for some patches of fibrous thickening.

*Myocardium.* — Very abundant infiltrations of small polymorphic cells at the margin underlying the pericardium and the endocardium. Diffuse or patchy infiltrations of small polymorphic cells in the mesial portion of the myocardium. Cardiac fibres turgid and slightly œdematous. Very few trypanosomes.

(Terminal infection with streptococci with commencing pleuro-pneumonic focus on the right.)

*Monkey 252.*

Strain from a patient, *Kavirondo* (region of Kenya, *G. palpalis*) ; 1st passage by cyclical transmission. Inoculation on November 17th, 1926 ; trypanosomes in the blood on December 20th, 1926 ; death on April 1st, 1927.

At autopsy, the pericardium contained 3 c.c. of turbid fluid; Rivalta reaction positive. Examination between coverslips showed numerous globular cells, no living trypanosomes. Heart dilated, pale and flabby, with opaque patches on the visceral pericardium.

*Smear Preparations :*

*Pericardial fluid.* — Mononuclears and globular endothelial cells, lymphocytes, leucocytes and altered erythrocytes. Doubtful, altered trypanosomes. Microfilaria.

*Myocardium.* — Small and large mononuclears with basophile protoplasm; few and altered trypanosomes.

*Sections :*

*Parietal pericardium.* — A few perivascular infiltrations with round and polymorphic cells.

*Visceral pericardium.* — Thickening in places; cellular infiltrations grouped in pseudo-nodules, with small polymorphic and round cells, very numerous in the vicinity of the myocardium.

*Myocardium.* — More or less marked oedema in some places; muscle fibres of different sizes, pigmented, turgid, with ill-defined striation. Interstitial tissue increased locally and studded with small spherical polyhedral cells, and small fibroblasts with elongated nuclei. Plasma cells very rare, capillaries well marked, with endothelial proliferation. A few trypanosomes close to the surface of the muscle fibres and in the spaces of the oedematous interstitial tissue.

(Terminal invasion of staphylococci without histological manifestations.)

D. CIRRHOSIS.

The evolution of trypanosomal myocarditis as observed by me has convinced me that the definite modification of the histological manifestations could have been obtained in treated or in more resistant monkeys.

In monkey No. 80, I had observed a well-marked cirrhotic state of the myocardium, but there was such a wide gulf between this structure, which was lacking in specific features, and even the most advanced forms observable in other monkeys, that they could not be correlated without having recourse to problematical conclusions.

The alterations found in monkeys 276, 126 and 208 have given me the data which were lacking to correlate the cirrhoses with the other manifestations of trypanosomal myocarditis.

*Monkey 276.*

Strain from the patient *Awasi* (region of *G. palpalis*); 2nd passage by direct transmission. Inoculation on January 17th, 1927; trypanosomes not found in the blood; killed on June 10th, 1927.

At autopsy, the pericardium contained  $\frac{1}{2}$  c.c. of turbid fluid; on examination between coverslips, the fluid showed many globular cells and living trypanosomes. Heart of normal size, with opaque patches on the visceral pericardium.

*Smear Preparations :*

*Pericardial fluid.* — Small and large mononuclears, lymphocytes and leucocytes; a few trypanosomes of typical form.

*Myocardium.* — Rare polymorphic and plasma cells; a few trypanosomes.

*Sections :*

*Parietal pericardium.* — Perivascular infiltrations of round and polymorphic cells.

*Visceral pericardium.* — Thickening and cellular infiltrations in places, grouped in pseudo-nodules, consisting of polymorphic and plasma cells.

*Myocardium.* — Hypertrophic muscle fibres, increased interstitial tissue studded with polymorphic cells, fibroblasts and plasma cells. A few trypanosomes in the interstitial tissue. (See Fig. 1, Plate VI.)

*Monkey 126.*

Strain from the patient *Adjambo* (region *G. palpalis*, of Kenya); 1st passage by cyclical transmission. Inoculation on June 30th, 1926; trypanosomes in the blood on July 17th, 1926; death on May 1st, 1927.

At autopsy, the pericardium contained a little limpid fluid, with a weakly positive Rivalta reaction. Heart a little œdematous at the base, brown "dead leaf" colour.

*Smear Preparations :*

*Pericardial fluid.* — Nothing specific.

*Myocardium.* — A few polymorphic cells. Doubtful trypanosomes.

*Sections :*

*Parietal pericardium.* — Perivascular infiltration of round and polymorphic cells. Vessels with thickened walls.

*Visceral pericardium.* — Marked thickening of connective tissue. Some infiltration of fibroblasts and plasma cells.

*Myocardium.* — Patches and islets of well-marked cirrhosis, with atrophy and dissociation of the muscle fibres. The connective tissue is fibrous, studded with fibroblasts and with a very few deformed plasma cells in small heaps, close to the vessels. Cardiac fibres atrophied in places, or hypertrophied. No demonstrable trypanosomes. (See Fig. 6, Plate IV and Fig. 2, Plate VI.)

*Monkey 208.*

Strain from the Damba Island antelope *Situtunga* (*G. palpalis*); 12th passage by direct transmission. Inoculation on October 23rd, 1926; trypanosomes in the blood on November 16th, 1926; death on May 17th, 1927.

At autopsy, the pericardium contained 4.5 c.c. of turbid fluid, with positive Rivalta reaction, which coagulated in a few hours and became gelatinous in consistence. Heart showing a little œdema at the base, myocardium consistent, of "dead leaf" colour.

*Smear Preparations :*

*Pericardial fluid.* — Numerous large and small mononuclears, lymphocytes, leucocytes; a few altered trypanosomes.

*Myocardium.* — Nothing specific.

*Sections :*

*Parietal pericardium.* — Thickening and small round-celled infiltration.

*Visceral pericardium.* — Thickening of the subendothelial tissue.

*Myocardium.* — Infiltration of plasma cells and commencing cirrhosis.

Monkey 80.

Strain from the patient *Walubani* (region of *G. palpalis*, Samia); 3rd passage, direct transmission. Inoculation on May 11th, 1926; trypanosomes in the blood on June 28th, 1926; death on February 22nd, 1927.

At autopsy, the heart presented no macroscopic changes.

*Smear Preparations :*

*Pericardial fluid.* — Some endothelial cells; no trypanosomes.

*Myocardium.* — Nothing specific.

*Sections :*

*Parietal pericardium.* — Some very limited cellular infiltrations close to the vessels.

*Visceral pericardium.* — Unequal thickening of the subendothelial connective tissue; infiltrations of polymorphic cells close to the myocardium.

*Myocardium.* — Increase of connective tissue between the muscle fibres and close to the vessels. Some isolated infiltrations of small polymorphic cells, very rare.

These observations make the histological features of trypanosomal myocarditis sufficiently clear. We are dealing with a more or less chronic inflammatory process, essentially interstitial, determined by the deposition and multiplication of the parasites outside the blood-vessels, as well as by an exudative, hyperplastic reaction of granulo-matous type, capable of a fibrotic evolution.

\* \* \*

Clinical observations of sleeping-sickness frequently indicate disturbances of cardiac function by sudden deaths and a premature fatal issue before the lesions of the nervous system are sufficiently advanced.

If the nervous manifestations are capable of indirectly explaining the symptoms and of distracting attention from the myocardium, it must be recognised that the pathological anatomy has for long been teaching important lessons.

The observations made by THOMAS AND BREINL in three autopsies on the state of the heart followed by histological examinations, and also earlier less-detailed investigations noted in the early work of MOTT, CASTELLANI and LOW, show that chronic myocarditis is not rare in sleeping-sickness of the *gambiense* type.

If one has not attached more importance to these observations, it is partly because trypanosomes could not be demonstrated in the lesions, partly because other factors were associated with the trypanosomiasis and also because bacteria were found in the myocardial tissue. But these lesions represent advanced phases of cardiopathies due to infections of the *gambiense* type in human beings, and correspond closely to the lesions produced by the same type of infections in monkeys. This also is a mark of the remarkable resemblance existing between trypanosomal lesions in human beings and in monkeys.

I have not been able to obtain any information on the pathological histology of infections of human beings by the *rhodesiense* type; but the result of my observations justifies the conviction that, in human infections of this type, cardiac disturbance must be extremely severe and must sometimes outweigh those of the nervous system.

#### CONCLUSIONS.

Trypanosomal myocarditis frequently causes death in inoculated monkeys. The lesions are due to massive deposits of trypanosomes, which multiply in the myocardium, external to the vessels.

They become manifest in the first stages by infiltrations which are rich in macrophages, in mono- and polynuclear cells and in leucocytes. In advanced and chronic conditions one finds infiltrations of polymorphic, lymphocytic and plasma cells, and confluent foci of fibrosis.

These forms of myocarditis are always associated with exudative pericarditis. The gravest and most frequent forms are due to trypanosomes of human or animal origin conveyed by *G. morsitans* and kindred species and to trypanosomes of animal origin conveyed by *G. palpalis*.

Less acute forms are found in infections caused by trypanosomes of human origin conveyed by *G. palpalis*.

These latter forms correspond without doubt to the human lesions described in the earliest publications on the pathological anatomy of sleeping-sickness, ascribed to the *gambiense* strain.

In view of the cardiac disturbances, sudden deaths and premature fatal issue of the malady noted in human infections due to strains of *rhodesiense*, it is extremely probable that in these forms the cardiac changes are very severe and sometimes more important than the changes in the nervous system.

Researches on this subject would surely yield interesting results.

Entebbe, June 1927.

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4.

CHANGES IN THE CHOROID PLEXUSES AND ENTRY OF  
TRYPANOSOMES INTO THE CEREBRO-SPINAL FLUID

The experimental pathology of sleeping-sickness has very rarely demonstrated characteristic lesions in the nervous system; the most detailed reports only refer to neuroglial proliferations and rare cellular infiltrations surrounding the vessels and these only in the last stages of the most prolonged infections.

In more rapid forms, examination of the nervous system has until now given nothing but almost negative results.

In my autopsies, I only found really characteristic and well-marked meningo-encephalitis in infections, which were severe and at the same time prolonged, due to trypanosomes of human and animal origin conveyed by *G. palpalis*, type *gambiense*, and strains from the Damba antelopes.

In infections where the course was rapid, due to trypanosomes of human and animal origin conveyed by *G. morsitans* and kindred species (*rhodesiense* and *brucei*), the lesions of the nervous system were, with certain rare exceptions, appreciable only with difficulty.

It is generally believed that trypanosomes only infect the nervous system and the cerebro-spinal fluid in the last stages of infections whose course is slow. Nevertheless, my findings in examining the cerebro-spinal fluid and the choroid plexuses have revealed unexpected facts which are singularly interesting in this respect and which throw some light on the relations between the vascular lesions and the alterations of the cerebro-spinal fluid in trypanosomiasis of the nervous system from the point of view of pathological anatomy.

Here are some of the most significant observations :

*Monkey 365.*

Inoculated with an animal strain from *G. palpalis* (Damba Island antelope Situtunga) on May 23rd, 1927, by direct transmission at the 5th passage. Duration of infection, 8 days.

Severe infection with hæmorrhagic lesions and exudative pericarditis. Death followed a hæmorrhagic ulcer of the intestine and peritonitis.

*Examination of the nervous system.* — Negative.

*Examination of the cerebro-spinal fluid.* — Negative.

*Choroid plexuses.* — Early signs of infiltrations of mononuclears and lymphocytic cells. Trypanosomes very rare.

*Monkey 351.*

Inoculated with a human strain from *G. swynnertoni*, type *rhodesiense*, at the 4th passage by direct transmission on May 8th, 1927. Duration of infection, 14 days. Severe infection with hæmorrhagic lesions, exudative pericarditis, massive deposits of trypanosomes in the myocardium.

*Examination of the nervous system.* — Moderate hyperæmia at autopsy; no appreciable change on examination of sections.

*Examination of the cerebro-spinal fluid.* — Rivalta reaction negative; no cytological change; no demonstrable trypanosomes.



*Choroid plexuses.* — Well-marked dilatation of interstitial spaces ; very numerous trypanosomes of typical shape and short and globular, some in compact masses, some dispersed throughout the areolar tissue. Some also are visible on the surface of the epithelium between the folds, proving that they have reached the ventricular cavity. Inside the vessels, trypanosomes are rare or absent. In the interstitial tissue there is moderate proliferation of polymorphic cells ; rare mononuclears or leucocytes are the first elements to herald the very beginnings of an histological reaction. (See Fig. 1, Plate VIII.)

We are not dealing with a simple passage of trypanosomes through the vessel walls, but with a true localisation by invasion and extravascular multiplication which is not repeated in the perivascular spaces of the meninges or nervous tissue.

*Monkey 354.*

Inoculated with a very virulent human strain of doubtful *G. palpalis*, origin *Bambu*, at the 2nd passage by direct transmission on May 12th, 1927. Severe infection, with hæmorrhagic manifestations, pericarditis and myocarditis. Duration of infection, 28 days.

*Examination of the nervous system.* — Moderate hyperæmia ; beginnings of meningo-encephalitis with neuroglial proliferation.

*Examination of the cerebro-spinal fluid.* — Results doubtful (traces of blood).

*Choroid plexuses.* — Widely infiltrated with mononuclears, leucocytes and lymphocytic cells. Extremely numerous trypanosomes as in Monkey 351. (See Fig. 2.)

*Monkey 337.*

Inoculated with a human strain from *G. morsitans*, type *brucei*, at the 2nd passage by direct transmission on April 27th, 1927. Duration of disease, 31 days. Severe infection, with incipient myocarditis.

*Examination of the nervous system.* — Moderate hyperæmia, incipient meningo-encephalitis with cellular infiltrations, neurological and endothelial proliferations at the level of the basilar nuclei, and of the protuberance and of the bulb.

*Examination of the cerebro-spinal fluid.* — Rivalta reaction weakly positive ; spherical cells with small nuclei of ventricular origin, mononuclear and lymphocytic cells ; rare, much-altered trypanosomes.

*Choroid plexuses.* — Large deposits of pleomorphic trypanosomes, scattered and in compact masses. Very abundant infiltrations of mononuclears and leucocytes. (See Fig. 3, Plate VIII.)

*Monkey 311.*

Inoculated with a human strain from *G. swynnertoni*, *rhodesiense*, at the 5th passage by direct transmission on March 25th, 1927. Duration of the infection, 34 days. Severe infection with polyserositis, myocarditis and nephritis.

*Examination of the nervous system.* — Moderate hyperæmia, incipient meningo-encephalitis with neuroglial proliferation.

*Examination of the cerebro-spinal fluid.* — Rivalta reaction negative ; cells from the ventricular walls and mononuclears.

*Choroid plexuses.* — Infiltrations very rich in mononuclears, in leucocytes and in lymphocytic cells ; very numerous pleomorphic trypanosomes.

*Monkey 265.*

Inoculated with an animal strain from *G. palpalis* (Damba Island antelope Situtunga) at the 3rd passage on January 20th, 1927. Duration of infection, 64 days. Very severe infection with myocarditis and nephritis, complicated by malaria.

*Examination of the nervous system.* — Moderate hyperæmia ; incipient infiltrative meningitis.

*Examination of the cerebro-spinal fluid.* — Rivalta reaction negative ; cells of ventricular origin ; rare mononuclears.

*Choroid plexuses.* — Beginnings of perivascular infiltrations of lymphocytic cells ; trypanosomes rare.

*Monkey 276.*

Inoculated with a strain of human origin from *G. palpalis*, type *gambiense*, at the 1st passage on January 17th, 1927. Duration of infection, 173 days. Killed for diagnosis. Latent infection observed at autopsy with a moderate degree of splenomegaly and exudative pericarditis.

*Examination of the nervous system.* — Negative.

*Examination of the cerebro-spinal fluid.* — Quantity increased ; Rivalta reaction negative ; lymphocytic and mononuclear cells.

*Choroid plexuses.* — Desquamation of the epithelium ; beginnings of infiltration of lymphocytic cells.

*Monkey 208.*

Inoculated with a strain of animal origin from *G. palpalis* (Damba Island antelope Situtunga), at the 12th passage by direct transmission on October 23rd, 1926. Duration of infection, 206 days. Severe infection with myocarditis and exudative pericarditis.

*Examination of the nervous system.* — Advanced meningo-encephalitis.

*Examination of the cerebro-spinal fluid.* — Amount increased ; weakly positive Rivalta reaction ; abundant lymphocytic and mononuclear cells.

*Choroid plexuses.* — Moderate infiltration of polymorphic, lymphocytic and plasma cells. Interstitial fibrillary substance visibly increased. Rare trypanosomes in the spaces of the fibrillary tissue.

*Monkey 180.*

Inoculated with the same strain as monkey 208 at the 11th passage on August 31st, 1926. Duration of infection, 208 days. Severe infection, complicated by a terminal streptococcal septicæmia.

*Examination of the nervous system.* — Advanced meningo-encephalitis.

*Examination of the cerebro-spinal fluid.* — Rivalta reaction weakly positive ; lymphocytic and mononuclear cells. Trypanosomes rare and extremely altered (doubtful observation of nuclei alone).

*Choroid plexuses.* — Perivascular infiltration of lymphocytic and plasma cells ; rare trypanosomes. (See Fig. 4, Plate VIII.)

*Monkey 3.*

Inoculated with a strain of animal origin from *G. palpalis* (Damba Island antelope Situtunga) at the 19th passage by cyclical transmission on December 21st, 1925. Duration of infection, 426 days. Severe infection, with lethargic symptomatology.

*Examination of the nervous system.* — Very advanced meningo-encephalitis.

*Examination of the cerebro-spinal fluid.* — Amount increased; Rivalta reaction strongly positive; lymphocytic and mononuclear cells very numerous; muriform cells; trypanosomes.

*Choroid plexuses.* — Fibrous thickening and pronounced infiltrations of lymphocytic cells.

*Dog (Autopsy XXXVIII).*

Natural, very advanced infection, originating in Mbarara, region of *G. morsitans*, in Uganda. Brought to the laboratory dying on April 10th, 1927; killed by chloroform. Severe infection with paresis of the hind-quarters, keratitis and exudative pericarditis.

*Examination of the nervous system.* — Very advanced meningo-encephalitis.

*Examination of the cerebro-spinal fluid.* — Rivalta reaction strongly positive; numerous mononuclear and lymphocytic cells; altered trypanosomes.

*Choroid plexuses.* — Fibrillary thickening and infiltration of polymorphic and lymphocytic cells.

*Calf 13.*

Inoculated with a strain of human origin of the *rhodesiense* type on January 12th, 1927. Killed after four months, in a grave condition; œdemas, effusions, glandular hypertrophies.

*Examination of the nervous system.* — Advanced meningo-encephalitis.

*Examination of the cerebro-spinal fluid.* — Numerous mononuclear and lymphocytic cells; traces of blood.

*Choroid plexuses.* — Fibrous thickenings and moderate infiltration of lymphocytic cells; rare trypanosomes.

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RELATIONSHIP BETWEEN THE CHANGES IN CEREBRO-SPINAL FLUID AND VASCULAR SYSTEM AND THE PRESENCE OF TRYPANOSOMES.

*Changes in the Cerebro-spinal Fluid.*

I have examined the cerebro-spinal fluid recovered post mortem by suboccipital puncture after dissection of this region. This procedure has nearly always enabled me to obtain the fluid without foreign elements, except in some animals punctured immediately after death, in which it was difficult entirely to avoid traces of blood.

The very limited quantity of fluid which was all that I could usually obtain was not enough for precise chemical researches. I was obliged to confine myself to simple and rapid examinations, which were always possible, carried out during the autopsy; that is why I employed the Rivalta reaction, as for the serous fluids. In these less

favourable cases, a drop of cerebro-spinal fluid was enough to give comparable information on its albuminoid content. One drop was likewise sufficient for the examination between coverslips and for small preparations.

When the cerebro-spinal fluid contained no trace of blood, the Rivalta reaction was almost always negative ; it was clearly positive only in very advanced meningeal and nervous lesions.

The Rivalta reaction and microscopic examination between coverslips and of smear preparations stained with Giemsa facilitated the determination of different degrees of alteration which accorded with those of the histological lesions.

#### *Early Changes.*

I have been able to convince myself that the cerebro-spinal fluid frequently presents noteworthy changes in infections whose course is rapid, caused by trypanosomes of human or animal origin from *G. morsitans* and *G. swynnertoni*, *rhodesiense* and *brucei* types.

In the cytological picture, mononuclears and the cells from the walls of the ventricles almost always predominate, associated with lymphocytic cells and a very few leucocytes. Exceptionally there were red cells whose doubtful origin could be excluded.

The chemical changes observable in the Rivalta reaction were much rarer.

These early changes were evident when the duration of the infection had surpassed two weeks.

The presence of trypanosomes in the cerebro-spinal fluid was observable in monkey 337 ; parasites were extremely rare and much changed ; sometimes reduced to nucleus alone, more easily recognised when engulfed in phagocytes.

In this monkey, the Rivalta reaction had been positive, though not strongly so.

#### *Late Changes.*

In infections whose progress was slow, caused by trypanosomes of human and animal origin from *G. palpalis* (*gambiense* type and strains derived from the Damba Island antelopes), I found changes in the cerebro-spinal fluid to be rarer but much more pronounced.

These changes were characterised by the predominance of lymphocytic cells and by the occasional presence of muriform cells. The chemical changes were manifest in a strongly positive Rivalta reaction.

It was often easy to observe the presence of trypanosomes. The parasites were always motionless, free, morphologically altered, with a stiff flagellum, or else well phagocytosed, of globular form.

#### *Histological Changes.*

The histological changes of the nervous system in rapid infections were almost insignificant, except in monkey 337. They consisted in a dilatation of the lymphatic spaces and in a certain proliferation of the neuroglia. In monkey 337 there was a true beginning of meningo-encephalitis, characterised by cellular infiltrations like cuffs round the arteries at the level of the basilar nuclei, the protuberances and the bulb. On the other hand, the choroid plexuses of all these monkeys were the seat of important inflammatory changes, always associated with the presence of trypanosomes, always numerous and occasionally in massive quantities.

We find the first stages of these lesions in monkeys 351, 354 and 337.

In monkey 351, the infection lasted 14 days. The histological reaction is in its very earliest phase. There are enormous numbers of trypanosomes in the interstitial spaces of the plexuses beneath the epithelium.

In monkey 354, the infection lasted 28 days. The reaction is manifest in proliferative and exudative phenomena; the trypanosomes are extremely numerous.

In monkey 337, the infection lasted 31 days. The histological reaction is already advanced, but the trypanosomes are still present, sometimes scattered, sometimes in massive deposits. In these preparations, then, we find localisation of the parasites at the level of the choroid plexuses, the steps of their progress to the ventricles and the phases of a local reaction in the interstitial tissue; the latter is characterised by a proliferation of histiocytes and an emigration of leucocytes.

These are the predominating elements in the cerebro-spinal fluid associated with epithelial cells from the surface of the plexuses and from the walls of the ventricles.

Yet trypanosomes were not found except in monkey 337.

The histological changes in the nervous system in slow infections were extremely severe.

In some monkeys and in the dog infected naturally, they gave pictures of the most advanced forms of meningo-encephalitis characteristic of sleeping-sickness, and trypanosomes were evident in the perivascular lymphatic spaces.

The choroid plexuses were the seat of fibrous thickenings and perivascular infiltrations with lymphocytic cells; the vessels of the nervous tissue were surrounded by rich infiltrations of lymphocytic cells and mononuclears, sometimes associated with muriform cells.

These histological elements associated with trypanosomes predominate in the cerebro-spinal fluid.

The relation between the findings is striking: meningo-encephalitis, increase in the albuminoid content of the cerebro-spinal fluid and demonstrable presence of trypanosomes: these are the three phenomena which one finds united in the most advanced forms of cerebral trypanosomiasis. On the other hand, the most massive deposits of trypanosomes in the choroid plexuses and their passage in large numbers into the cerebro-spinal fluid are not necessarily associated with meningo-encephalitic manifestations.

These trypanosomes, whose passage is easily observable, are not found in the fluid recovered by suboccipital puncture.

The conclusion is that normal cerebro-spinal fluid does not favour the survival of the trypanosomes. Those which escape from the choroid plexuses are rapidly destroyed. The cerebro-spinal fluid is therefore a defensive mechanism against the invasion of trypanosomes, until the moment when its albuminoid content becomes increased. If we examine, with this in mind, the tables given by BRODEN AND RODHAIN with regard to their numerous examinations of cerebro-spinal fluid, we find that, when trypanosomes were demonstrable, the albuminoid content was at least 0.5 per thousand. Below this figure, no trypanosomes were demonstrable.

The interest of these findings extends to some therapeutic questions, as, *e.g.*, to that of treatment of the patients by serum injections into the cerebro-spinal fluid.

The pathogeny of trypanosomal meningo-encephalitis hardly differs from the pathogeny of other lesions. The parasites can settle in the choroid plexuses and there produce important histological lesions; there are very common early manifestations of the power of invasion possessed by strains from *G. morsitans* and from *G. swynner-toni*, but these deposits are not in themselves the cause or the beginnings of true meningo-encephalitis. This shows itself in more extensive vascular changes,

characterised at the beginning by endothelial and neuroglial proliferations, associated with chemical modifications of the cerebro-spinal fluid which allow of the survival and possibly also of the multiplication of trypanosomes which have escaped from the vessels.

#### CONCLUSIONS.

The choroid plexuses are the site of early extravascular deposits of trypanosomes of human and animal origin from *G. morsitans* and *G. swynnertoni*.

These penetrate into the cerebro-spinal fluid in large numbers, but are rapidly destroyed, not finding conditions favourable to their survival.

The degree of these lesions is not proportional to the vascular lesions of the meninges and of the nervous tissue. The cerebro-spinal fluid is a defensive mechanism against the invasion of trypanosomes until its albuminoid content is raised. It contains demonstrable trypanosomes when the phenomena of meningo-encephalitis are definitely established. This happens particularly, but not exclusively, in advanced forms of chronic trypanosomal infections, caused by strains of human or animal origin from *G. palpalis*, and also when the changes in the cerebro-spinal fluid are very pronounced.

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5.

## ON THE ORIGIN AND HISTOLOGICAL RELATIONS OF THE MORULAR CELLS IN TRYPANOSOMAL MENINGO-ENCEPHALITIS

From the earliest researches on sleeping-sickness onwards, the changes in the cerebro-spinal fluid have attracted the greatest share of attention. The cytological picture, clearly mononuclear, was pointed out by CASTELLANI, even before his discovery of the trypanosome.

It is particularly in diagnosis of the chronic forms and in evaluating the nervous lesions that the cytological changes in the cerebro-spinal fluid can provide the most useful information. Among the cellular elements of the cerebro-spinal fluid, the morular cells play the most important rôle. In fact, their appearance is considered as a sign of very advanced lesions.

Morular cells (mulberry cells, Mott's cells, maulbeerförmige Zellen) observed by Christy in the cerebro-spinal fluid, have been studied from the histological point of view by MOTT, SPIELMAYER and STEVENSON, while others have studied their clinical significance in the cerebro-spinal fluid (BRODEN, RODHAIN, REICHENOW).

According to MOTT's researches, the morular cells represent degenerative manifestations of the plasma cells, or of the endothelial cells of the vessels.

SPIELMAYER considers them to be a product of degeneration attacking the vessel walls.

BRODEN and RODHAIN think they are derived from the large mononuclears of the cerebro-spinal fluid.

REICHENOW considers them as lymphocytes undergoing vesicular degeneration.

The cerebro-spinal fluid indeed often contains vacuolated cells of different sizes, which may simulate initial forms of morular cells. But histological researches prove that these cells have a deeper origin, in the interior of the nervous tissue; and, if one considers their protoplasmic and nuclear characteristics, no doubt is possible as to their histological significance: morular cells are elements attacked by grave degenerative changes; sometimes all trace of nucleus has disappeared and only a mass of refractile spherules remains, bound together by traces of protoplasm difficult to show up.

These features prove the impossibility of active movements. Yet morular cells have been pointed out in the most diverse situations even outside the nervous system. MOTT has described them among the elements of the cellular cuffs which surround the blood-vessels in the lymphatic spaces, and he has recognised in these cells the features of the Körnchenzellen which ALZHEIMER has described in his studies of general paralysis.

The same author has found morular cells in the lymphatic glands.

SPIELMAYER has seen them in the vascular walls in different organs.

STEVENSON has recently pointed them out in the midst of the white matter in a chronic case of sleeping-sickness; he has mapped out their position and has admitted that they were in relation to the walls of capillaries situated beyond the limits of the section.

VAN HOOFF found morular cells in the cerebro-spinal fluid of monkeys. I had the opportunity to perform the autopsy on one of these (No. 3); its nervous system presented

the most complete picture of meningo-encephalitis as found in the most advanced cases of sleeping-sickness in man.

I have had the opportunity to define some histological and cytological relations of the morular cells in making use either of material obtained at this autopsy or at the autopsies of other monkeys which had presented the same lesions, or of the cerebro-spinal fluid collected at these autopsies, or of the cerebro-spinal fluid of patients.

In examining the morular cells in the cerebro-spinal fluid of a patient and in the sections from monkeys Nos. 3 and 180, I noticed their resemblance to elements which were well known to me, *e.g.*, to cells with hyaline globules and to the Russel bodies; and, as I had recognised in my researches on certain granulomata that fuchsinophile hyaline cells were derived from plasma cells, I set myself to discover the initial forms of Mott's cells in order to gain a close acquaintance with these cells, and establish their identity. I had found the same cells in perivascular infiltrations in the liver (see Report No. 1), and fuchsin stains had given me the same very pronounced reaction, both in sections and in the smear preparations of the cerebro-spinal fluid in man and in the monkey.

There was, then, no doubt on this point: the cell of Mott is a cell with fuchsinophile hyaline globules, and these globules, after the destruction of the protoplasm and nucleus, have all the morphological characters of Russel's bodies.

The findings up to this point allowed of the identification of the cell of Mott, with very common and very widely spread elements in the most diverse granulomatous inflammations; but further researches, pursued in order to obtain information with regard to the earliest manifestations of this cellular degeneration, have given me results which most unexpectedly complicate the question; they have given me striking arguments in favour of a neuroglial origin of these morular cells, and of their pathogenic rôle, in consequence of the relation which they frequently bear to nerve cells.

I will choose, among the nervous lesions found in monkeys, a few which present more or less advanced lesions, given in the plates.

*Monkey 3.* — Inoculated in December 1925, with a strain from the Damba Island antelope *Situtunga (G. palpalis)*; 19th passage by cyclical transmission. Trypanosomes in the blood on January 11th, 1926; death on February 15th, 1927. The examination of the cerebro-spinal fluid made by VAN HOOFF on October 7th, 1926, showed 350 lymphocytes per c.mm.; large mononuclears and morular cells. During the last weeks of its life, this monkey had presented clear symptoms of lethargy.

At autopsy, the examination of the cerebro-spinal fluid gave the same cytological results which VAN HOOFF had found previously; moreover, trypanosomes were found to be present. At inspection, the pia mater was visibly thickened at the level of the bulbar protuberance, over the base and on the parietal lobes.

Histological examination revealed very advanced and characteristic meningo-encephalitic lesions.

*Monkey 180.* — Inoculated on August 31st, 1926, with a strain from the Damba Island antelope *Situtunga (G. palpalis)*; direct transmission at the 11th passage. Trypanosomes in the blood on September 7th, 1926; death on April 28th, 1927; it had appeared seriously ill on the previous day.

At autopsy, the cerebro-spinal fluid contained globular cells and lymphocytes; presence of trypanosomes doubtful.

At inspection, a pronounced hyperæmia of the meninges was noted.

Histological examination revealed advanced meningo-encephalitic lesions.

Death was caused by a generalised streptococcal infection with incipient pneumonia and localised pleurisy on the right.



*Monkey 311.* — Inoculated on March 25th, 1927, with a strain from the patient *Boga* (region of *G. swynnertoni*, Tanganyika), direct transmission at the 5th passage. Trypanosomes in the blood on April 5th, 1927. Death on April 29th, 1927.

At autopsy, the cerebro-spinal fluid contained some lymphocytes and mononuclear cells of ventricular origin. The meninges were very hyperæmic.

At the histological examination, pronounced lesions were found at the level of the choroid plexuses and the onset of lesions in the small vessels of the bulb, of the basal ganglia and of the covering of the hemispheres.

Death was caused by a specific myocarditis and nephritis.

*Monkey 222.* — Inoculated on October 28th, 1926, with a strain from the patient *Dabiro* (region of *G. palpalis*, Kenya); cyclical transmission at the 1st passage. Trypanosomes in the blood on November 30th, 1926; death on February 14th, 1927.

At autopsy, the cerebro-spinal fluid contained a few lymphocytes.

On inspection of the nervous system, no appreciable changes were found.

On histological examination, incipient encephalitis with neuroglial proliferation was found.

Death was due to peritonitis, probably caused by œsophagostomes.

The four monkeys in question present the different stages of the nervous lesions: very severe in monkey 3; severe in monkey 180; weak in monkey 311; at their onset in monkey 222.

In order to demonstrate the morular cells contained in the tissues, I employed acid fuchsine staining, which has an elective affinity for these elements and is an easy method of discovering them.

The most favourable method, which I employed also in researches on cells with hyaline bodies in granulomata, is the following:

Fix the material in formol or in sublimate in alcoholic solution;

Stain the sections first with hæmatoxylin, following the usual technique;

Stain for five minutes in an aqueous solution of 1 per cent acid fuchsine; after differentiation with alkaline water and washing with distilled water, pass directly into iodised alcohol; leave for three minutes.

Differentiate with alcohol at 86° C.; (187° F.); dehydrate; put through xylol; mount in balsam.

The figures of Plate IX show the appearance of sections treated thus.

The same staining may be applied to smear preparations of the cerebro-spinal fluid after moist fixation; but this colouration is specially useful for the study of morular cells in sections: their very visible colouration allows them to be easily picked out, and their initial forms and their relations to be observed.

We will study successively the histological relations of the morular cells in vascular, nervous and meningeal lesions.

### I. *The Morular Cell in Vascular Lesions.*

It is in the perivascular cellular cuffs that MOTT has described morular cells in man, and studied their relations with the other infiltrating elements.

This localisation is frequent if the lesions of the nervous system are advanced.

*Monkey 3.* — Morular cells are found in some perivascular cellular cuffs. They lie most frequently in the peripheral or mesial layers. I have only found them in relation with the intima when the infiltration was at its onset and represented by a single layer of cells.

The surrounding elements are polymorphic cells, among which one can identify lymphocytes, small and large mononuclears with displaced nuclei and small fibroblasts; some polynuclear leucocytes and sometimes erythrocytes are found there occasionally.

On the walls of the lymphatic space in the peripheral or mesial layers of the perivascular infiltrations, one nearly always sees cells whose nucleus is larger and paler, with two or three chromatic granules, protoplasm ramified in prolongations which occasionally preserve the relation of continuity with the adjacent neuroglial elements. All these elements are contained in a very delicate fibrillary network but easily stainable by Bielschowski's method. The stouter fibrils of this cementing network are arranged concentrically round the intima, which proves that they are newly formed, and superimposed on the rare radial filaments which normally support vessels in the lymphatic spaces.

These cellular cuffs do not represent only a cellular infiltration; they are a real tissue, composed of embryonic cells of diverse origin altogether comparable to that found in other organs. The specific elements are represented by cells of the lymphatic space and particularly by the neuroglial cells.

The initial forms of morular cells, which are difficult to encounter, are cells with hyaline protoplasm, or studded with fuchsinophile globules, with a nucleus closely resembling that of the neuroglial cells.

These initial forms are situated at the periphery of the cuff or on the walls of the lymphatic space.

These perivascular infiltrations attain their maximum development at the level of the bulb, of the protuberance and of the basilar nuclei; they are well marked round the larger vessels but they do not spare even the capillaries, where the structure of the cellular cuffs clearly reveals them as being of endothelial origin.

*Monkey 180.* — The perivascular infiltrations are less marked than in the preceding case.

The cellular cuffs present very characteristic features, but they are less rich in cement fibrils; the cells are freer and represented by small and large mononuclears and by neuroglial cells. Mott's cells are extremely rare and situated in the more peripheral layers.

*Monkey 311.* — The vascular changes are ill defined, but already characterised by an endothelial and adventitial proliferation of the smallest vessels. The new-formed elements are small pleomorphic cells with dark nuclei. In the lymphatic space, some proliferating neuroglial cells are extending (taking their point of departure from the limits of the nervous tissue) along the course of the radial filaments, which support the vessels and have reached the surface of these.

No morular cells, even in their earliest form.

*Monkey 222.* — Vascular changes even less marked. The intima of the small vessels does not present cellular proliferations; the lymphatic space is free; in the surrounding nervous tissue there is a moderate neuroglial proliferation. The larger vessels are the site of a weak adventitial proliferation. No morular cells.

## 2. *Morular Cells in Nervous Lesions.*

In very advanced cases, the nervous tissue is the site of important infiltrative phenomena. The appearance of some lesions might suggest the possibility of the emigration of mobile elements of vascular origin. In reality, it is a question of neuroglial proliferations.

*Monkey 3.* — The white matter as well as the grey is studded with cellular infiltrations, both diffuse and in definite foci. These infiltrations are frequently found close to the most altered vessels; sometimes a partial obliteration of the lymphatic

space and an apparent continuity of the vascular lesions with the cells of the nervous tissue are observed ; but these foci of cellular infiltration are likewise found in the midst of the nervous tissue without any continuity with the vascular structures, sometimes at the boundary between the white and the grey matter or along the lengths of the bundles of nerve fibres.

Judging by their appearances, one might confound the infiltrating cells with lymphocytes, plasma cells or endothelial cells. In fact, one finds all the transitional forms between neuroglial cells and the lymphocytic cells of chronic inflammations. It is impossible to make a precise distinction ; the blood capillaries certainly may give rise to lymphocytic cells, or to cells closely resembling plasma cells ; but it is more probable that the neuroglia is the origin of the small polymorphic and globular cells which form these infiltrations.

Morular cells are very numerous in the foci of infiltration, but may also be found isolated along the smallest capillaries or the bundles of nerve fibres.

Morular cells are frequently found side by side with nerve cells, which are on that account deformed and profoundly altered.

*Monkey 180.* — Less pronounced but still definite infiltrative lesions ; a few morular cells in the nervous tissue, usually limited to the neighbourhood of vascular structures, where neuroglial proliferation is most manifest.

*Monkey 311.* — Very limited neuroglial proliferations in the neighbourhood of the vessels ; morular cells absent.

*Monkey 222.* — Neuroglial proliferations localised in the neighbourhood of the vessels, at the boundary between the white and the grey matter, and along the fibres, in regular layers ; morular cells absent.

### 3. *Morular Cells in Meningeal Lesions.*

The presence of morular cells in the cerebro-spinal fluid was an indication for searching for them in the meningeal tissue in monkey 3.

I was able to find a few, but always free, although the vessels of the pia mater were the site of an extremely pronounced cellular infiltration in the form of cuffs and massive stratifications.

The very few muriform cells that I found were on the surface of the pia mater, or in the subpial spaces. The perivascular infiltrations of the pia mater comprised polymorphic elements very similar to those of the nervous infiltrations ; on the other hand, there was a larger number of typical lymphocytes, of plasma cells and of leucocytes. Amid the numerous free elements which were on the surface of the pia mater or in the subpial spaces, there were numerous globular cells with small, round nuclei ; these were even more numerous in the neighbourhood of the choroid plexuses and their folds, and presented all the features of epithelial cells of the plexuses and ventricles. It was a question of elements shed by the tissue limiting the lymphatic spaces which are frequently found in the cerebro-spinal fluid of monkeys, and which may or may not be associated with lymphocytic cells at the onset of nervous lesions.

In monkeys 180, 311, 222, the meningeal lesions were very limited and there were no morular cells, even at the level of the choroid plexuses, where the alterations were most pronounced.

These four cases give a résumé of the whole history of the cerebral changes during the course of trypanosomiasis.

The figures of Plate IX show its principal phases.

Fig. 1 (monkey 222) represents an initial stage. It is a question of proliferation and accumulation of neuroglial cells on the wall of the lymphatic spaces. No modification

is yet discernible in the endothelial lining of the vessel. The neuroglial cells are typical and in no way different from the cells studded throughout the surrounding tissue. They are star-shaped or triangular, with a tendency to pleomorphism.

A more advanced stage is represented in Fig. 2 (monkey 311). The perivascular lymphatic space is dilated, the endothelium proliferating and some neuroglial cells have reached the lymphatic space and the vessel wall, while yet conserving their typical features.

These figures show clearly that the neuroglial cells are the first elements that come into play in the production of nervous changes and behave in this respect like interstitial elements in other tissues.

In the majority of experimental observations, one notes only findings of this order.

A more pronounced proliferation of the neuroglia is evident in monkey 180, particularly along the course of the vessels. In the same case, morular cells make their appearance in the nervous tissue in the neighbourhood of the vessels, that is to say, in the same site as the neuroglial proliferation makes its first appearance in monkey 222.

Figs. 3 and 4 (monkey 3) show the lesions at a still more advanced stage; their topography is very nearly the same. Fig. 3 represents the first appearance of a small morular cell, whose nucleus preserves the features of neuroglial nuclei, while the protoplasm is studded with small granules and with fuchsinophile hyaline globules. The position of this young morular cell is characteristic; it lies on the wall of the lymphatic space, among some neuroglial cells which are proliferating *in situ*.

In the dilated lymphatic space, a blood capillary presents endothelial proliferation, initial stage of the formation of a cellular cuff. The position of the morular cell still corresponds to that of the neuroglial cells of Fig. 1.

Fig. 4 represents a cellular cuff of perivascular infiltration. It occupies the widely dilated lymphatic space almost completely, and is formed by polymorphic cellular tissue of compact little highly coloured cells, among which a typical morular cell is to be found, accompanied by a cell with fuchsinophile protoplasm and by some neuroglial cells with clear nucleus and ramified protoplasm. Their position corresponds to that of the neuroglial cells in the lymphatic space in Fig. 2.

The deduction from these findings is that the morular cells in the diverse phases of their evolution have certain relations with vascular structures which correspond with those which, at the onset of the lesions, are peculiar to proliferated neuroglial cells.

The other figures of the plate represent the most frequent position of morular cells in the grey matter of the basilar nuclei, of the protuberance and of the medulla oblongata (monkey 3).

The most exact definition of their relation to the nervous cells is that of morular satellites. The normal satellites of nervous cells are neuroglial cells. These are the same satellite cells which destroy the degenerate nerve cells in general paralysis and in senile atrophy. In the case which occupies us, it is, on the contrary, the satellite cells which suffer a degenerate process; this makes them no less dangerous to the adjacent nerve cells.

These findings are among the most convincing from the point of view of the neuroglial origin of the morular cells. It is well also to cite the observations made by EISATH, which contain some very interesting information on this point.

MOTT (who nevertheless regards morular cells as endothelial or plasmatic in origin) gives, on page 26 of his pathologico-anatomical report, the results of research on the neuroglial changes in sleeping-sickness, which he had intrusted to EISATH in view of his special competence on this subject. In the conclusions of the latter, these words are found: "Some of the glia cells have a uniform homogeneously stained

protoplasm, as if the nuclear substance had dissolved out or had disappeared. Such cells usually have a dark-brown stained nucleus, whilst others may have lost their processes and are converted into hyaline balls”.

I think that these words suffice to make the morular cells accepted as derived from neuroglial origin.

It still remains to us to consider their relation to the cells with hyaline bodies that one finds in the most diverse organs attacked by granulomatous inflammations, and even outside the nervous system in trypanosomiasis.

Evidently, morular cells and cells with hyaline globules are not specific cellular elements. They represent only a manifestation of degeneration, which may in any case quite well attack cells of different origin so long as these are capable of proliferative reactions in chronic inflammations and in processes of repair. These properties are equally characteristic of neuroglia, and no reason prevents our admitting that these elements may be endowed with an analogous susceptibility when confronted with this degenerative change.

The hyaline globules of these cells have the same optical and staining properties ; but in the cells with hyaline globules found in granulomata, as in Russel's fuchsinophile bodies, the Gram reaction is nearly always positive, while, in my efforts with morular cells, the results have nearly always been negative ; this might indicate that it is a question of analogous but not identical reactions.

As regards the pathogenic importance of morular cells, it is only necessary to consider their topographical relations with the nerve cells in Figs. 5, 6, 7, 8 and 9 of Plate IX. It is not only a question of profoundly degenerated cells, either inert or serving no useful function, but of elements rendered dangerous by reason of the atrophic and degenerative changes which they determine in the adjacent nerve cells. The chromatic alterations in the protoplasm, the loss of dendrites, the displacement and distortion of the nucleus indicate that the nerve cells are profoundly disturbed in their function and ready to disappear, later to be phagocytosed by other satellites. If one adds that these changes take place in the nerve cells in the centres of the bulb, protuberance and basal ganglia, which all preside over essential functions, one may judge of the importance of these phenomena in the production of the symptomatology.

The pathogenic rôle of the morular cells could not possibly be more striking than it is. Moreover, clinical practice has long since attributed a very grave significance to the appearance of these cells in the cerebro-spinal fluid.

#### CONCLUSIONS.

Morular cells are cells with fuchsinophile hyaline globules analogous to cells found in granulomata. They have, perhaps, in trypanosomal encephalitis a multiple origin, but the features and relationships they present in the different phases of their evolution indicate neuroglia as their most frequent origin.

The transformation of satellites of the nerve cells into morular cells determines grave changes in the nerve cells, particularly in the grey matter of the bulb, the protuberance and the basal ganglia.

Entebbe, June 1927.

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6.

CEREBRAL FILARIASIS IN FIVE MONKEYS INFECTED WITH  
TRYPANOSOMAL MENINGO-ENCEPHALITIS

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It is necessary to go back to the first studies on sleeping-sickness to recall researches into the relations of this disease with filariasis.

P. MANSON indeed had attributed an ætiological rôle in sleeping-sickness to *filaria perstans* after the study of some patients in London; and the first task of the Commission sent to Uganda by the Royal Society was to study the epidemiology of the two affections. The researches of CHRISTY and of LOW showed that filariasis and sleeping-sickness did not share the same distribution; moreover, CASTELLANI'S discovery showed the true ætiological agent in sleeping-sickness and MANSON'S theory was quickly abandoned. If the findings which I am about to expound had been observed at that period, they would perhaps have complicated the researches in a singular manner; to-day, they permit a view of a certain relationship between the two affections.

In fifty-three autopsies on monkeys of which forty-three were infected with different strains of trypanosomes, I fourteen times found microfilariae in the blood, in the pericardial fluid or in smear preparations of certain organs; in five cases, there were in addition adult filariae in the subcutaneous tissue and underlying the aponeuroses of the pectoral, abdominal or dorsal muscles. In five monkeys infected with trypanosomiasis and showing meningo-encephalitis with obvious lesions in the choroid plexuses, I found adult filariae, sometimes in great numbers, in the subarachnoid and cephalic spaces.

This unexpected localisation of adult filariae in the nervous system is worthy of mention; the morbid anatomical findings lead me to believe that the presence of adult filariae in the nervous system may bear a close relationship to the vascular lesions and alterations in the cerebro-spinal fluid due to trypanosomiasis. Moreover, some information in the literature would lead one to suspect that this association may also exist in man.

In some cases of sleeping-sickness, CHRISTY pointed out nematodal larvae and eggs resembling those of *Ankylostoma duodenale* in relation with the cerebro-spinal fluid.

BRODEN AND RODHAIN, in expounding their researches on the cerebro-spinal fluid, wrote: "It has happened in some very rare cases that we encountered a larva of *filaria*, but there were always a few red-blood corpuscles, which forced us to admit that the puncture needle had wounded a small vessel".

VAN HOOFF, in studying the cerebro-spinal fluid of two monkeys affected with trypanosomiasis, found *filaria* embryos (monkeys 41 and 112). The cerebro-spinal fluid of these monkeys contained very numerous lymphocytes, mononuclears, morular cells and "eggs in varying stages of maturity".

Here are my own observations.

*Monkey 3.* — Autopsy on January 15th, 1927. Inoculated in December 1925 with a strain from the Damba antelope, by cyclical transmission.

Examination of the cerebro-spinal fluid, recovered by suboccipital puncture by VAN HOOFF in October 1926, showed well-marked changes in the cytological picture (350 lymphocytes per c.mm., large mononuclears, morular cells); no microfilariae.

On the morning of February 15th, 1927, this monkey was found dead ; for several days previously it had been in a lethargic condition.

Examination of the cerebro-spinal fluid, recovered at the autopsy, confirmed the changes found previously by VAN HOOFF and showed trypanosomes.

On inspection of the nervous system, I found a pronounced lepto-meningitis, particularly at the base of the brain, at the level of the bulb and of the protuberance.

Despite a very attentive examination, made on account of these lesions in the monkey which are exceptional, I did not perceive any microfilariae ; later, in examining numerous sections to study the typical and very advanced meningo-encephalitic lesions, I found sections of filariae in the subarachnoid spaces and at the level of the bulb and protuberance. In other organs, I found chronic lesions typical of trypanosomiasis.

*Monkey 112.* — Autopsy on March 20th, 1927. Inoculated in July 1926 with a human strain of *gambiense* type, by cyclical transmission.

Examination of the cerebro-spinal fluid, recovered by suboccipital puncture by VAN HOOFF in October 1926, had shown very pronounced cytological changes in the cerebro-spinal fluid (85 lymphocytes per c.mm., mononuclears and filaria embryos).

On the morning of March 20th, this monkey was found dying. Examination of the blood made before death showed microfilariae.

At autopsy, I find numerous adult filariae in the subcutaneous tissue and under the aponeuroses of the abdominal wall.

In the cerebro-spinal fluid recovered by suboccipital puncture, there were also microfilariae, lymphocytes and mononuclears.

On examination of the nervous system, I notice a slight opacity of the leptomeninges at the base of the brain and at the level of the protuberance. Numerous adult filariae interlaced into a meshwork are spread in the subarachnoid spaces, near the base of the brain, round the protuberance of the bulb ; some isolated filariae are also found at the level of the cervical medulla.

In smear preparations of the pericardial fluid and of the salivary and suprarenal glands, I also find filariae.

The specific lesions of the myocardium, spleen, liver and other organs justify the attribution of the not very serious meningo-encephalitis and changes in the choroid plexuses to trypanosomiasis ; I can therefore assign no pathogenic rôle to filariasis in this case.

*Monkey 237.* — Autopsy May 22nd, 1927. Inoculated in November 1926 with a human strain of *gambiense* type, by direct transmission ; treated (atoxyl) in December. Relapse in January 1927 ; frequently repeated crises made us believe that this monkey was about to die.

I found death apparent on the afternoon of May 22nd, although it had presented no alarming symptoms that morning. Intending to do a subinoculation, I first opened the thorax in order to puncture the heart after examining the pericardium. The heart was beating feebly and continued its pulsations for several minutes ; it was therefore a case of apparent death. In continuing the autopsy, I found only very limited lesions. The cerebro-spinal fluid gave the Rivalta reaction and contained lymphocytes and microfilariae.

On examination of the nervous system, the meninges did not appear visibly thickened, but at the base of the brain, round the protuberance of the bulb and the cervical medulla, there were large numbers of adult filariae, exactly as in monkey 112.

Histological examination showed chronic lesions of the choroid plexuses, of the myocardium and of the spleen.

It is very probable that the crises and the apparent death were in relation to the localisation of the parasites, which authorises the supposition that even in monkey 112 the cerebral filariasis had played some part in the causation of death.

*Monkey 335.* — Autopsy on May 29th, 1927. Inoculated in April 1927 with an animal strain, type *brucei*, by direct transmission; death on May 29th.

At autopsy, the cerebro-spinal fluid presented many lymphocytes, globular cells, altered trypanosomes and microfilariae; it gave a feeble reaction to Rivalta's test. On inspection of the brain, one observed at first nothing but a pronounced hyperæmia of the meninges. In making a careful search at the level of the protuberance and of the bulb, I could not altogether assure myself of a few adult filariæ, although the lepto-meninges were transparent. I fixed the bulb and protuberance without sectioning them.

On histological examination, I found a remarkable cellular infiltration of leucocytes and mononuclears in the pia mater and even more marked in the choroid plexuses, with very numerous trypanosomes between the vessel walls and epithelium; besides these, the preparations showed sections of adult filariæ at the level of the protuberance and bulb.

*Monkey 41.* — Autopsy on June 24th, 1927. Inoculated in March 1926 with a strain of human origin of the *gambiense* type by cyclical transmission. After June 2nd, no trypanosomes were demonstrable in the blood and one considered this monkey cured of trypanosomiasis. Nevertheless, examination of the cerebro-spinal fluid, recovered by suboccipital puncture by VAN HOOFF in October 1926, had shown grave alterations (112 lymphocytes per c.mm., mononuclears, morular cells) and some microfilariae.

On June 24th, 1927, I killed this monkey with chloroform and demonstrated microfilariae. At autopsy, the cerebro-spinal fluid still presented microfilariae, but I had not been able to avoid traces of blood and the findings were doubtful. On opening the cranium, I found a localisation of very numerous adult filariæ, as in monkeys 112 and 237. They were particularly numerous on the posterior surface of the bulb and beneath the cerebellum. On opening the ventricles, I found the choroid plexuses visibly thickened. Dissection of the skin showed deposits of adult filariæ in the subcutaneous tissue. The lymphatic glands were enlarged, but the juice showed no trypanosomes.

In continuing the autopsy, I found splenomegaly with incipient cirrhosis. The pericardium contained 0.8 c.c. of turbid fluid, rich in lymphocytes and mononuclears, with living trypanosomes and microfilariae.

Dr. LAVIER undertook the investigation of the parasite; I entrusted the material to him in view of his competence in this matter. On examining the microfilariae discovered by VAN HOOFF in the cerebro-spinal fluid of monkey 112, he had noted some embryos corresponding to various stages of intra-uterine development.

The investigation of the parasite will tell us whether we are dealing with a new species or with an exceptional localisation of a known species. Nevertheless, the morbid anatomical observations lead me to certain considerations.

The histological examinations have shown meningo-encephalitic lesions and lesions at the level of the choroid plexuses. The three examinations of the cerebro-



spinal fluid made by VAN HOOFF and my own findings at the autopsies demonstrate changes which are absolutely typical of trypanosomiasis. So far as the local changes are concerned, no pathogenic rôle can, for the moment, be assigned to the filariasis, but the observations on monkey 237 make us believe that the presence of a large number of nematodes, living and multiplying at the level of the protuberance and bulb, may play a mechanical rôle by at least causing disturbances in the roots of the bulbar nerves, and that they may thus determine grave trouble.

As regards the localisation of adult filariæ in the nervous system, the following points must be considered.

In fifty-three monkeys that were autopsied, ten of which were negative or not inoculated, I found adult filariæ in the nervous system of five which were infected with trypanosomiasis and all of which presented pronounced changes in the cerebro-spinal fluid meningo-encephalitic phenomena and important lesions in the choroid plexuses.

The absence of microfilaræ in the cerebro-spinal fluid of monkey 3 at the moment when the puncture was made by VAN HOOFF and the small number of adult filariæ, revealed only by histological examination, showed that the infection was recent in this monkey, which, however, had been inoculated with trypanosomes in 1925, and showed very grave changes in the cerebro-spinal fluid in October 1926; all this proves that the cerebral localisation of filariæ took place during the course of the trypanosomal infection.

It also happened that I found in eight other monkeys, of which two were negative and one not inoculated, microfilaræ in the blood and pericardial fluid and adult filariæ in the subcutaneous tissue; yet none of these monkeys nor any other that came to autopsy presented cerebral localisations of filariæ even on histological examination.

Given the notable differences in the numbers of parasites in monkeys 3 and 335 and in monkeys 212, 237 and 41, and the rôle of insect carriers hitherto found to be obligatory in filariasis, it must be admitted that the most numerous deposits (hundreds of adults of different sizes) are due to inoculations frequently repeated in the same animal in captivity. The percentage of cases in which I have verified the occurrence is singularly limited, given the large number of animals all living in the same conditions; on the other hand, it would be difficult to admit the possibility of such a localisation in normal conditions without its having attracted attention heretofore.

It must therefore be presumed that this localisation was at least facilitated by the special circumstances, and these circumstances might well be the vascular changes and the alterations in the cerebro-spinal fluid determined by trypanosomiasis. Indeed, these lesions, which are very frequently associated with small hæmorrhages, might facilitate the passage by the hæmatogenous route for the parasites, while the biochemical alterations and particularly the increase in the albuminoid content of the cerebro-spinal fluid might so change its properties as to allow of a localisation which would not occur in normal conditions.

The localisation of the filariæ corresponds singularly with the habitual site of the most pronounced vascular lesions of the nervous system in trypanosomiasis.

I think that the arguments that I have here set forth are sufficient to justify the opinion that cerebral filariasis was caused or facilitated by trypanosomiasis in these five monkeys.

This opinion may be confirmed or modified after observing cerebral filariasis and its percentage in normal monkeys. In the meantime, the observations of CHRISTY, BRODEN and RODHAIN may also justify research into the occurrence of similar relations between trypanosomiasis and filariasis in man.

CONCLUSIONS.

A cerebral and bulbar localisation of adult filariæ was observed in five monkeys (*Cercopithecus*) infected with vascular lesions and alterations in the cerebro-spinal fluid due to trypanosomiasis. The percentage of this localisation, the relation between the quantity of parasites and the duration of the trypanosomiasis, and the results of examination of the cerebro-spinal fluid at different periods would lead us to suspect that the localisation took place during the captivity of these monkeys and in the course of the trypanosomiasis, caused or facilitated by the alterations in the cerebro-spinal fluid.

No pathogenic rôle can for the moment be assigned to the cerebral filariasis so far as concerns the local lesions, but grave bulbar phenomena and apparent death occurred in a case where the parasites were very numerous.

Entebbe, June 1927.

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7.

## IMMUNITY AND PROTECTIVE ACTION OF BABOON SERUM

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The attempts made to provoke experimental trypanosomiasis in baboons have not in general led to any results except the finding of a well-marked immunity peculiar to these monkeys. This immunity has long since attracted the attention of experimenters, because it is thought to have some relation to the protective properties of the serum of these animals and of man, which relation was established by LAVERAN in 1904.

If these properties were really related to each other, man would be, like the baboons, naturally resistant to infection by trypanosomes; which is not borne out by experience.

The researches carried out on the protective properties of the sera of primates have thrown no light on the method by which this immunity has been vanquished on occasions, which are very rare, yet serve to prove this possibility (THOMAS AND BREINL). Meanwhile, very interesting facts have come to light.

Human or baboon serum injected into naganised mice determines the temporary or lasting disappearance of trypanosomes (LAVERAN).

Baboon sera are on an average ten to twenty-five times more active than human sera, with remarkable individual differences (MESNIL AND LEBEUF).

There are trypanosomes which resist this action (*T. gambiense*, *T. dimorphon*, *T. congolense*), and trypanosomes which are susceptible (*T. brucei*, *T. evansi*, *T. rhodesiense*); these latter, moreover, may be rendered resistant by treatment with these sera (MESNIL AND LEBEUF, LAVERAN).

Protective sera do not act *in vitro* but only *in vivo* in infected animals (LAVERAN AND MESNIL); their action does not show itself by following a mechanism similar to that of any known anti-bodies (GOEBEL) and has no relation to phagocytosis; but it has some relation to the leucocytic function and to lipæmia (JACOBY).

Heated to 64°, protective sera lose their activity and cannot be reactivated (GOEBEL).

The protective action is very feeble in the fœtus; it is diminished or destroyed by changes in the hepatic function and by blockage of the reticulo-endothelial system (EHRlich, ROSENTHAL, KLEUMANN).

Protective sera have an indirect action comparable to that of some therapeutic substances which only act *in vivo* (MESNIL AND NICOLLE). Trypanosomes rendered artificially resistant to protective sera retain this property through a certain number of passages, but may lose it suddenly (JACOBY).

*T. rhodesiense* is susceptible to the protective sera of man and baboons, but may be rendered serum-resistant, and this resistance appears particularly quickly (LAVERAN AND NATTAN-LARRIER).

Starting from these data, I undertook some research in baboons (*Cynocephalus tersellatus*) with the object of obtaining some trypanosomal infections (*T. rhodesiense*, *T. brucei*, Damba trypanosome) and of studying the mechanism of their resistance.

These researches enabled me to observe the protective action of these monkeys' serum in guinea-pigs, but I was not able to provoke any demonstrable infection. However, some experiments that I will describe have given results worthy of discussion.

MESNIL AND LEBEUF could not provoke infection of a cynocephalic monkey with a strain of *T. brucei*, which proved resistant in the mouse, though treated with 2 c.c. of serum from the same species of animal. I proposed to repeat the same experiment with a strain of *T. rhodesiense* serum-resistant in the guinea-pig.

The strain employed was from the patient *Boga* (region of *G. swynnertoni*, Tanganyika), sent to the Commission laboratory by KLEINE in January 1927.

I inoculated two guinea-pigs (Nos. 298 and 299) with 0.5 c.c. of the same mixture of blood and citrated water (2 drops of blood from an infected rat with 1 c.c. of citrated water) on February 21st, 1927. Trypanosomes appeared in the blood of both guinea-pigs on March 2nd, 1927.

On the following day, I began the treatment of both guinea-pigs with baboon serum.

The serum was derived from a well-nourished healthy female; I employed serum from the same animal for all the experiments; it was obtained by cardiac puncture on March 2nd, 5th and 12th. Each puncture gave 30 c.c. of blood, which supplied about 10.5 c.c. of serum, which I kept in flasks sealed in the flame. The collection and distribution of the serum were done with all aseptic precautions.

Not knowing the protective power of the serum, I began treatment of the guinea-pigs with ½ c.c. given into the thigh by subcutaneous injection; I increased the doses successively as will be seen in the following tables.

At the beginning of treatment, the two guinea-pigs (weight about 250 grammes) had the same number of trypanosomes in the blood (3 per field; x 400 diameters).

*Guinea-pig 298.*

March 1927	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Trypanosomes for field x 400	3	1/5	0	0	0	0	0	1/5	2	0	0	0	0	1	0	0	1/5	1	0	1	2	4	2	3	2	3	3	Found dead in the morning, still warm
Serum injected in c.c.	0.5								1					1.5				2.5				3.5				4.5		Effusion of blood in the mediastinum; sub-pericardial hæmorrhages

*Guinea-pig 299.*

March 1927	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Trypanosomes for field x 400	3	2	4	3	4	3	4	4	2	3	4	3	3	2	4	4	3	3	4	5	3	3	5	4	4	5	4	Found dead in the morning
Serum injected in c.c.	0.5		1								1.5			2.5				4				4.5				4.5		Hæmorrhagic effusion in the mediastinum; sub-pericardial hæmorrhages and rupture of the heart at the apex.

The very different response to treatment of the two guinea-pigs must be noted rather than the duration of the infection and hæmorrhagic lesions, which caused death in both almost simultaneously.

Two guinea-pigs of the same size, inoculated simultaneously with the same quantity of parasites of the same strain, equally infected, react very differently. The same trypanosome reveals itself susceptible to treatment in guinea-pig 298, while showing itself naturally resistant in guinea-pig 299.

The sero-sanguineous effusion is in both guinea-pigs very rich in living trypanosomes.

I used  $\frac{1}{2}$  c.c. of the effusion from guinea-pig 299 to inoculate two others ; and I inoculated 5 c.c. of the same, mixed with 5 c.c. from guinea-pig 298, into baboon 2. I proposed to continue the treatment in the new guinea-pigs 298*a* and 299*a*. With this object, I inoculated both with the strain which had proved naturally resistant in guinea-pig 299.

However, the findings of the facts observed in the first two guinea-pigs made me suspect that the reaction to the serum might represent a function of the infected guinea-pigs rather than a variable quality in the same strain.

I decided then to use guinea-pigs 298*a* and 299*a* in a new experiment, with the object of studying the action of baboon serum in the presence of guinea-pig organs, normal and infected, treated and untreated.

I began treatment in guinea-pig 299*a* with a dose of 0.5 c.c. to test its susceptibility, and I left 298*a* without treatment.

*Guinea-pig 298a.*

April 1927	8	9	10	11	12	13	14
Trypanosomes per field x 400	3	2	3	4	3	4	Found dead that morning; hæmorrhagic effusion in the mediastinum
Serum injected	—	—	—	—	—	—	

*Guinea-pig 299a.*

April 1927	8	9	10	11	12	13	14
Trypanosomes per field x 400	2	0	0	0	$\frac{1}{2}$	$\frac{1}{3}$	Used in following experiment
Serum injected	0.5	—	—	—	—	2.5	

The same strain which had resisted the same serum in monkey 298 showed itself susceptible in monkey 299 *a*.

Guinea-pig 298*a*, found dead on the morning of April 14th, was replaced by guinea-pig 290, which Dr. PRATES was kind enough to cede to me, as it was in analogous conditions to the one which I had lost on the very day fixed for the experiment.

The object of the experiment was as follows : To examine *in vitro* in the same strain of *T. rhodesiense*, derived from a heavily infected monkey, the action of baboon serum alone and in the presence of different tissues, and of serum obtained with aseptic precautions from living guinea-pigs, infected and normal, treated and untreated.

The following material was at my disposal for this experiment, which was very laborious and demanded more than twenty-four hours' continuous work and observation.

*Baboon Serum.* — On the morning of April 13th, I collected 25 c.c. of blood, which gave 10.5 c.c. of serum, by cardiac puncture from a baboon. I kept it in flasks sealed in the flame, working with the greatest aseptic precautions.

*Trypanosomes.* — Monkey 311 had been inoculated with the same strain on March 25th; he had been positive since April 5th, and showed numerous trypanosomes in the blood (five to six per field; magnification: 400 diameters).

*Guinea-pigs.* — Guinea-pig 330, normal; 290, infected; 329, normal: 2.5 c.c. of baboon serum inoculated on previous evening; 299a, infected, treated like 329. For the treatment of the guinea-pigs, I used serum collected some weeks previously, which, however, was derived from the same baboon.

I had besides: physiological sterile 0.8% solution; 4 centrifuge tubes; 60 test-tubes; graduated pipettes; syringes and a little boiler for sterilisation of the instruments necessary at the operation.

The operations succeeded each other in the following order, according to the plan indicated in the tables:

1. Distribution of the physiological saline solution into the test- and control-tubes.
2. In each guinea-pig I performed the following operations:
  - A. Cardiac puncture to the point of exhaustion; I obtained about 12 c.c. of blood, which was immediately defibrinated in a centrifuge tube, by beating with a sterilised metal wire.
  - B. After disinfection and lavage of the skin of the still living animal, the skin was dissected; on the operating-table the axillary and inguino-crural glands were taken out and placed in tubes 1 and 2 of each series; the abdomen was opened and two fragments of omentum (tubes 3 and 4), of the suprarenals (tubes 5 and 6), of the liver (tubes 7 and 8), and of the spleen (tubes 9 and 10), were cut out and 0.5 c.c. of bile was aspirated from the gall-bladder (tubes 11 and 12). Each operation was performed with instruments sterilised by boiling in the small boiler, which was close at hand, and was executed with the greatest rapidity by the aid of an assistant who held the tubes.

Thus I got four series of tubes (A, B, C, D) containing fragments of organs and bile, placed in physiological saline, about 0.5 grammes of each.
  - C. To each test-tube and to the controls, I added 0.25 c.c. of baboon serum.
  - D. Cardiac puncture of monkey 311; 15 c.c. of defibrinated blood, mixed with 18 c.c. of physiological saline and distributed to the amount of 0.5 c.c. to each tube.
  - E. The blood of the four guinea-pigs having been centrifuged, I placed 0.5 c.c. of it in each of the eight tubes of a fifth series (*d*) with the physiological saline and the other ingredients mentioned above.

I left the five series of tubes at room temperature and began the observations an hour later.

Each series demanding about half-an-hour for examination between coverslips, the examinations succeeded each other always in the same order (E, C, B, A, D).

TABLES OF EXPERIMENTS AND RESULTS.

*Series, A, B, C, D.*

Tubes		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Trypanosomes (blood from monkey 311) c.c.:		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cynocephalic serum c.c.:		0.25	phys. saline	0.25	phys. saline	0.25	phys. saline	0.25	phys. saline	0.25	phys. saline	0.25	phys. saline	0.25	phys. saline
Physiological saline 0.8 % c.c.:		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Organs from guinea-pigs A, B, C, D, gramme 0.5		Lymph. glands		Omentum		Supra-renal		Liver		Spleen		Bile		Physiolog. saline	
<i>Series A:</i>	Observed After 3.30 h.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+oo	+oo	+++	+++
Normal Guinea-pig 330	» 7¾ h.	++	+++	+++	+++	+++	+++	++	+++	+++	+++	+	+	+++	+++
	» 22 h.	—	+++	+	++	+	++	o	+o	—	o	—	—	+++	+oo
	» 48 h.	—	—	o	+	o	+	o	o	—	—	—	—	oo	oo
<i>Series B:</i>	» 3 h.	+++	+++	+++	+++	+++	+++	oo	+++	+++	+++	++	++	+++	+++
Infected Guinea-pig 290	» 7 h.	+	+++	+++	+++	++	+++	oo	+++	+	+++	o	o	+++	+++
	» 21 h.	—	+++	—	++	+	++	+	+o	—	—	—	—	+++	++o
	» 46 h.	—	+o	—	o	—	—	o	++	—	—	—	—	++	++o
<i>Series C:</i>	» 2½ h.	+o	+++	+++	+++	+++	+++	+o	+++	+++	+++	++	++	+++	+++
Normal guinea-pig. treat. 329	» 6 h.	+	++	o	+++	+++	+++	o	+++	++	+++	++	++	+++	+++
	» 20 h.	—	+oo	—	++	+	+++	o	++	—	++	—	+	+++	++o
	» 44½ h.	—	ooo	—	oo	o	ooo	—	++	o	++	—	o	ooo	oo
<i>Series D:</i>	» 4 h.	++	+++	+++	+++	+++	+++	o	oo+	+++	+++	—	—	+++	+++
Infected guinea-pig. treat. 299 a	» 8h.50m.	oo	+++	+o	+++	+o	+++	o	oo+	++	+++	—	—	+++	+++
	» 24 h.	—	+++	+	+++	+	+++	—	ooo	+	++o	—	—	++	++o
	» 50 h.	—	—	—	—	—	o	—	oo	—	—	—	—	oo	oo

*Series E.*

Tubes	1	2	3	4	5	6	7	8	9	10
Trypanosomes (blood from monkey 311) c.c.:	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cynocephalic serum c.c.:	0.25	phys. saline	0.25	phys. saline	0.25	phys. saline	0.25	phys. saline	0.25	phys. saline
Guinea-pigs' serum A, B, C, D	A	A	B	B	C	C	D	D	phys. saline	phys. saline
Observed after 1 h.	+++	+++	+++	+++	+++	+++	o	+++	+++	+++
» » 4½ h.	+o	+++	o	+++	oo	+++	o	+++	o++	+++
» » 19 h.	+o	+++	o	+++	—	+++	—	++o	ooo	oo+
» » 42 h.	o	+++	o	+++	—	+++	—	++	+	+

Symbols: + = Trypanosomes normal and mobile.  
 o = Trypanosomes vacuolated, with slow movements.  
 oo = Trypanosomes much altered, immobile.  
 — = Trypanosomes disappeared.

*Results.*

*Series E.* — After an hour, trypanosomes are vacuolated and not very motile in tube 7. After four and a-half hours, they are altered globular, tadpole-shaped, vacuolated and non-motile ; after nineteen hours, they have disappeared. This tube contains the serum of guinea-pig D, infected and treated (299a). The same modifications are observable, though more feeble and delayed, in tubes 1, 3, 5, 7, while in the control-tubes the trypanosomes are intact and strongly motile.

There is, then, an immediate action of baboon serum on the trypanosomes, which is stronger and appears earlier in the tube containing the serum of the infected and treated guinea-pig.

The weaker and more delayed reactions observable in the general controls are due to the greater amount of normal saline.

*Series A, B, C, D.* — The first action on the trypanosomes is manifest in tubes 11 and 12 ; it is due to the bile.

Among the other tubes, No. 7 presents a very rapid action in series D, *i.e.*, where the tube contains the liver of the infected and treated guinea-pig (299a). The same action, though less marked, is shown in Nos. 7 of series C and B, *i.e.*, in the tubes containing fragments of the livers of the infected and of the treated guinea-pigs ; the feeblest action appears in No. 7 of series A, which contains liver of the normal guinea-pig.

A trypanocidal action, though weaker and more delayed, is also manifest in the other tubes, notably No. 9, containing spleen.

The controls functioned regularly, except in some of the tubes containing fragments of spleen. After fifteen hours, I inoculated some gelatine tubes with the contents of each tube, and after four days I made new smear preparations to stain them by Giemsa.

*Remarks.*

The results of this experiment show three pronounced phenomena :

- (1) An action of baboon serum *in vitro* ;
- (2) An immediate action in the tubes containing baboon serum and guinea-pig's liver ;
- (3) A delayed but very pronounced reaction in the tubes containing lymphatic organs.

The action of baboon serum was manifest in all the tubes of series E, including No. 9, which did not contain guinea-pig serum ; but it contained, though in smaller amount, the serum of the monkey from which the trypanosomes were derived.

This serum, which I was not able to eliminate, may have minimised the differences between tubes 1, 3, 5, 7, but this does not diminish their importance.

The gelatine cultures gave negative results. In the smear preparations made at the end of four days, the control-tubes (No. 4) contained bacteria (a bacillus), but also well-preserved trypanosomes.

In series A, B, C, D, baboon serum showed itself active in the presence of living tissue, particularly liver, in which case the action was very rapid.

The cultures from tubes 1 and 9 of series A and Nos. 2, 9 and 10 of series B gave bacteria. The other tubes with fragments of lymphatic tissue were sterile.



The trypanocidal action manifest in these tubes may have the same significance as the phenomena verified at the autopsies, as regards the early alterations of trypanosomes in the spleen, which take place after death. On the other hand, the alterations noticed in the tubes which contained liver may be related to EHRlich's observations and ROSENTHAL's researches on the diminished protective power of human sera in hepatic insufficiencies.

The object of my researches was to verify some differences in the action of cynocephalic sera under differing conditions. The results obtained authorise the supposition that the protective action of baboon serum is only a manifestation specific to the infected animal, with individual differences which may be produced artificially; and that is the reason why attempts to infect cynocephalic monkeys have no more success with serum-resisting than with normal strains.

It is very probable that serum resistance is manifest only in treated animals and that the same strain transferred to the baboon possesses no greater infective power than any other.

In any case, this property is natural to *T. gambiense*, towards which cynocephalic monkeys exhibit the same resistance.

#### ATTEMPTS TO INFECT BABOONS.

##### *Baboon 2.*

Strong male, weight 11 lb.; fasting for twenty-four hours; bled by radial section (130 c.c. of blood) on March 20th, 1927. Inoculated with 10 c.c. of the hæmorrhagic effusion from guinea-pigs 298 and 299, which was very rich in trypanosomes (serum resistant *T. rhodesiense*).

Inoculation into hindquarters, into the calves and into the scrotal sac.

The monkey is kept fasting for five days, and after that sustained on reduced rations.

After six hours, puncture of the calf and aspiration of the serous fluid:

*Examination between coverslips.* — Elements from the blood with a large number of polynuclears; still living trypanosomes.

*Preparations stained by Giemsa.* — Numerous trypanosomes morphologically normal or altered, clumped and tadpole-shaped.

After twenty-four hours, puncture of the calf and aspiration of serous fluid:

*Examination between coverslips.* — Negative.

*Preparations stained by Giemsa.* — Some altered trypanosomes, blood elements, numerous poly- and mononuclear cells.

After forty-eight hours, puncture of the calf and aspiration of the serous fluid:

*Examination between coverslips and of stained preparations.* — Result negative.

After three days, puncture of a crural gland:

*Examination between coverslips and of stained preparations.* — Result negative.

*Examination of the blood between coverslips and in stained preparations.* — Negative.

On April 7th, 1927, inoculation subcutaneously into the tail of 1.5 c.c. of pericardial fluid from monkey 312, containing numerous living trypanosomes (same strain).

After six hours, aspiration of the serous fluid :

*Examination between coverslips and of stained preparations.* --- Abundant leucocytes, no trypanosomes.

On May 5th, 1927, intracardiac inoculation of 12 c.c. of heart blood from monkey 338, containing numerous living trypanosomes (same strain). The inoculation provokes disturbances for a few minutes (pallor of the mucous membranes, cough, bristling of the hairs).

After an hour and a-half, examination of the peripheral blood :

*Preparations between coverslips.* --- Living trypanosomes with slow movements.

*Smear preparations stained by Giemsa.* --- Altered trypanosomes.

After two hours and a-half, examination of the peripheral blood :

*Preparations between coverslips.* --- Negative.

*Stained smear preparations.* --- Clumped trypanosomes, vacuolated, with flagellum.

After twenty-four hours, examination of the peripheral blood : negative.

On May 22nd, 1927, inoculation into the right tunica vaginalis, of 3 c.c. of pericardial fluid from monkey 351, very rich in living trypanosomes (same strain).

After an hour and a-half, puncture of the tunica vaginalis and examination of the fluid :

*Preparations between coverslip.* --- Numerous mononuclears and lymphocytes, leucocytes ; no living trypanosomes.

*Smear preparations stained by Giemsa.* --- Numerous trypanosomes, either free or phagocytosed by numerous mononuclears.

On May 20th, 1927, cardiac puncture, inoculation of 4 c.c. of blood into the subcutaneous tissue of monkey 362.

On May 22nd, 1927, peritoneal and pleural puncture ; examination of the fluid, negative.

#### *Baboon 6.*

Strong female, weight 12 lb.

On April 10th, 1927, intraperitoneal inoculation of 25 c.c. of pericardial fluid from a moribund dog, natural infection, coming from the region of *G. morsitans* (Mbarara). The fluid was very rich in living trypanosomes.

Frequently repeated blood examinations gave negative results ; nevertheless, the animal wasted appreciably and had palpable glands in the inguino-crural region.

On May 15th, 1927, glandular and peritoneal puncture :

*Examination between coverslips and of smear preparations stained by Giemsa.* --- Results negative.

On May 19th, 1927, renewed examination of the blood and glandular juice, results negative.

On May 20th, 1927, cardiac puncture and inoculation of 3.5 c.c. of blood into the subcutaneous tissue of monkey 363.

On June 25th, 1927, cardiac puncture :

*Blood examined between coverslips after centrifuging and staining by Giemsa.* --- Results negative.

#### *Baboon 7.*

Strong male, weight 11 1/4 lb.

On April 22nd, 1927, intramyocardial and probably intrapericardial inoculation of 2 c.c. of the pericardial fluid from monkey 292, very rich in trypanosomes (strain from the Damba antelope).

After five hours, attempt to aspirate the pericardial fluid :

*Examination of the serous fluid between coverslips and of smear preparations stained by Giemsa.* — Results negative. Frequent blood examinations with negative results.

On May 20th, 1927, cardiac puncture ; inoculation of 3.5 c.c. of the blood into the subcutaneous tissue of monkey 364 ; peritoneal puncture :

*Examination of the fluid between coverslips and of smear preparations stained by Giemsa.* — Results negative.

Monkeys 362, 363, 364 have shown no trypanosomes up to date. I did not take the baboons' temperatures. All three had malaria and Nos. 6 and 7 were infected with filariasis.

In any case, even in baboon 6, which, by the glandular swellings and the loss of 2 lb. in weight, led one to suspect a latent infection, I did not expect a positive result from inoculations into monkey 363.

*T. brucei* is as susceptible to baboon serum as *T. rhodesiense* is to human serum, if not more so. It would be necessary to admit that the trypanosomes had acquired serum-resistance in the baboon, to believe in the possibility of transmission to the monkey without the protective power of the blood being manifest. On the other hand, the great differences that one often observes in the susceptibility of small animals to the first passage of human strains by direct transmission authorise the belief that trypanosomes derived directly from man are not serum-resistant ; and that the blood inoculated with the trypanosome plays a protective rôle in the inoculated animal, with individual differences which are comparable to those obtained in guinea-pigs 298, 299, 299a of my experiments.

#### CONCLUSIONS.

A strain of *T. rhodesiense* behaved differently towards treatment with baboon serum in two guinea-pigs of the same size, inoculated at the same time and with the same quantity of virus at the 2nd passage ; a further difference appeared when this strain, which in one guinea-pig appeared to be naturally serum-resistant, was transferred to another.

Serum-resistance, then, would appear to be a property of the host rather than of the trypanosome.

A trypanocidal action *in vitro* is manifest when baboon serum is in the presence of hepatic tissue taken from still-living infected animals ; and in these circumstances is more rapid and intense than when we are dealing with infected animals which have been previously treated with the same serum.

The numerous attempts to transmit to cynocephalics a strain of *T. rhodesiense* serum-resistant in the guinea-pig, a strain of *T. brucei* derived directly from a naturally infected dog, and a trypanosome from *G. palpalis* derived from a Damba antelope gave negative results. Inoculations of exudates very rich in living trypanosomes were made into the serous membranes and into the myocardium, where trypanosomes develop most easily in predisposed animals.

Nevertheless, the survival of trypanosomes for more than twenty-four hours in the baboon, the local reactions observable in the subcutaneous tissue and in the *tunica vaginalis* together with phenomena exactly similar to those in predisposed animals, as also the differences found according to the site of inoculation, show that the limits of immunity have been reached.

Further researches on serum-resistance of human strains at the first passage, and on the infection of cynocephalics by direct or cyclical transmission starting from man, are still necessary.

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PLATE I.



Figure 1.—Pericarditis (monkey 121).

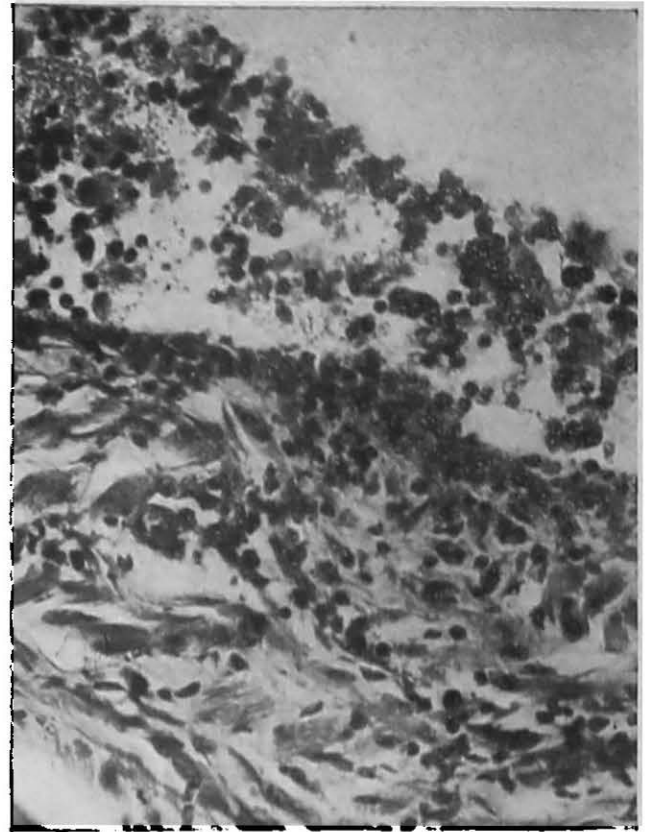


Figure 2.—Vaginalitis (monkey 265).

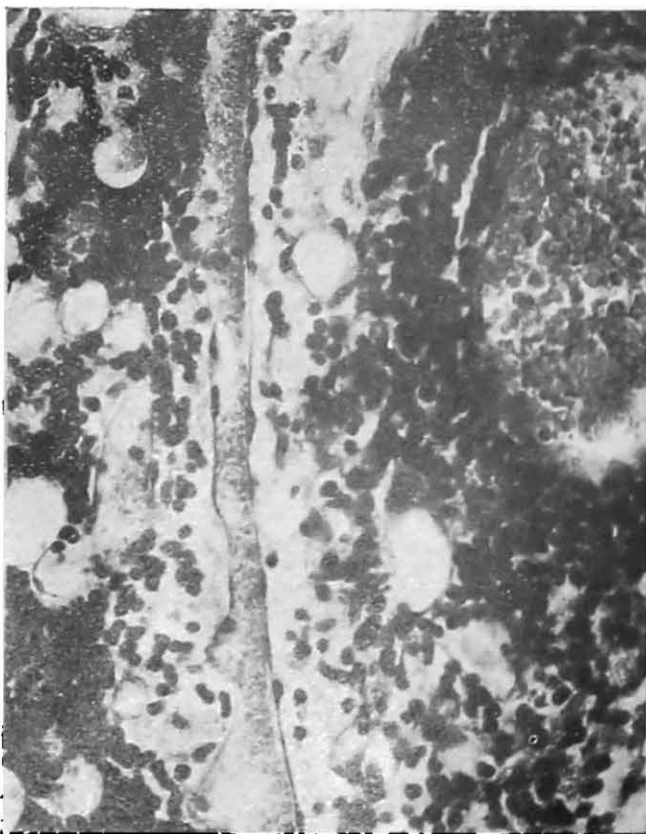


Figure 3.—Peritonitis, epiploön (monkey 180).

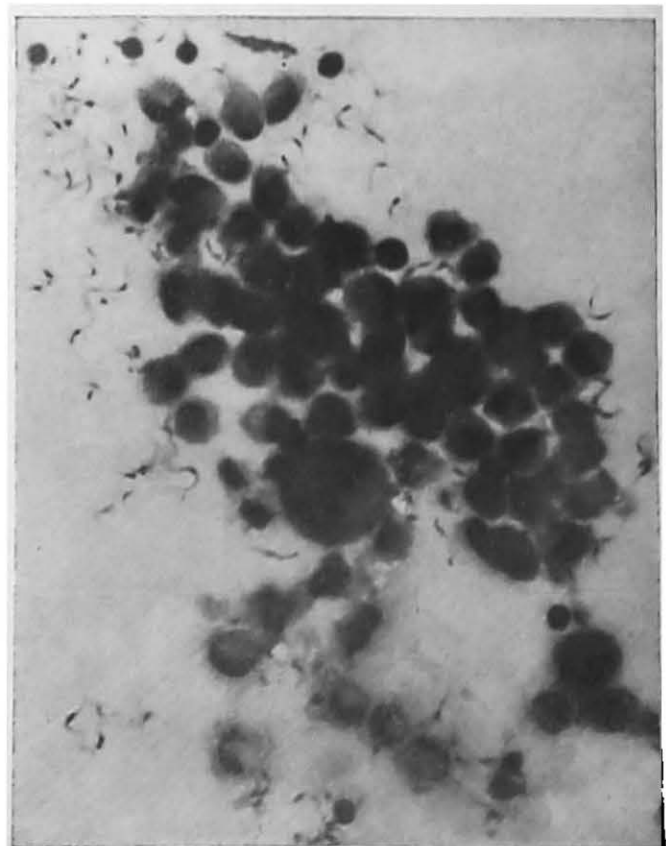


Figure 4.—Pericardial exudate (monkey 121).

PLATE II.

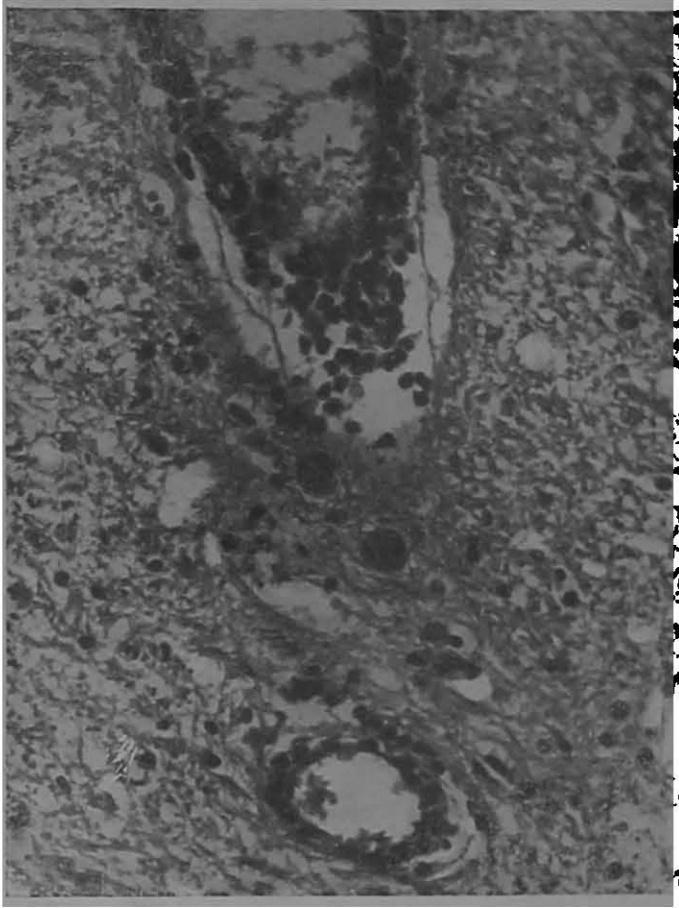


Figure 1. —Meningo-encephalitis; two morular cells in the nerve substance (monkey 180).

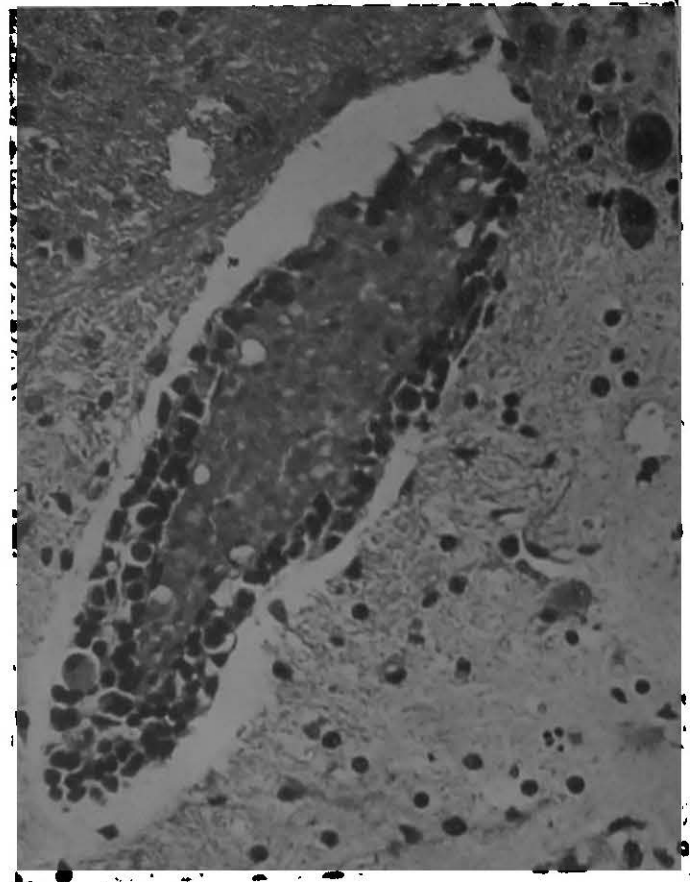


Figure 2. —Meningo-encephalitis; morular cell in a cuff of perivascular infiltration (monkey 3).

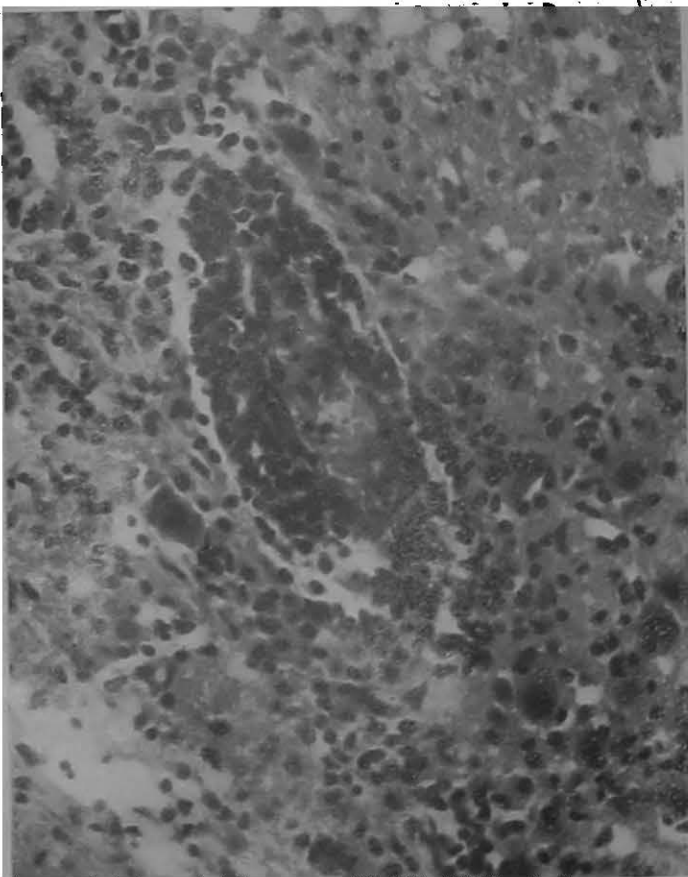


Figure 3. —Meningo-encephalitis; cellular



Figure 4. —Meningo-encephalitis; general



PLATE III.

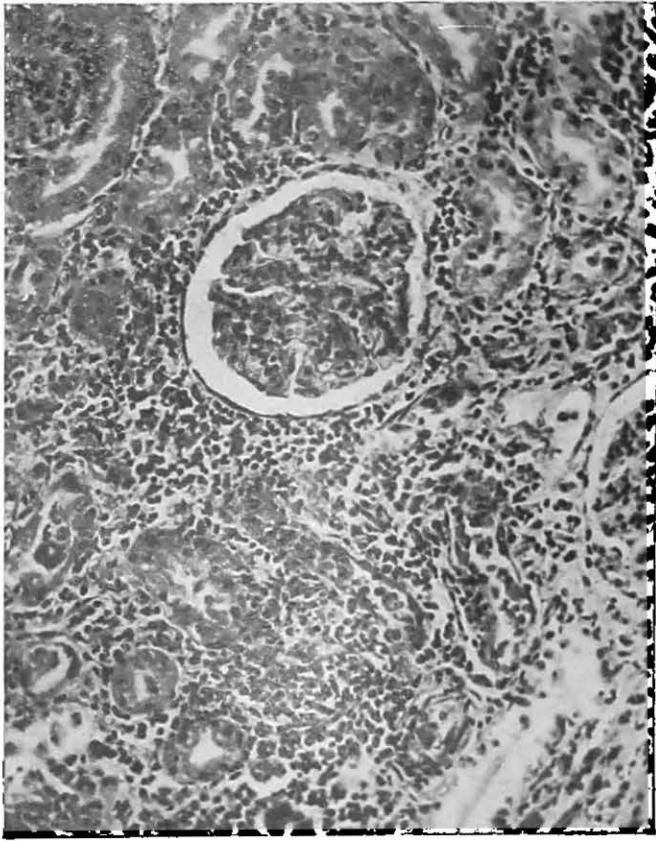


Figure 1.—Interstitial nephritis of large white kidney (monkey 313).

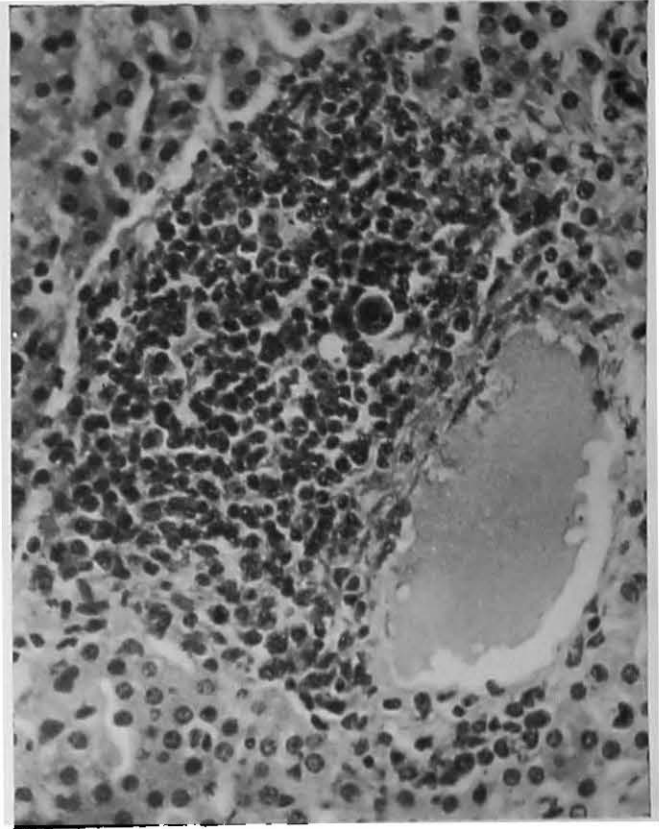


Figure 2.—Hepatic lesions ; cellular infiltration, with a morular cell (monkey 222).

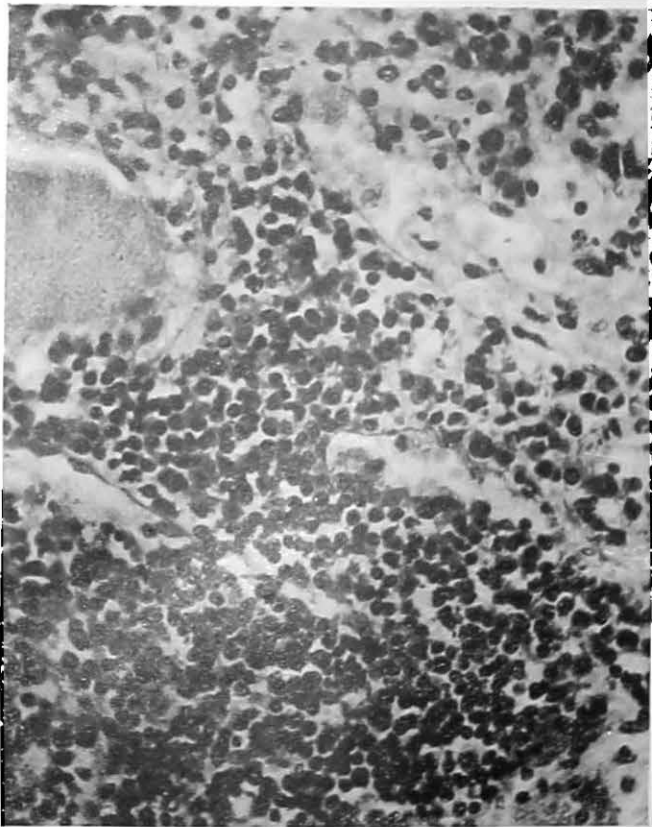


Figure 3.—Cellular infiltrations of the medullary substance of the suprarenal (monkey 180).

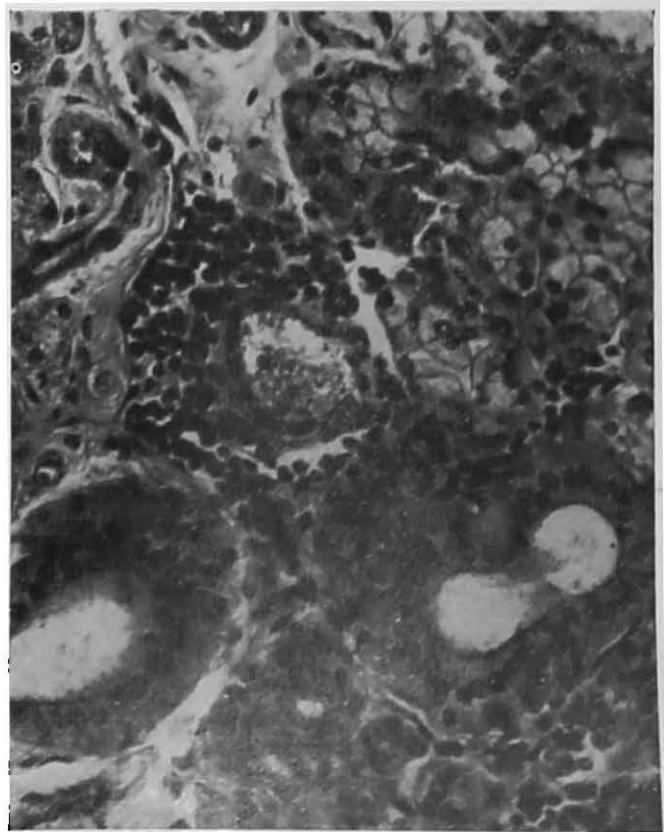
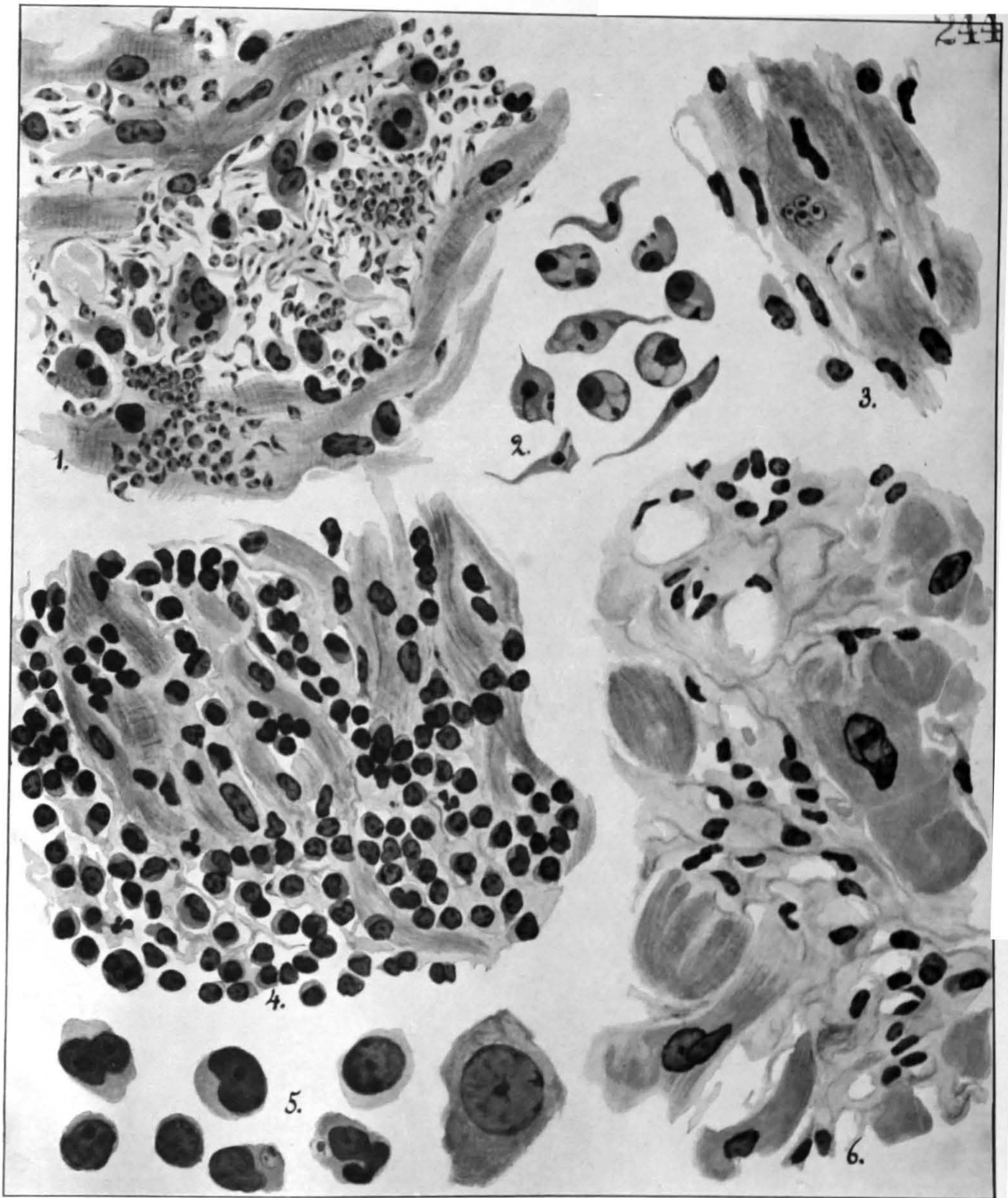


Figure 4.—Perivascular cellular infiltration of a salivary gland (monkey 260).



- Figure 1. — Trypanosomal myocarditis; massive deposits of parasites and lesions in their first phase (monkey 309). (See page 284.)
- Figure 2. — Some aspects of trypanosomes in the myocardium (monkey 309). (See page 284.)
- Figure 3. — Trypanosomes inside a cardiac fibre (monkey 311). (See page 284.)
- Figure 4. — Trypanosomal myocarditis; advanced phase (monkey 265). (See page 288.)
- Figure 5. — Some infiltrating cells in a smear preparation of the myocardium (monkey 265). (Stained with Giemsa.) (See page 288.)
- Figure 6. — Cirrhotic myocarditis (monkey 126). (See page 292.)



PLATE V.

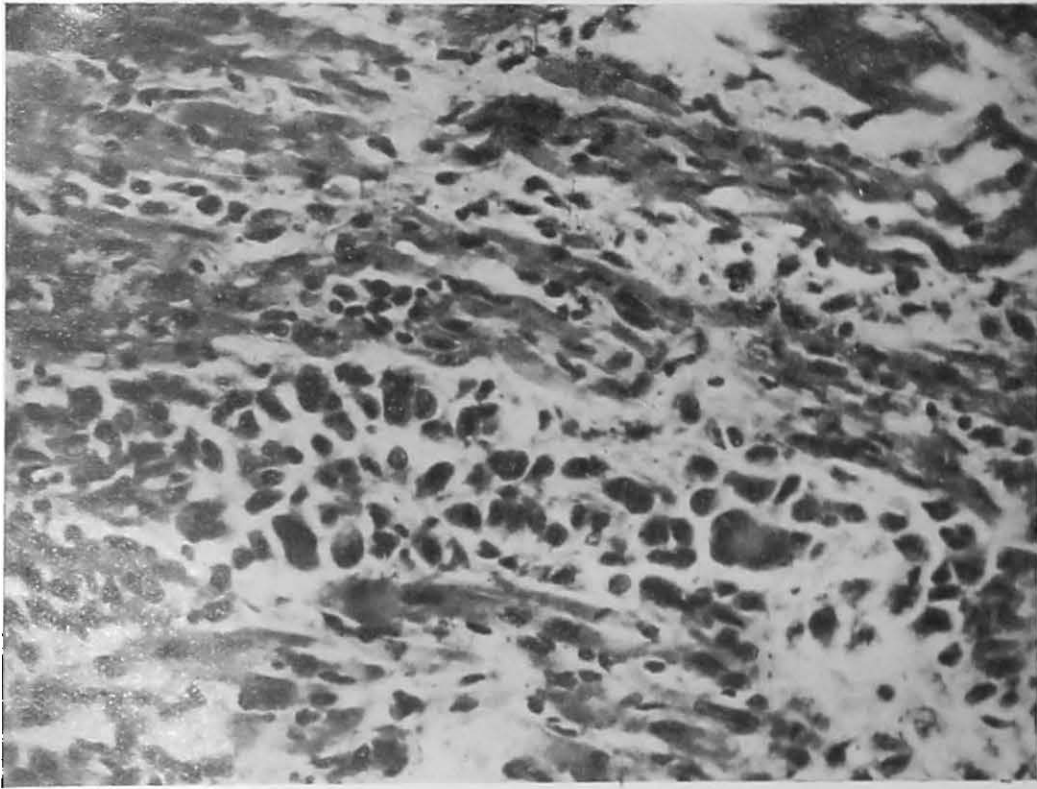


Figure 1.—Trypanosomal myocarditis; early lesions; infiltrations of macrophages, mononuclears and polynuclears (monkey 337). (See page 285.)

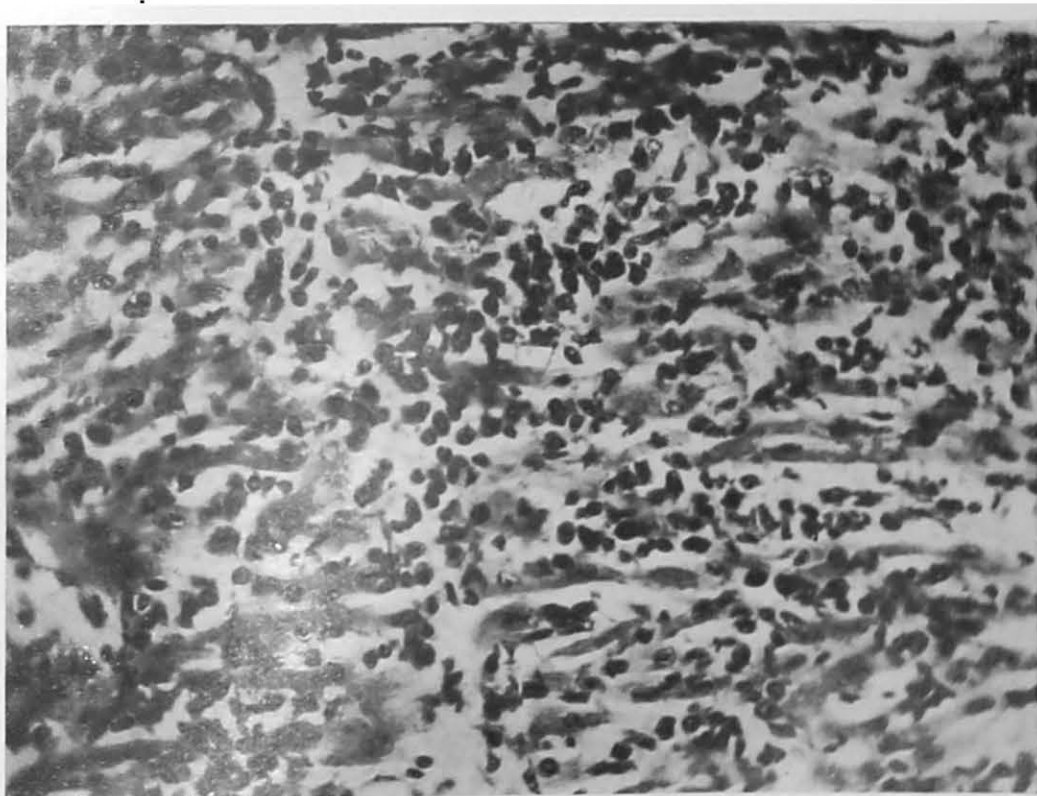


Figure 2.—Trypanosomal myocarditis; advanced lesions (monkey 265). (See page 288.)

PLATE VI.

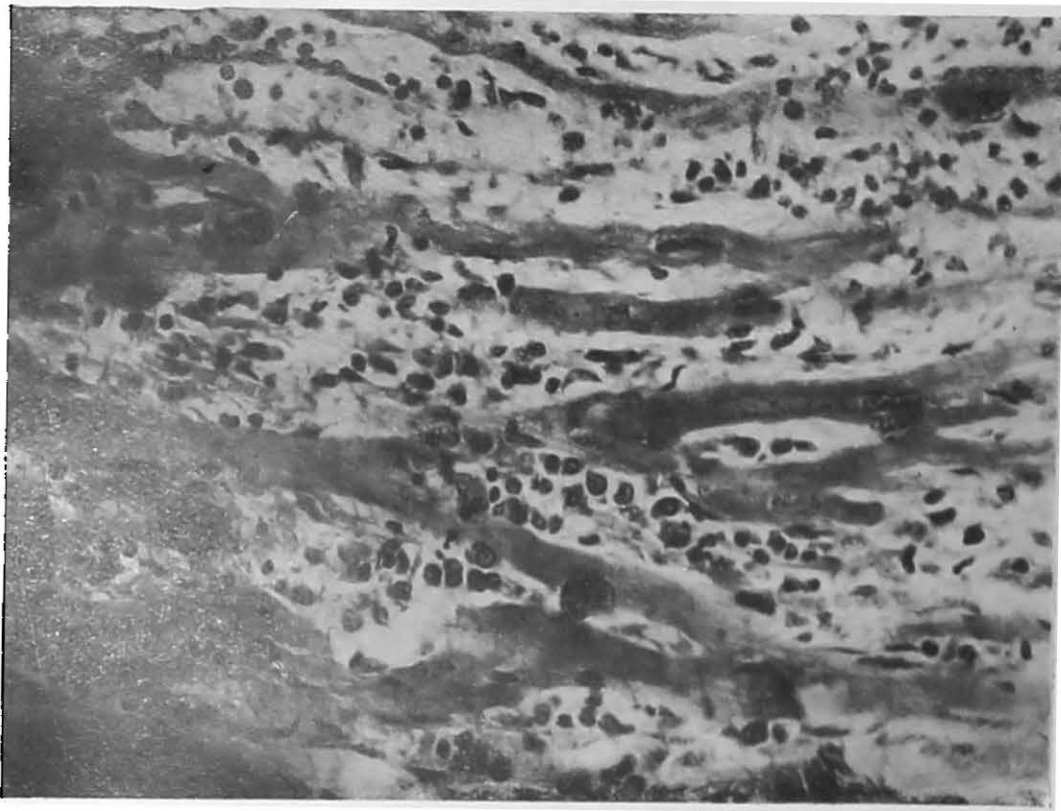


Figure 1.—Trypanosomal myocarditis ; early fibrosis, infiltrations of plasma cells (monkey 276).  
(See page 292.)

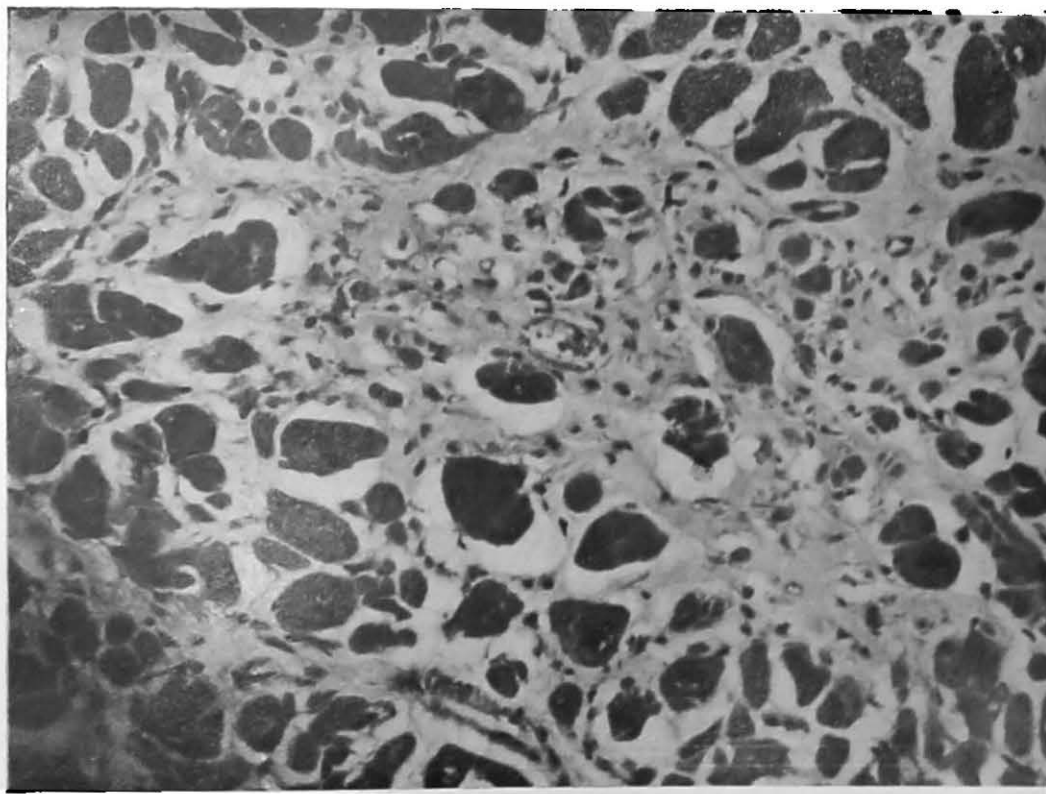


Figure 2.—Trypanosomal myocarditis; advanced fibroses (monkey 126). (See page 292.)

PLATE VII.



Figure 1.—Deposits of typical trypanosomes in the myocardium (monkey 309).

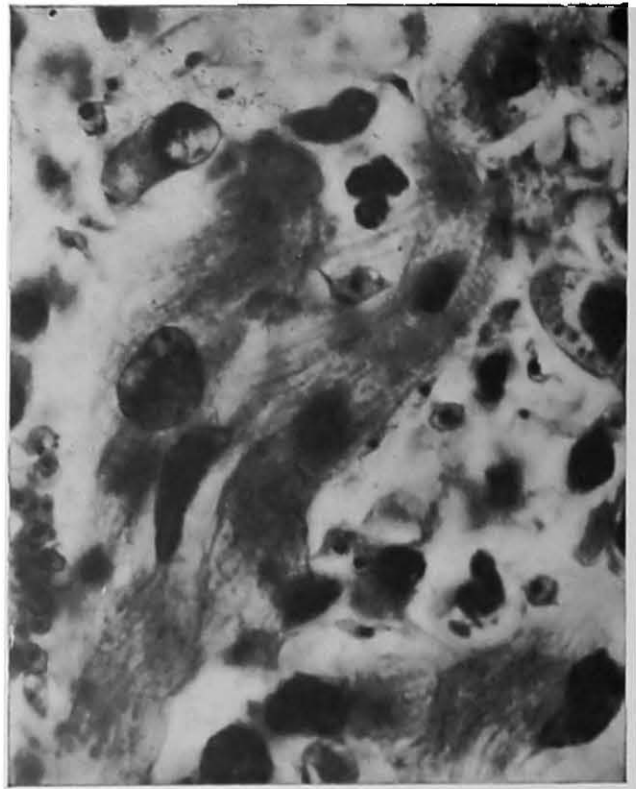


Figure 2.—Deposits of pleomorphic trypanosomes in the myocardium (monkey 309).

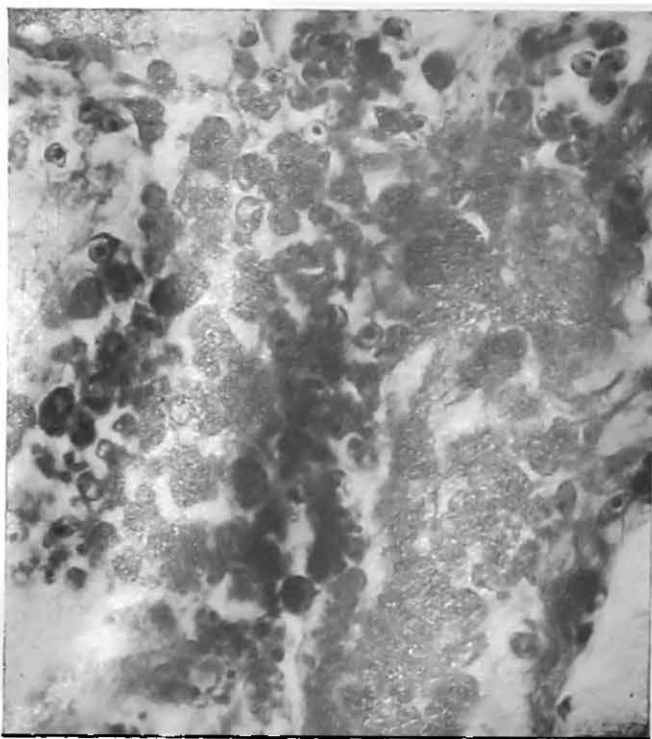


Figure 3.—Deposits of leishmanoid trypanosomes in the myocardium (monkey 312).

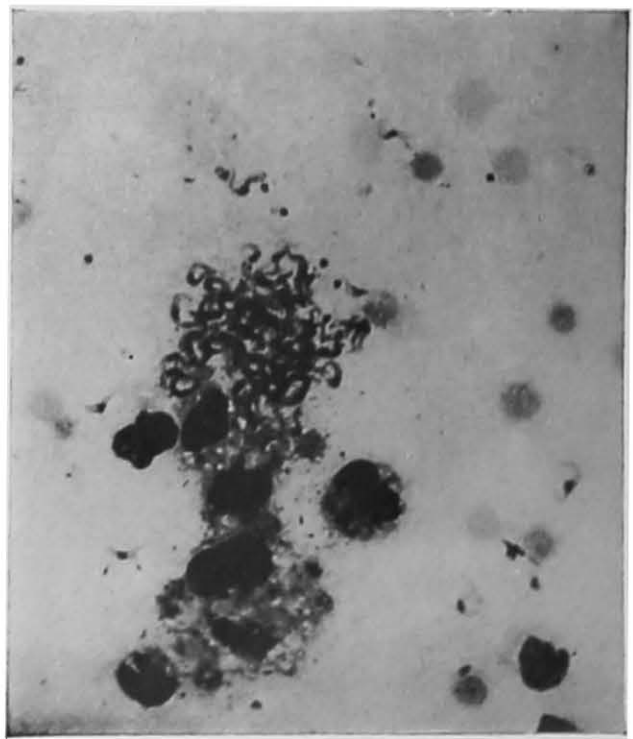


Figure 4.—Trypanosomal myocarditis ; smear preparation of the myocardium, infiltrating elements and trypanosomes (monkey 309).

PLATE VIII.



Figure 1.—Choroid plexuses (monkey 351). Dilatation of interstitial spaces; commencing proliferation of polymorphic cells and infiltration of mononuclears and leucocytes. (Deposits of numerous trypanosomes.)



Figure 2.—Choroid plexuses (monkey 354). Very extensive infiltrations of mononuclears, leucocytes and lymphocytes. (Deposits of numerous trypanosomes.)

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PLATE VIII (Continued).

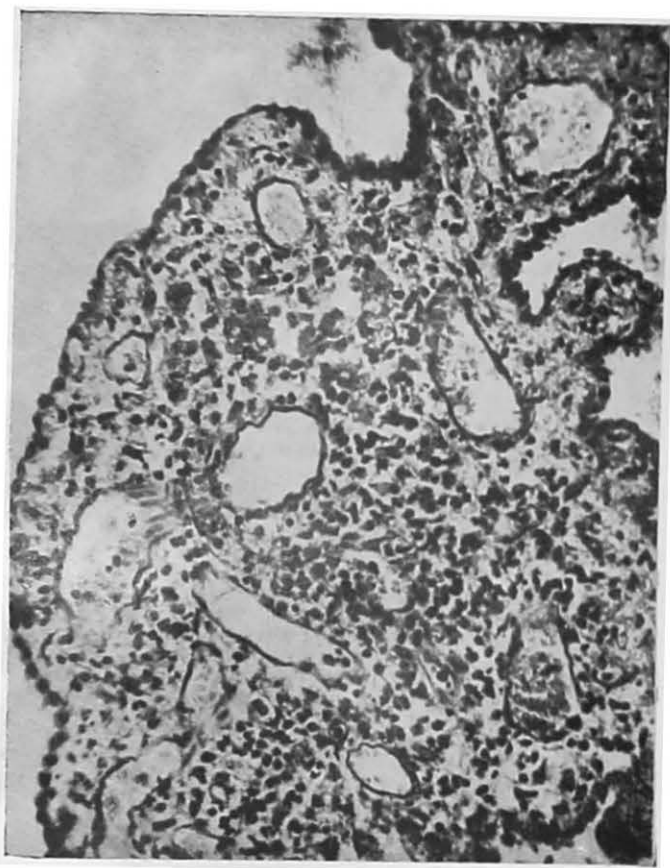


Figure 3.—Choroid plexuses (monkey 337). Very extensive infiltrations of mononuclears and leucocytes. (Deposits of numerous trypanosomes.)



Figure 4.—Choroid plexuses (monkey 180). Perivascular infiltrations of lymphocytic and plasma cells. (Trypanosomes few in number.)

(All preparations stained with hæmatoxylin and eosin.)

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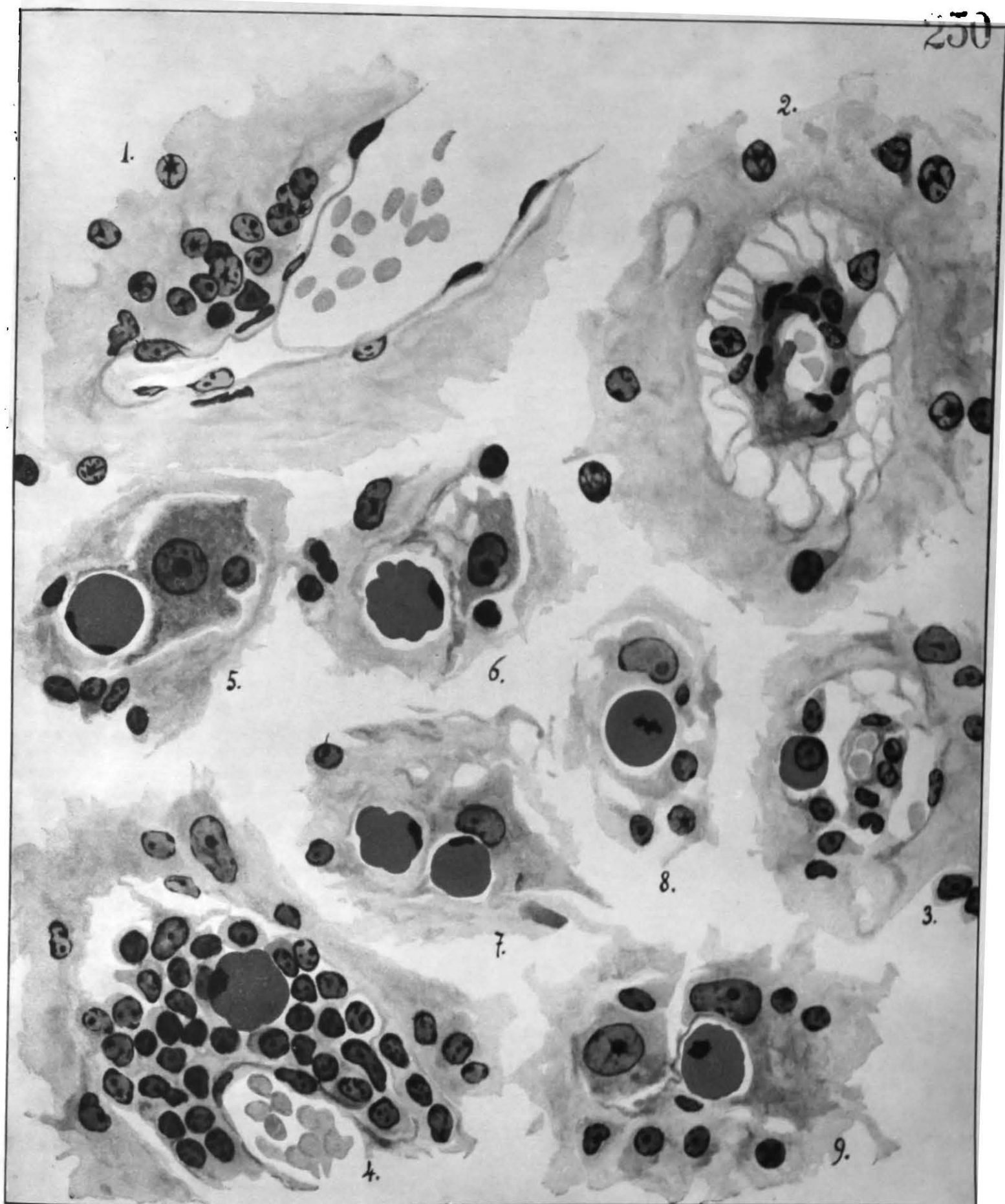


Figure 1. — Proliferation of neuroglial cells near blood-vessel (monkey 222).

Figure 2. — Endothelial proliferation, dilatation of lymphatic space, containing two neuroglial cells (monkey 311).

Figure 3. — Endothelial proliferation, dilatation of lymphatic space, containing parietal neuroglial cells in proliferation and a morular cell in its earliest stage (monkey 3).

Figure 4. — Cellular cuff in the lymphatic space, composed of small polymorphic and neuroglial cells, containing a morular cell (monkey 3).

Figures 5 to 9. — Morular cell satellites of nerve cells (monkey 3).

**VI.**

**EPIDEMIOLOGY OF SLEEPING-SICKNESS IN THE  
G. PALPALIS AND T. GAMBIENSE AREAS**

**AND VARIOUS OBSERVATIONS ON TREATMENT.**

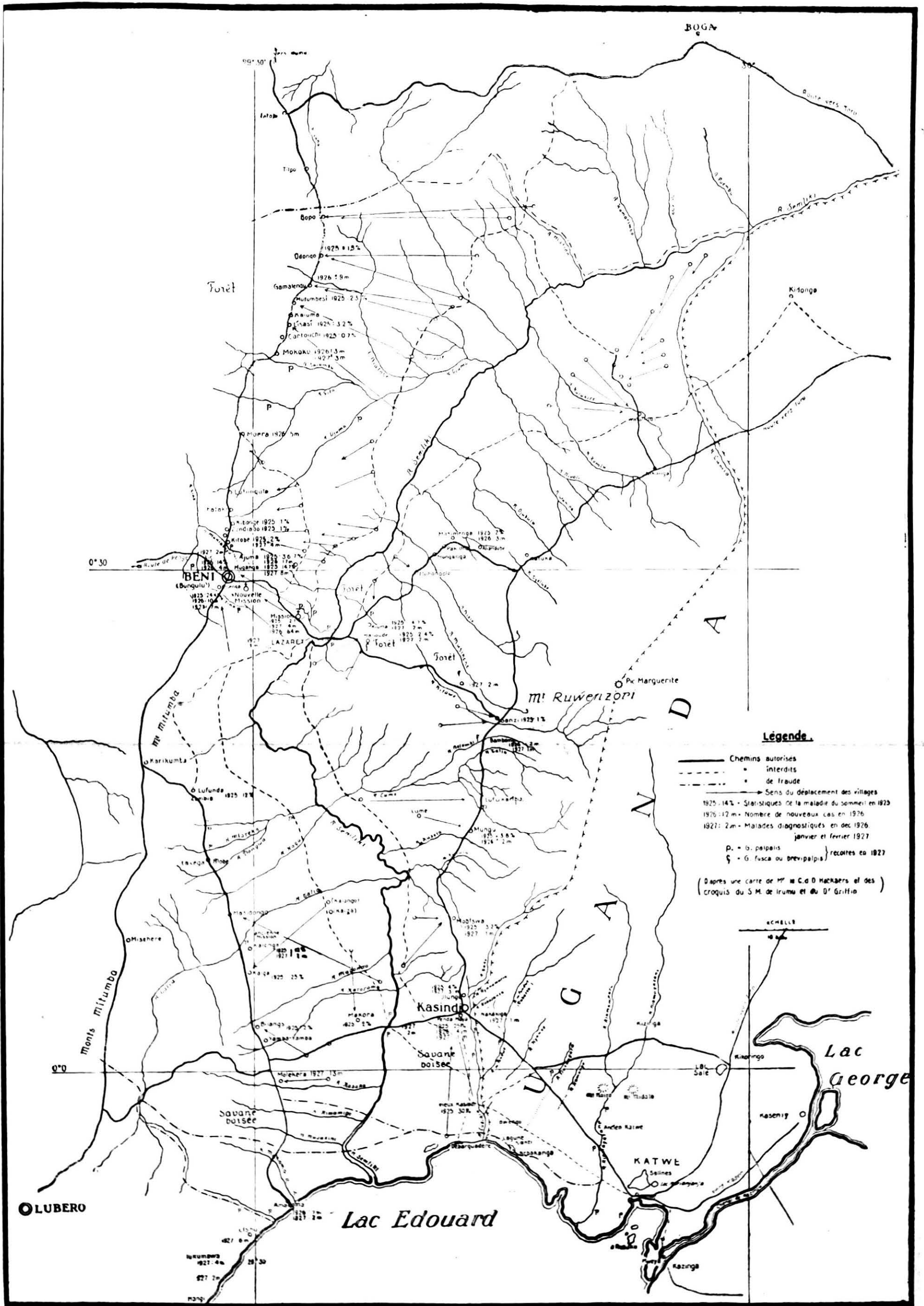
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**Légende.**

- Chemins autorisés
  - - - interdits
  - de fraude
  - Sens du déplacement des villages
  - 1925-14% - Statistiques de la maladie du sommet en 1925
  - 1926-12 m - Nombre de nouveaux cas en 1926
  - 1927: 2 m - Malades diagnostiqués en dec 1926, janvier et février 1927
  - p. = b. palpis
  - § = G. fusca ou brevipalpis } recotes en 1927
- (D'après une carte de M<sup>r</sup> C. D. Hackaers et des croquis du S. M. de Irumu et du D<sup>r</sup> Griffin.)

ÉCHELLE  
1:100,000

1.

SLEEPING-SICKNESS IN THE SEMLIKI VALLEY

(Belgian Congo).

BY DR. L. VAN HOOF.

---

[*Translation.*]

On December 7th, 1926, I left Entebbe with Dr. G. LAVIER to visit the foci of human trypanosomiasis which in the last few years has decimated the southern half of the fertile valley of the Semliki. Our investigations took us as far as the northern banks of Lake Edward and the frontier of Ankola. We reached Irumu, the furthest point on our journey, on February 20th, 1927.

This few weeks' tour through the infected zone was obviously too short for us to carry out a regular epidemiological enquiry. Nor was this my object. Knowing that at certain points infection had reached high percentages which might even be described as epidemic, it seemed to me desirable to investigate the causes of this state of affairs and to establish a comparison with zones where endemicity is low.

I wish to take this opportunity of expressing my grateful thanks to all those who assisted me in these enquiries, and particularly to my travelling companion, Dr. LAVIER, who frequently helped me with the examination of patients and microscopic work. The cordial reception given us by the Government authorities relieved us of many difficulties and we obtained most valuable information, which we were able to confirm on the spot, in the reports of M. HACKAERS, the District Commissioner, and of the Medical Service. I have made extensive use of these sources in framing the present report and have also drawn on the information supplied by Dr. GRIFFIN, Acting Specialist Officer, Sleeping-Sickness, Uganda, after a visit to the endemic zones in the neighbourhood of Lake Edward, Mount Ruwenzori and Lake Albert.

The inspection of the whole of the Semliki valley would have required a visit extending over several months. The itinerary we selected took us directly to the worst foci in the south of the valley. The road which is to link up Irumu and New Beni is in process of construction. We followed it from one workers' camp to another, through the dense primeval forest of Ituri, examining the natives who have recently settled there and the majority of whom come from the low-lying plains near the river. From New Beni, we went down into the valley, which is almost completely evacuated, and followed the lower spurs of the Mitumba mountains as far as Lake Edward; we then crossed the valley, following the shores of the lake for about 15 kilometers, and reascended the eastern side at the foot of Ruwenzori, recrossing it finally in the direction of Old Beni. The map facing this page will, we hope, be of assistance to the reader; it has been charted according to information obtained from a recent report of M. HACKAERS, District Commissioner, sketches by the Medical Service and personal notes.

\* \* \*

The part of the valley through which we passed is comparatively flat, and is broken by the winding course of the Semliki river and the innumerable tributaries, both large and small, which come down from Ruwenzori or the Mitumba mountains to swell the volume of the river. Streams, torrents and smaller rivulets flow through

forest belts of varying density and usually at the bottom of deep ravines. The waters rise very suddenly with the torrential rains, by as much as several meters in a few hours. Conditions are favourable for the development of *G. palpalis*, which is generally found here and breeds in the low scrub on the sandy river promontories above high-water level. There are other streams, generally small, whose banks are free from scrub, and which are hidden behind a thick screen of elephant grass and form marshes at certain points. Tsetses are still to be found there.

The Semliki itself is not subject to these sudden floods. It has a gravel bottom and flows between well-wooded banks infested with glossinæ. Its course is broken by numbers of rapids. Crocodiles are unknown in the south, and the same applies to Lake Edward.

The shores of Lake Edward are sometimes well wooded and sometimes marshy and overgrown with tall reeds. To the west, at the foot of the Mitumba range, they form steep cliffs, while to the east they remain low and wooded as far as the Kazinga channel, being broken by the inflow of the Semliki and the mouths of the Lubilia, Niamagasani, etc.

The altitude of the plain is about 900 meters. It must have changed very much as the result of the gradual disappearance of the inhabitants. The alluvial soil, which is very fertile, has been left entirely uncultivated, and is now overgrown with elephant grass, except for a few stretches of wooded savanna on the lower slopes of the mountains, the grazing land in the Molekera district, and the hills of Manaunde, Musenene and Kilima, which are covered with thick forest.

\* \* \*

This fertile plain and possibly also the Ruwenzori range, whose gorges contain rich soil, have from time immemorial attracted migrant native tribes. To quote M. HACKAERS : " According to native tradition, long before the arrival of the white man, sleeping-sickness decimated in succession numbers of tribes forming part of the big migratory movements which have always converged upon the Semliki, attracted apparently by the lofty slopes of Ruwenzori, whose snow-capped summits are sometimes visible from a distance of close on 300 kilometers.

" The Mabudu, for example, who now live in the Nepoko, were settled over two hundred years ago on the Semliki, to the north of Ruwenzori ; they abandoned that part of the country, so they say, in order to escape the ravages of sleeping-sickness.

" Similarly, the Bakumu or Babira, whose vanguard, coming from the east, passed round to the south of Ruwenzori and then spread northwards and westwards, to the districts of Irumu and Mombasa, only remained for a short time in the Semliki valley, where the dreadful disease killed more of their people than the wars they had had to wage in order to force their way across the high plateaux dominating the fatal valley."

The European occupation of this territory assumed an administrative character in 1896. Its first task was to pacify the tribes and to get the natives to settle down. Once trade became possible, an active traffic set in between the various occupied posts, and this contributed greatly to the spread of the disease. Then as the population of the plain seemed too scattered, some of the tribes which had settled on the spurs of Mounts Ruwenzori and Mitumba, and were as yet untouched by the ravages of the disease, were urged to move nearer to the administrative centres, " which became the most dreadful foci of infection, since it was impossible to approach them from any side without crossing countless streams and marshes infested with tsetse. It is not surprising, then, that practically nothing remains of the population which had

settled round these stations, and that in some cases, especially in the Kasindi plain, whole villages should have disappeared ”.

At the first news of the epidemic, the Belgian medical services organised a systematic campaign and an enormous lazaret, built of durable materials, was established close to Beni. The atoxylisation of patients and isolation of advanced cases, however, did little to improve the situation. The contact between the natives and the glossinæ and the opportunities of infection remained unchanged and defeated all prophylactic measures.

In the course of a few years, from 1915 to 1923, M. HACKAERS was able to estimate the extent of the catastrophe. In the Kasindi plain, whole villages had disappeared and a native chief who in 1923 had buried twenty-seven out of his one hundred and seventy-five subjects, including women and children, asked permission to settle elsewhere. Of the regular boatmen in the Lake Edward boat service, every single one almost was suffering from trypanosomiasis and, in spite of atoxyl treatment, five or six out of every twenty died each year. Those who took their place soon became infected in their turn and the regular lake transport service to Rutshuru had to be suspended. The salt trade on Lake Katwe was responsible for an active native traffic, both along the shores of the lake and by canoe and constituted an additional source of infection.

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The situation was most alarming and it may be well to determine the causes of the failure which attended the early efforts of the Medical Service.

To quote M. HACKAERS, “ settlements were strung out all along the valley on the banks of the Semliki, where the people lived by fishing, and along the tracks close to rivers infested with glossinæ ”. There was an active canoe traffic on the Semliki and on Lake Edward.

The lazaret itself was situated in the midst of villages where at least 40 per cent of the population were suffering from the disease. The census of population was very incomplete, many tribes indeed refusing submission. In this dense scrub, it was impossible to hope for any supervision over traffic without the assistance of a large staff or recourse to coercive measures. All this time there was a shortage of labour, the upkeep of the ways of communication became exceedingly precarious, and both for the members of the Medical Service and for the administrators of the territory the supervision of the native districts involved not only hard work but also considerable danger.

In view of the urgency of the situation, it was essential to move the settlements and to close down the administrative and commercial centres which were serious foci of infection and a constant inducement to the natives to visit the danger zones, and to expose themselves to the risk of infection.

This was decided upon at the end of 1923 and the decision was immediately carried out. The general plan was to move the population from the villages on the spurs of Mounts Mitumba and Ruwenzori to the east and west of the valley—that is, to an area not infected with glossinæ. Innumerable difficulties were encountered, however, and all credit is due to those who since 1924 have devoted themselves to preparing new plantations for the natives, often right in the depths of the Ituri forest, who have supplied them with food to tide them over the initial period, and have visited the old villages with untiring zeal so as to make sure that the former occupants were not continuing, in their ignorance, to expose themselves to the disease. Uganda traders obtained concessions in the old settlements, such as Beni and Kasindi, and could not be moved against their will. Sometimes the people of the villages were

dissatisfied and returned in a body to their old settlement. The Catholic Mission at Old Beni, which had already been driven by sleeping-sickness from the banks of the Talia river, could not be evacuated until other quarters had been built. Lastly, the salt trade, which seems in these parts to be a vital necessity, and the communications with Uganda through the country north of Ruwenzori meant the maintenance of very considerable traffic over the danger-infested routes.

There would appear to be few endemic areas where the problem of prophylaxis has presented such difficulties. The distance from all the big centres (there are still eight stages, averaging 20 kilometers, between Irumu and Beni); the lack of means of communication; the dangers of the climate; the diversity of races and native languages; the restless mentality of the nomadic negro tribes, who are hardly settled or only provisionally established; their frequent hostility towards Europeans; their attachment to certain customs, such as fishing and the use of hemp; their allegiance to hereditary chiefs who may sometimes live at a great distance, out of reach of the administrative authorities or even in a different country (Unyoro, Toro, Ankole in Uganda) — all these factors have rendered the campaign against sleeping-sickness an overwhelming and thankless task.

Notwithstanding, a big effort has been made and the work now proceeding may be summarised as follows :

1. *Census.* — In addition to the administrative census and the issue of identity books including a medical passport, the Medical Service has carried out a census according to families, and there exists for every village a register showing the number of huts, the number of natives according to families and tribes, and the dates and results of examinations for sleeping-sickness. This census is still very incomplete.

2. *Treatment.* — The patients present themselves once a week, with a special card, at the treatment centres assigned to them. A treatment centre has been established for every group of villages, so that out-patients may have only a short way to go to receive their weekly injection. A European or native hospital orderly attends there on fixed dates. Advanced cases and sometimes recalcitrant cases are kept at the lazaret, a huge collection of brick huts to the south-west of Beni. The condition of the lazaret became very unsatisfactory, especially after the evacuation of the neighbouring settlements, and it was decided accordingly to transfer it to New Beni (Bungulu). A village for tripanosomiasis cases (isolation camp) had been established in 1922 between the Mission and Old Beni — at one time housing over 250 cases and their families — in the neighbourhood of a number of glossinæ breeding-grounds, on the road to Watelinga, north of Ruwenzori. It was evacuated at once. It constituted a danger to natives from the east of the valley, who were obliged to follow this, the only route, to reach the capital of the territory.

As regards the details of the treatment, for a long time atoxyl injections of 0.50 gramme to 1 gramme, according to the condition of the patient, were administered once a week over a period of three or four months, or even longer if the clinical condition seemed to require it. Potassium emetic and "Bayer 205" were also given simultaneously with the atoxyl cures, but produced only mediocre results. Latterly, tryparsamide and tryponarsyl have been substituted for atoxyl.

3. *Movement of Inhabitants.* — As I have explained above, the situation made this impolitic measure necessary, chiefly owing to the impossibility at the time of preventing contact between the scattered natives and the innumerable glossinæ breeding-grounds in the plain. It was a matter of urgent necessity to save the survivors. Chemical prophylaxis, which had been tried for some years, had proved ineffective.

For it to have been successful, it would have been necessary first to take a census of the *whole* population, to diagnose *all* cases of sickness, and to subject *all* patients to treatment which would sterilise peripheral blood for a sufficient length of time. This was out of the question, and, supposing that the necessary medical and administrative staff had been available, before even a part of the programme could have been carried out, the epidemic would have spread still further, and perhaps extended all round the focus, especially towards the north and east.

The map between pages 328 and 329 shows the transplantation of villages as carried out by M. HACKAERS and the modifications made as regards the ways of communication discovered to be infested with glossinæ.

We found the new capital established on the Bungulu plateau, about 300 meters above the valley, and a considerable part of the population settled in comparatively healthy parts round the new post. The Mission was just completing its removal to another plateau near New Beni. There were now only twenty-three patients left in the lazaret, and it was decided to move it at once to Bungulu. The new post at Kasindi is outside the glossinæ radius, and forms a centre for the survivors of the settlements formerly situated between the Semliki and the Lubilia river. There remain in the plain only a few settlements of Banande and Bahimas, those left behind from the Mission and the lazaret. New villages have been established on both slopes of the valley and in the forest, close to the Irumu road; the new plantations are flourishing, but trypanosomiasis has not yet disappeared; new infections still occur, chiefly among natives, who persist in visiting their old fields and banana plantations in the plain or in fishing in the Semliki.

4. *Deforestation: Supervision over Traffic.* — The principal deforestation work is that carried out at the fords over the main rivers. It would have been impossible to undertake such work on the old *safari* routes, and it seemed preferable to move the latter to the foot of the mountains so as to escape the glossinæ. This measure has not proved very efficacious, as the new roads are still badly infested, and the old routes (see map) are still much frequented. Despite the strict police measures, many natives manage to slip into the prohibited areas, fishing and planting and settling down, securely hidden in the impenetrable elephant grass; on the slightest alarm they flee into Uganda, where there is the risk that they may introduce the virus.

This work was begun in 1924, and is still being carried on.

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It may be of interest, after these various upheavals, to examine the political and economic situation of the inhabitants of the Semliki. The races most strongly represented there are the Bambubas, the Banande and the Bahimas, all more or less closely related to the natives of Unyoro and Ankole.

*Crops* flourish exceedingly in the rich soil of the valley; the rice recently introduced has produced good harvests among the Watelingas, and wheat is doing well on the high plateaux.

*Fishing* is engaged in by all the races, even the Bahimas, who are a pastoral people. Clandestine fishing is still carried on all along the Semliki and in Lake Edward, both of which are full of fish.

*Game* is plentiful. Elephants and red buffalo are common. Bush buck are found near the lake. Hippopotami are very numerous in the Semliki and on the shores of Lake Edward, though crocodiles, on the contrary, are never found in the



part of the valley which we visited. They do not become common until north of the Beni rapids, where they are found in considerable numbers, even in the smallest tributaries. Okapi are found only in the marshy depths of the great forest. Wart-hogs ravage the plantations all over the country and river-hogs are particularly destructive. None of the natives are hunters, except the Mambuti or pygmies, who keep to the forest and often hunt on behalf of other tribes. Game plays a part in the biology of glossinæ, and from this point of view the absence of reptiles, which elsewhere constitute a considerable item in the food of the flies, is an important factor.

*Work and Portage* are also important factors here. Although the population of the area visited is very much reduced (about 10,000 according to the census, and probably at least 15,000), transport depends entirely on porters, since the roads for wheeled traffic are not yet completed. Outside the valley, the danger is not so acute, the forest roads to Irumu and Boga and to Penge being comparatively safe. The road to Lubero and Rutschuru climbs rapidly up to high plateaux, which are absolutely free from infection. In the valley there are only three authorised roads, namely, the Boga road, which is reputed to be free from danger, and the roads branching to Beni-Watelinga and Beni-Kasindi-Katwe. The last two are fortunately but little used. The only industrial concern in the area, the Great Lakes Mining Company, does not use them, and they are employed simply for the conveyance of foodstuffs and salt, and by the carriers of administrative officials and medical officers on tour, hunters, etc. Generally speaking, the taxes levied on the natives are very light, and this applies both to personal taxes and to labour levies; the efforts of the Administration are concentrated on the extension of food crops in the healthy areas, with a view to preventing the inhabitants from moving to other parts.

The *Salt Trade* at Katwe and in the Kasinga channel has frequently been cited as one of the chief causes of the spread of sleeping-sickness. These saltworks have been exploited from time immemorial, and STANLEY speaks of them in his diary. The waters of Lake Edward and the small neighbouring lakes contain a variable proportion of chlorides, sulphates and carbonates of lime, potassium, magnesia, etc. The salts, obtained by natural evaporation, are regarded by the natives as indispensable for their food, and are used as a stimulant and remedy for their cattle. There is a continual stream of porters from the Belgian Congo and different parts of Uganda, who come to buy salt at Katwe and in the neighbourhood. These natives often pass through badly infested areas, and frequently have with them patients who have not been treated; they sometimes have to work in order to pay for their salt, and put in a few days wood-cutting on the shores of the lake, which are an active focus of trypanosomiasis. Lastly, a number of them are independent itinerant traders, who go from village to village through the valley along the Semliki selling their load of salt in small quantities; when it is considered that any one of them may be the carrier of a highly contagious virus, it is obvious what a part he can play in the dissemination of the disease.

*The Migration of Natives across the Frontier* is an additional danger from the point of view of the conveyance of the virus to distant parts. Such migrations are promoted by ethnical ties and are occasioned by the most varying causes: the attraction of high wages, the desire to escape from the authority of the Administration, the fear of sleeping-sickness or the census, or, again, of treatment, and sometimes the mere fancy or superstitious instinct of essentially unsettled and migratory negro races. On many occasions already, groups of Bahimas have crossed the frontier in one direction or another after unusually heavy mortality among their cattle.

We shall see later what a danger this represents for the country to which they migrate, especially Uganda, where many of the cases diagnosed are fugitives from the Semliki <sup>1</sup>.

The *General Pathology* is that of the whole of Central Africa. In addition, however, should be noted a comparatively recent epidemic of relapsing fever. I have referred to it elsewhere in connection with the therapeutic experiments with "Spirocid". The *ornithodoros moubata* and the infection which it conveys are a regular scourge for the convoys passing through the country; the huts and even the lazarets are rendered uninhabitable, and what is more, the disease is assuming a gravity and causing a mortality greater than we found anywhere in the Congo or in Tanganyika Territory.

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The progress of the endemic during the last few years may be summarised as follows :

Sectors	Natives included in census from 1923 to 1925	Cases of the disease diagnosed.					
		In 1923-24	In 1925	In 1926	Our tour in 1927	Total	Percentage
New Beni . . . . .	2,049	85	323	32	18	458	22.3
Beni-Irumu Road	2,684	—	36	14	5	55	2
Old Beni, Mission	886	14	195	63	4	276	31.15
Eastern spur :							
Ruwenzori	2,205	—	102	4	11	117	5.3
Western spur :							
Mitumba . . . . .	412	—	26	7	16	49	11.8
Isolated groups . . . . .	107	—	—	—	13	13	12
Total . . . . .	8,243	99	682	120	67	968	11.6

These statistics are obviously inaccurate. As a matter of fact, they do not include old cases diagnosed before 1923, and, moreover, omit the mortality during 1926. At the same time, the column for isolated groups constitutes a check, or at all events a basis of comparison, and the percentage at which we arrived during our visit is very close to the general endemic percentage for this area.

The census figures are also lower than the facts warrant. The population is really far more numerous, especially on the slopes of Mounts Mitumba and Ruwenzori; to take an example, in the villages of the Molekera district alone, the number of inhabitants we saw in four days was higher than the census figures for the whole of that part of the valley from 1923 to 1925. It would have been a difficult matter, moreover, to carry out a satisfactory census in view of the transfer of the natives to other sites, the establishment of new settlements, the feeding of negroes who had been

<sup>1</sup> Dr. GRIFFIN, Acting Specialist Officer, Sleeping-Sickness, Uganda, reports that, in the course of a visit in August and September 1926 to the endemic zones of Katwe and Bwamba, out of five fresh cases in these areas, three at least had recently become infected in the Semliki area. He also bears witness to the intractability of the natives, who make off on the approach of the authorities and fish and move about in the prohibited zones, and reports the introduction of trypanosomiasis into the very interior of Uganda. He states in conclusion, when speaking of Katwe, that "danger of the disease assuming epidemic proportions exists". In 1926, close upon 600 fugitives are said to have arrived in the endemic area of Bwamba from the west. He examined 460 and found one suffering from the disease, in addition to suspect cases.



obliged to abandon their crops, etc. Lastly, unsubmitive tribes are by no means the exception and the members of the Medical Service required a certain amount of tact in order to penetrate among them by pacific means. The same difficulty was found in introducing ordinary measures of hygiene, new agricultural processes, etc.

The average percentage for the whole area is less instructive than the local statistics, which show the real foci, often very limited in radius, where it is possible, without much difficulty, to analyse all the biological and economic conditions which have served to promote the spread of the disease. We give in the map between pages 328 and 329 the percentages noted at the end of 1926. They were already high and have since been exceeded. For example :

	Per cent
Old Beni and Mission. . . . .	32.7
Zumbia-Lufunda. . . . .	12
Masidongo . . . . .	25
Kolongo (above Talia) . . . . .	12
Populations moved from Beni. . . . .	14 to 36.7

The wave of infection spread from the south northwards. The Mission formerly established on the Talia river had to be evacuated owing to the disastrous mortality caused by sleeping-sickness. In 1916 it settled near Old Beni, where a fairly dense population had collected and where cases of trypanosomiasis were already by no means rare. The site chosen was in the neighbourhood of a stream and two springs, where *G. palpalis* are always present, although only in small quantities. Further, the Semliki river was only half-an-hour's distance away, and the Mission itself and the administrative post formed centres towards which large numbers of natives were always converging. At the end of 1925, then, there were already 189 cases of trypanosomiasis undergoing treatment, out of a census population of close on 600. Forty-seven children, including a number of infants, had been found to be infected, and on this occasion the source of infection was proved to be the neighbouring springs where the women go to fetch water, perhaps carrying one child, strapped to a piece of bark, and leading another by the hand. In 1926, out of 98 persons who had previously been certified free from infection, 63 were now discovered to be infected, making 64 per cent fresh cases. On this visit I was only able to make a hasty examination of 50 persons, and among them I found four suffering from the disease, *i.e.*, 8 per cent fresh cases, after a very short interval. This result must be attributed to the failure of chemical prophylaxis in a case when the virus reservoir in the flies was left untouched and kept in close contact with the inhabitant.

As a contrast to the fate of the Mission may be mentioned that of certain native settlements which had been moved to sites reputed to be healthy.

The present village of Mokoko was formerly established on the land between the Djuma river and the Semliki and belonged to a tribe which suffered terrible losses. No definite figures can be given as regards the percentage of infection on the Djuma, but, according to the natives, by the time they were moved they had been reduced by the disease to one-third of their original number. In 1924, plantations were prepared for them in the forest, beside the Irumu-Beni road. Old cases were treated, but none of recent infection were discovered until 1926 (May), when three fresh cases were diagnosed, out of 250 inhabitants. On December 26th, 1926, I examined the population and found three cases as follows :

*Observation 201.* — *Kosamwana*, a man of 35. Son of the chief, settled for the last two years in the new village. Recent infection, possibly dating from two or three months back. Probably infected on the Djuma river (in the tribe's old plantations) east of Moera.

*Observation 202.* — *Falaki Henetzula*, a man of 30. Also recent infection, contracted during a short stay away from the village (the only one for two years) at Manaunde, near the Semliki, opposite Old Beni.

*Observation 203.* — *Kadwida*, a girl of 6. Accompanied case 201 to the plantations in the prohibited area beside the Djuma river. Recent infection.

Of the three old cases, two mentioned in their replies that they had been in the evacuated danger zones; the third was an advanced case, suffering from a relapse and infected before the removal of the village.

It seems clear, then, that this village is quite safe, although *G. palpalis* exist on the Tarambu and the Gita, small neighbouring streams. Here the measures taken have sterilised the human virus reservoir, which has been kept out of reach of the infecting flies, and the new cases have contracted the infection elsewhere.

The new villages grouped round Kasindi showed the same results, but in this case there are no glossinæ breeding-grounds in the immediate vicinity.

The New Beni settlement is a far more complex case. This post was established by the artificial settlement of populations which had suffered severely from sleeping-sickness, belonging to the districts in the neighbourhood of Old Beni. There are a number of streams round the new post, where we soon discovered glossinæ breeding-places. The streams are frequented by the natives, and one quarter of the village even extends to the Muniaberu river, the worst infested part. There are close upon 2,500 inhabitants, who still have many opportunities of contracting trypanosomiasis on account of portage, clandestine fishing and visits to their old fields, and to the Mission, etc. They settled at New Beni (Bungulu) in 1923 and 1924 with their sick members, and, in 1925, 323 were undergoing treatment. In 1926, 32 fresh cases were discovered: these were imported cases, persons who had become infected elsewhere, or more advanced cases not previously examined. Among them, however, was a woman who, it was suspected, might have contracted the affection on the spot. The question immediately arose: Did breeding-grounds of infected glossinæ already exist; had the infection got a footing? This appeared improbable at first sight, and Dr. TAVERNARI, who had just carried out an enquiry into the matter, adduced a number of facts to disprove the suggestion; the natives under the Chief Kiniambarara, who had been settled for over six years at Bungulu, had never had any but sporadic imported cases; the disease was everywhere decreasing rapidly, glossinæ were comparatively rare, and further points were the extreme variations in temperature, which were unfavourable to their development and power of infection, the fact that the large majority of recent cases were supposed to have become infected elsewhere, and so on.

To form an opinion, it was necessary to question a certain number of recent cases so as to determine the probable place of infection. An examination of the natives at Bungulu produced the following information:

*Observation 205.* — *Maliabwana*, a man of 35. Left the infected banks of the Semliki seven months previously; very recent infection; the glands are barely puncturable, and the clinical state is normal. Lives quite near a tsetse breeding-ground, beside the Muniaberu river.

*Observation 206.* — *Kakungu*, a young man of 18. Settled fifteen months before in the village of Kiniambarara (Bungulu), previously under Masidongo, in the plain. The various examinations of this patient during the year 1926 gave negative results. On December 30th, 1926, the result of puncture of a small cervical gland was positive. A case of recent infection, no clinical symptoms. Does not appear to have ventured during the last few months into the valley foci.

*Observation 207.* — *Busemba*, a girl of 10. A recent case, evacuated two years previously. Gland puncture positive on December 30th, 1926; lumbar puncture showed two lymphocytes per cubic mm. It is impossible to determine the probable place of infection. The child frequented the neighbourhood of Bungulu, going as far as the Mission.

*Observation 208.* — *Soko*, a boy of 12. A very recent case, but infected at the old settlement of Kaniama, in the valley.

*Observation 209.* — *Mukira*, a girl of 16. A recent case, diagnosed on December 31st, 1926. Previous examinations, including one in August 1926, proved the absence of enlarged glands. C.S.F. : 4.1 lymphocytes per cubic mm. Note : The patient does not visit the valley or the Mission. She lives near the stream to the north of Bungulu, where the fly-boys have found glossinæ.

*Observation 210.* — *Kabaya*, a woman of 25. A recent case, but evidently infected at the Mission a few months previously.

*Observation 211.* — *Kambere*, a boy of 12. Has lived for a long time in the village of Kiniambarara, but appears to have become infected during the course of a month's visit to the old settlement of the Chief Kisenge, not far from Old Beni.

*Observation 212.* — *Kaisi*, a man of 50. Has lived at Bungulu for the last two years, but may have become infected elsewhere, perhaps at the lazaret, where his wife is undergoing treatment. C.S.F. : 182.5 lymphocytes per cubic mm., big mononuclears and muriform cells.

*Observation 213.* — *Muswage*, a woman of 25. Has lived at Bungulu (Kiniambarara) for the last ten years, but may have become infected elsewhere, particularly at the old settlements at Kisenge and Monganga, near Old Beni. Case of medium gravity. C.S.F. : 85 lymphocytes per cubic mm. and big mononuclears.

*Observation 216.* — *Kisuki*, a man of 30. A fairly advanced case. The patient engages in clandestine fishing in the Semliki.

*Observation 217.* — *Mopedahé*, a man of 40. A fairly advanced case. Infection contracted in the forest cutting of the Semliki, near Namingu, where he used to build canoes.

*Observation 218.* — *Mukinga*, a woman of 25. Has lived at Bungulu for the last two years ; it is impossible to prove that she has visited other breeding-places. A very recent case ; lumbar puncture : 5 lymphocytes per cubic mm. The nearest glossinæ breeding-ground is the Lina river, where there are a number of *G. palpalis*.

*Observation 219.* — *Muswaga*, a woman of 25. A case like the preceding one. The woman is a native of the hill where the Kisenge survivors have been established. No connection with the valley, and no other reason appears to have taken her to any known foci. Lives quite near the Lina river, where there are glossinæ.

*Observation 220.* — *Kommada*, a boy of 4. Infection recent, but certainly contracted in the neighbourhood of the lazaret.

*Observation 221.* — *Mopira*, a woman of 40 from Kitobe, who left the plain over two years ago. A recent case ; the patient leaves her home only on regular trips (to carry food) between Kitobe, Kiniambarara and Karibumba. Frequents the tributaries of the Tina river.

*Observation 222.* — *Musukali*, a woman of 45. A case of medium gravity ; probably contracted infection in the old plantations in the valley.

Of the sixteen cases which I have just quoted, six were probably infected in the immediate vicinity of the new post. The replies given by the natives, who are unwilling to confess that they have set foot on the roads prohibited by the European authorities, must obviously be accepted with some caution, but I was assisted in these investigations by an old medical orderly who knows the haunts and habits of most of these natives, and by the village chiefs and notables. Nearly all the cases, it may be noted, are quite recent. Cases shown by the modification of the cerebro-spinal fluid to be in an advanced stage were all infected elsewhere. It must be admitted, then, that the infection has already gained a footing in the new post, though there is reason to anticipate the extinction of this focus before long as a result of measures now being taken.

In any case, the system of moving the population is open to the objection that there is the danger that new centres of infection may be created while the old ones may be, as it were, extended. It may appear strange to anyone who has no experience in such matters that natives should have been moved to plateaux where the glossinæ exist, even if only in small numbers, but all who have been through this part of the country will realise how difficult it is to find a site which is really free from this dangerous

diptera and is at the same time suitable for the growing of crops and possessed of a water supply.

In this connection I must mention the appearance of a new focus in a zone which there was good reason to regard as immune during a certain number of years.

The Bahima tribe is settled on the southern bank of the Talia river, in the angle formed by its junction with the Semliki river. These natives are a pastoral tribe, owning numbers of fine cattle. Sleeping-sickness had claimed several victims among them, as they are very fond of fishing in the Semliki. Accordingly, part of the tribe had been moved to the grassy plains at the foot of the Mitumba mountains, and since 1921 no further case of sickness had been diagnosed among them. The remainder, under the Chief Makora, were not moved until 1924, when the cases of sickness amounted to 2 per cent. It may be noted, further, that these natives, who are a superior race, hire the service of the Banande for most of their heavy work, and it is these servants who are in reality the most exposed, when fishing, watering the cattle, etc. The Banande, moreover, are proud and do not mix with the neighbouring races.

After two years' immunity, sleeping-sickness reappeared among the Bahimas. On November 22nd, 1926, the medical orderly discovered three cases, in which the clinical symptoms showed the disease to be in the first stage. On January 17th, 1927, and the following day, we examined the natives, and found that all three cases were in a state of relapse, despite the recent treatment, while there were eleven other cases, all recent, which had given negative results when examined two months previously. On investigating the causes of infection, we learned that nine of these cases belonged to a group of agricultural workers, under the orders of the same deputy chief, not far from the Semliki, where they get their water and water their cattle. Lack of time prevented our continuing the investigations, but I may add that a number of glossinæ caught in this place were dissected and that no flagellates were found, even in the proboscis. At the same time, the cattle throughout the area are badly infected with *T. vivax*.

This is the present position as regards sleeping-sickness in the Semliki territory. To sum up, all the measures taken up to the present time have had the effect of transforming a very deadly epidemic into an endemic which cannot yet be described as benign, and, given a conjunction of unfavourable conditions, as among the Bahimas, the disease shows a definite tendency to spread as before.

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I can find no better way of defining the Semliki virus than by giving the following characteristics, which will permit of a comparison between it and the *T. gambiense* in other areas.

1. *Evolution in Man.* — There is no appreciable difference, either in the symptoms or in the rate of evolution, as compared with the *T. gambiense*, which I observed on the shores of Lake Victoria. In the many recent cases I have diagnosed, an examination of the fresh blood specimens did not reveal the abundance of trypanosomes frequently found in cases infected by *T. rhodesiense*. Dr. LAVIER examined a good number of patients for the purposes of research into the morphology of the trypanosome, and only in two or three cases did he find parasites in any quantity in the blood-films.

The first symptoms of nervous or psychic troubles hardly appear earlier in these cases than elsewhere. For example, case No. 213, whose cerebro-spinal fluid already contained 85 lymphocytes per cubic mm. and big mononuclears, exhibited neither trembling, reflex troubles nor psychic troubles. Case No. 212, whose fluid was in a more advanced stage and contained muriform cells, had been suffering from the disease

for eight months, and this tallies with the evolution of cases on Lake Victoria. Case No. 264, diagnosed in 1925 and treated with atoxyl, emetic and "Bayer 205", was found to have in his spinal fluid 1,015 lymphocytes, muriform cells and trypanosomes, but he works every day at the deforestation on the Semliki ford.

In the early periods of the disease, the regular symptoms are fever, anæmia and asthenia. Neither œdema nor gastro-intestinal troubles are found. Enlarged glands are the rule and do not occur any earlier.

2. *Distribution according to Age and Sex.* — The following statistics cover the last few years :

Year	Men Per cent	Women Per cent	Children	
			Male Per cent	Female Per cent
1923-24. . . . .	50.1	37.2	7.3	5.4
1925. . . . .	50	29	12.7	8.3
1926. . . . .	85.4	7.3	2.9	4.3
1927 (our cases) . .	53	20	17	10

As elsewhere, male adults constitute the largest proportion of cases, and here again the explanation is to be found in their occupations, especially fishing and portering, which expose them to infection more than women and children. At the same time, as we have seen in certain foci, such as the Mission, children and even infants may be contaminated at the glossinæ breeding-grounds in the vicinity of the huts. The missionaries, who lived at Old Beni for many years, and one of whom is trained in the microscopic diagnosis of sleeping-sickness, have found trypanosomes in infants only a few months old, the evolution of the disease in such cases being rapid and fatal.

Again, the percentage of adult men infected increased to 85 per cent after the evacuation of the valley, owing to the fact that men more than others continued to frequent that part, either as porters or clandestinely. The latest figures which were obtained on this tour approximate much more nearly to the previous averages, as our tour of inspection extended without distinction to all the inhabited points in the endemic zone.

3. *Index of Mortality.* — It is impossible to give an exact estimate of the mortality from sleeping-sickness and, moreover, as the majority of the cases are treated, these figures possess no real significance. For example, to take the cases diagnosed in 1923, 1924 and 1925, the annual death rate was about 10 per cent for the total number, and 25 per cent for certain groups of natives, such as the dwellers on the shores of Lake Edward, who had suffered more severely. For certain cases diagnosed in 1925 and 1926 which had not been treated, the annual death rate was found to be one-third of the number. On the other hand, for a group of natives moved to the New Beni (Bungulu) district, it has remained at 3 per cent during the last two years. As compared with other countries — for example, the Upper Uele, where in 1926 the rate was 8 per cent — these figures are in no way exceptional.

4. *Proportion of Advanced Cases.* — Lack of time prevented me from carrying out an exhaustive examination of all the cases I encountered, and I was frequently obliged to omit the lumbar puncture as a means of checking the clinical examination. The following, however, are the statistics obtained from about 60 cases, shown side by side with the results obtained in other epidemic or endemic areas :

	Mayombe (Belgian Congo) Per cent	Budama and Kavirondo Per cent	Upper Uele (Belgian Congo) Per cent	Semliki (Belgian Congo) Per cent
Recent cases . . . . .	74.95	23.8	14	54.5
Cases in the middle stage .	15.90	28.5	} 86	} 30.3
Advanced cases . . . . .	9.15	47.6		
To be isolated . . . . .	—	—		4.6

The proportion of recent cases, as I have explained elsewhere, is a factor in what might be called the index of endemicity. As regards the Semliki, this index is midway between that of Mayombe, where I saw a regular epidemic of trypanosomiasis, and that of the Upper Uele, where the disease is extremely sporadic in character.

4. *Efficacy of Regulation Treatment.* — From the outset, the medical service has employed atoxyl. The treatment consists of weekly injections, extending over a period of at least four months, of 0.50 to 1 gramme of atoxyl, smaller doses being administered to patients who appear clinically in a more advanced stage, and larger injections to those whose clinical state points to the infection being of recent date.

Despite this treatment, which is certainly efficacious in a large percentage of cases, I have been surprised by the frequency of relapses. I give below a number of examples relating to various methods of treatment, and one case of cure.

*Observation 204.* — *Bambu*, a woman of 25. Diagnosed by gland puncture on October 14th, 1924. On that date the disease was of average clinical gravity; the lumbar puncture was not carried out. Treatment: 20 injections of 0.50 gramme of atoxyl and one of 1 gramme: 5 injections of 0.05 gramme potassium emetic. Under observation since April 20th, 1925. Appears to be recovering, no longer visits the valley where she became infected. Negative examinations up to December 30th, 1926, when there was a relapse as shown by blood and lymph.

*Observation 214.* — *Kabaya*, a man of 30, a recent case. On December 24th, 1924, received an injection of 0.50 gramme and 22 injections of 1 gramme of atoxyl, and 5 injections of 0.05 gramme of emetic. Relapse on January 4th, 1927, unexplained by any suspicion of reinfection.

*Observation 215.* — *Bangana*, a boy of 6, a recent case. Diagnosed on September 12th, 1926, and treated with weekly injections of 0.35 gramme of atoxyl, suited to his weight. Relapse discovered on January 4th, 1927, after treatment had been suspended for six weeks.

*Observation 259.* — *Mupanda*, a man of 45. Diagnosed one year previously; the patient fled to Uganda after having received 4 injections of 1 gramme of atoxyl. Relapse discovered, and patient found in a desperate state on January 27th, 1927.

*Observation 264.* — *Limbori*, a young man of 19. Was a recent case in 1925 and received 3 injections of 1 gramme of atoxyl, 2 injections of 0.10 gramme of emetic and 3 injections of 1 gramme of "Bayer 205". The lumbar puncture, carried out on February 1st, 1927, revealed 1,015 lymphocytes per cubic mm., large mononuclears, muriform cells and trypanosomes. Clinical state desperate.

*Observation 265.* — *Malosi*, a woman of 20. A recent case in 1925; received 13 injections of 1 gramme of atoxyl, 2 of 0.10 gramme of emetic and one of 1 gramme of "Bayer 205". Condition excellent on February 1st, 1927. C.S.F.: 2 lymphocytes per cubic mm.

This last case, in which an apparent cure was obtained by a treatment only slightly more intensive than that applied to the others, shows how the reaction of the trypanosomes to the medicament varies as between one patient and another. When inspecting some hundreds of patients undergoing treatment at the Bungulu post, I noted chiefly those who had been diagnosed at least one year previously. The annual death rate of 10 per cent had already reduced their numbers and operated a selection. Judged simply by the external clinical signs, 8 out of 45 — that is, close on 18 per cent — justified an unfavourable prognosis.

Taking into account the large proportion of recent cases, which are more readily curable, these facts appear to me to indicate very clearly that the Semliki virus offers a strong resistance to our usual therapeutic methods.

6. *Resistance in natura to Atoxyl.* — When examining the natives in the Molekera district, I observed three cases diagnosed two months previously in which the atoxyl treatment had not sterilised the peripheral blood.

*Observation 229.* — *Lubuta*, a man of 35. A Banande in the service of the Bahimas, infected on the Semliki, where he fishes and works.

Diagnosed on November 22nd, 1926, by examination of the glandular lymph.  
November 22nd, 1926 : 1 gramme atoxyl ;  
January 7th, 1927 : 1 gramme atoxyl ;  
January 17th, 1927 : Trypanosomes in the glandular lymph.

*Observation 230.* — *Komonda*, a man of 35. A Banande in the service of the Bahimas, infected like the preceding case.

Diagnosed on November 22nd, 1926, by examination of the glandular lymph.  
November 22nd, 1926 : 1 gramme atoxyl ;  
December 13th, 1926 : 1 gramme atoxyl ;  
December 26th, 1926 : 1 gramme atoxyl ;  
January 7th, 1927 : 1 gramme atoxyl ;  
January 17th, 1927 : trypanosomes in the glands.

*Observation 231.* — *Kasolomo*, a woman of 20. Pure Muhima, infected on the Semliki ; lives near the river.

Diagnosed on November 22nd, 1926, by examination of the glandular lymph.  
November 22nd, 1926 : 1 gramme atoxyl ;  
December 13th, 1926 : 1 gramme atoxyl ;  
December 28th, 1926 : 1 gramme atoxyl ;  
January 7th, 1927 : 1 gramme atoxyl ;  
January 17th, 1927 : trypanosomes in the glands.

In these cases, the lumbar puncture was not made at the beginning of the treatment and it was only by the external symptoms that infection was judged to be recent.

These three cases all came from the same part of the Semliki and were probably infected by the same germ. Does their trypanosome offer natural resistance to arsenic or has it acquired this property in the course of passage through natives who did not receive sufficient treatment ? In any case, this fact is most serious and it remains for us to draw conclusions therefrom regarding the regulation methods of treatment.

The trypanosomes of all three patients were inoculated into the muscles of a monkey, which did not become infected.

7. *Transmissibility.* — I endeavoured to bring back a good number of strains to the Entebbe laboratory by inoculating animals or sending back patients which had not been treated. Unfortunately, only one strain arrived. Dr. H. L. DUKE found it of medium transmissibility.

8. *Pathogenicity for Small Laboratory Animals.* — Five inoculations were made on guinea-pigs and three on a monkey. None of the animals became infected.

9. *Morphology of the Trypanosome.* — Professor G. LAVIER examined a good number of trypanosomes obtained directly from the patients, and detailed particulars are given in his reports. Morphologically this trypanosome belongs to the species *T. gambiense*.

The distribution of glossinæ in the Semliki territory and neighbouring zones would repay exhaustive investigation. During the course of this short tour, I could only note facts directly connected with my researches ; at the same time, I was greatly struck by the large variety of species and the extent of the area inhabited by each of them.

In this area, which is in reality badly infected with trypanosomiasis, it would be quite possible for an unsuspecting traveller to follow our route without noticing the glossinæ. Nevertheless, *G. palpalis* exist practically everywhere, on all the rivers, even the smallest, and possibly even at high altitudes during the hot seasons.

The following are a few notes concerning catches, which for the most part can be traced on the map attached to this report<sup>1</sup> :

*Irumu*. — *G. palpalis* present on the Chari river, north of the post. Note that among the breeding-stock *T. congolense* and *T. vivax* were found.

*G. fusca* or *brevipalpis* seen by Dr. G. LAVIER in the town.

*Loyo river, Geleza camp*. — *G. palpalis*.

December 20th, 1926, 1 boy, ½ hour : 4 males, 3 females.

February 18th, 1927, 1 boy, 1 hour : 10 males, 7 females.

*N'Domba camp*. — *G. fusca* or *brevipalpis*.

February 17th, 1927, wooded spring to the west, 1 boy, 1 hour : 1 male, 1 female.

*52 km. south of Irumu*. — *G. pallidipes*. 1 female caught on the way, in the forest. Game abundant, buffaloes.

*Tributaries of the Gita river*, on the road in process of construction, on the way to the villages of Moera and Lufungula. — *G. palpalis* and *G. fuscipleuris* Austen.

*Muniaberu river*, 2 or 3 kilometers north of Beni (Bungulu or new post). East and west of the bridge-road. — *G. palpalis*.

January 1927, 1 boy, 1 hour : 3 males, 1 female.

1 boy, 1 hour : 1 male, 1 female.

February 1927, 2 boys, 1 hour : 5 males, 5 females.

*Stream south of New Beni*, Lina river, tributary of the Tina river. — A few *G. palpalis* on February 7th and 8th, 1927, and one *G. fuscipleuris*.

(Note. — The Administration is at present carrying out important deforestation work, which is being pushed on energetically, all round the New Beni post. Not only have the forest belts been entirely cut down, but food crops, more particularly sweet potatoes, rice and manioc, have been planted there.)

*Old Beni Mission*. — *G. palpalis*.

Spring, to the north, February 2nd, 1927, 1 boy, 1 hour : 5 males, 5 females.

Spring, to the west, February 2nd, 1927, 1 boy, 1 hour : 1 male, 3 females.

The following are the rivers where *G. palpalis* was noticed as our party crossed : Dongwa, Kaga, Talia (west), Karoroma, Kasaka, Tambwe.

*Tambwe river*. — *G. palpalis*.

1 boy, 1 hour : 18 males, 10 females.

*Semliki river*, ford, to the south, near Lake Edward. — Deforestation has been carried out on the banks beside the main road ; there are no longer any tsetse. About 100 meters from the part not cleared : *G. palpalis*. January 19th, 1927, 1 boy, 1 hour : 15 males, 27 females.

*Lubilia river*. — Deforestation carried out in the neighbourhood of Nakahiga. North of the Nakahiga ford, no glossinæ found. In the south, they are rare. Above the falls : *G. palpalis*. January 23rd, 1927, 1 boy, 1 hour : 4 females, 3 males. *Palpalis* numerous at the old ford at Old Kasindi ; also at the marshy mouth of the river, among the wooded slopes.

*Shores of Lake Edward*. — *G. palpalis*. Number of flies varies very much according to the density of the trees. At Amakoma, there are no glossinæ, except near the mouths of the Makoma and the Tambwe.

*Tributaries of the Lume*, near Mungu. — *G. palpalis*.

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<sup>1</sup> I desire to express my gratitude to Mr. Austen, who was good enough to identify several specimens of glossinæ caught during this tour.



*Talia river* (east). — *G. palpalis*, rare, attacked our party as it was crossing. Note that these observations were carried out along the authorised Beni-Kasindi road.

*Bolombi river, Bambome camp.* — *G. fuscipleuris*.

*Bitawe river.* — Crossed twice; on each occasion a few *G. palpalis*.

*Musenene camp.* — *G. fuscipleuris*.

*Manaunde camp.* — *G. fuscipleuris*.

Both these camps are situated at a fair altitude, in a wooded patch in the middle of the Semliki plain (Mount Kilima). A steep slope leads down to the Semliki river, broken by a few torrents and stagnant pools where *G. palpalis* is rare. At the foot of the hill, the natives have plantations not far from the Semliki breeding-grounds.

*Semliki river*, opposite Old Beni. The ford has been but only partially cleared. — *G. palpalis*, March 1st, 1927, 1 boy, 1 hour: 11 males, 9 females.

The Old Beni post was abandoned over two years ago and is already overgrown and invaded by game. Elephants have destroyed the guava avenues and hippopotami and buffaloes have laid waste the gardens and plantations. The elephants are breaking down the brick walls of the old houses. A number of marshy streams flow round the old post, and glossinæ are by no means rare.

A number of glossinæ were dissected; others have been kept for purposes of identification. Dissection did not reveal any flagellates; the absence of *T. grayi* may be noted and, in conjunction with this fact, the absence of crocodiles in the whole area<sup>1</sup>.

#### GENERAL CONSIDERATIONS AND CONCLUSIONS.

As may be seen from this account, sleeping-sickness in the Semliki valley has assumed the form of a severe endemic. My object was to determine the causes which had led to its propagation and, at the same time, to establish prognostics as to its subsequent evolution and possible extension in the neighbouring districts.

Certain known biological and economic factors will assist in solving the question.

1. *Abundance and Distribution of Glossinæ.* — Although not very numerous, *palpalis* are found on all the rivers, lakes, etc., their habitat extending without interruption as far as the areas of Irumu, the Chari-Ituri river, the Ituri forest, Lake Edward and Lake George, Katwe, Bamba and Wasa, east of Ruwenzori.

2. *Density of Population and Natural Resistance.* — Conditions have changed since the evacuation of the valley, where only isolated groups are now left. On the slopes, however, there are thickly populated settlements over which it is difficult to exercise supervision and, chiefly towards the frontier, the natives, who are intractable and often insubordinate, move about on the slightest pretext from one *palpalis* zone to another. There, wherever the compact groups of Bahimas are settled, a dense population is to be found round certain definite tsetse breeding-grounds. Their natural power of resistance, so far as it counts, is comparatively low in regard to all forms of infection,

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<sup>1</sup> *G. morsitans* has been frequently reported in the vicinity of the Semliki endemic area, among others by Dr. H. L. DUKE, in the neighbourhood of Lake George and of the Wasa area. We did not obtain any specimens, although at certain points in the valley the appearance of the wooded savanna and the abundance of game would appear to offer a perfect environment for the development of this particular fly.

despite the fertility of the soil and their numerous and plentiful crops. A mixture of very different races is found, from the Bahimas to the forest pygmies. Their standards of morality and intelligence are below the average.

3. *Circumstances which favour Close Contact between Man and Glossinæ.* — (a) Dwellings in the vicinity of glossinæ breeding-places. Notable examples, namely, Old Beni and the Mission, have already been quoted, in which the natives and even the Europeans are exposed every day.

(b) Existence of breeding-grounds of glossinæ which feed exclusively on man. There are springs and streams where the natives go to fetch water all day long and where neither reptiles nor game exist to divert the fly. These conditions already exist in some of the new sites (for example, Bungulu) to which settlements have been moved.

(c) Native industries and occupations. Reference was made to these above, the most important being fishing, canoe building, and the cultivation of plantations near the rivers; all are prohibited by the Administration, but are none the less practised clandestinely. To these must be added the following: the house-to-house trading in salt; cattle-breeding in the plain; trade with neighbouring territories; exchanges of labour; the purchase of women between Bwamba and the Banande; the traffic in hemp; the exchange and migration of cattle between the Bahimas of Molekera and those of Bwengo, and perhaps of many other parts of Ankole; administrative, scientific and also hunting expeditions, the native porters being obliged to enter the old focus in the plain, often without any provision for medical supervision when they are exposed to infection.

4. *The Virulence of the Trypanosome*, so far as it can be estimated from the clinical evolution of the disease, the index of mortality, the efficacy of the regulation treatment and, in certain cases, natural resistance to atoxyl.

Other factors, and most important ones, are still uncertain and would have required considerable additional research both on the spot and in the laboratory. We do not possess data to show the degree of infectiousness of the virus or the "index of transmissibility", which Dr. H. L. DUKE might have established at Entebbe, if it had been possible to bring back a sufficient number of strains. Lastly, we have only very vague ideas as to the number of virus-carriers in this area, where chemical prophylaxis has been employed for many years with so little success.

From the purely practical standpoint, however, I feel justified in drawing the following conclusions:

1. The endemic in the Semliki area is serious, the virus may be described as of average strength and to-day, as in the past, shows a tendency to spread, creating fresh foci in the *palpalis* distribution area.

2. The nature of the virus, the biological conditions of the glossinæ and the opportunities for contact between them and man are responsible for this. There is every likelihood that the endemic will spread beyond its present limits and that the virus, which is sometimes arsenic-fast, may spread through the neighbouring territories.

3. In the present case, the natives possess no safeguard against infection, but will, on the contrary, be the first to spread it, by reason of their migratory habits and racial ties. The effects of chemical prophylaxis are thus bound to be checked by the continual importation of fresh strains of trypanosomes, until supervision over the movements of persons and over traffic in general becomes the rule and the foci where the glossinæ depend exclusively on man can be evacuated or cleared by means of deforestation.

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## EPIDEMIOLOGY OF SLEEPING-SICKNESS IN THE UPPER UELE

(*Belgian Congo*).

BY DR. H. LYNDBURST DUKE AND DR. L. VAN HOOFF.

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The endemic zone of Upper Uele was chosen for this brief enquiry because this district, while easily accessible, constitutes a group of foci well separated from those of Uganda and of the Semliki valley and could supply us with different clinical observations and different strains of trypanosomes.

Little is known of the history of the disease in this district. It has, however, been in existence there for a long time. Ponthier<sup>1</sup> observed cases among the Arabs defeated by him in 1891 near the Bomokandi river. This river forms the boundary of the Azande tribes, who are well acquainted with sleeping-sickness, and at present it is practically the southern limit of the focus of infection. A single case imported from the north has been discovered on this river, which is thickly infested with glossinæ. The populations to the south and on the banks have hitherto remained immune.

It appears that in the Zande language the word "pupë" is the true term designating sleeping-sickness and also signifying mental confusion, agitation and delirium. Since the European occupation, which has led to the use of the rudimentary lingala as the current language, the word "pongi" or "poingi" is usually employed and signifies "sleep"<sup>2</sup>.

It is probable that formerly the endemic wave spread from the north-west to the south and east, *i.e.*, from the districts most favourable biologically to the development of the disease to those less favourable. The basin of the French Ubangui and of its affluents, chiefly, as far as we are concerned, the Bomu and its tributaries coming from the watershed to the north-east, together with the Bahr-el-Ghazal, appear to us to be still the cradle of the disease. In the Belgian Congo, the disease has in the last ten years been propagated by native traffic from this frontier towards the main rivers and along the chief lines of communication. The territories in the neighbourhood of the river Bomu, *viz.*, Gwane, Gatanga and Bangaro, were infected first and with greatest intensity. The main lines of communication towards the south are the native roads towards Dakwa, Amadi and Niangara. A network of paths also leads towards the east, *i.e.*, towards the large kingdom of Renzi, by way of Doruma, Bafuka, Gilima, Wando and Tombo, and thus forms a connection with the territories of Faradje and Aba. The map of the Doruma district gives a definite impression that the infection followed this route, along which a quantity of minute foci have been formed.

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<sup>1</sup> According to information kindly supplied by Professor E. VAN CAMPENHOUT.

<sup>2</sup> The Zande language is rich in names of diseases, these often having a particular signification testifying to more or less exact observation or indicating a fanciful ætiology. Thus, according to Mgr. LAGAE'S dictionary, leprosy = "kaza uru" = sun sickness; elephantiasis of the lower limbs = "hima gido mbara" = sickness of the elephant's heel; smallpox = "bakofu", from "kofo", to pierce, etc.

The routes of river communication are the Uele and the Kibali river, whose glossina-infested banks have become gradually infected, the disease travelling upstream and creating here and there persistent foci of trypanosomiasis, such as Nakassa on the Kibali, certain points on the Yebu tributary, and the vicinity of Van Kerkhoveville near the confluence of the n'Zoro, which have had to be evacuated on account of trypanosomiasis.

The district of Aba may also have been contaminated owing to its proximity to the Sudan and the Mongalla, but to the east and south the hills which form the watershed between the Congo and the Nile have acted as an insuperable barrier. On the first slopes towards the Nile and Lake Albert, however, the Walendu and Alurs people have paid heavy tribute to the disease; this focus, however, belongs to the Uganda group.

On this journey we came into contact with the anti-trypanosomiasis medical organisation, the M.M.S.U.<sup>1</sup> which the Government of the Belgian Congo has established in the districts of Upper and Lower Uele. The details of its work and the results obtained are published in the *Annales de la Société belge de médecine tropicale*, to which we refer the reader. The general programme of the M.M.S.U. is as follows:

1. *Complete Census of the Population and of Patients.* — This work, which is partly administrative, requires a large staff, among which responsibility for the various foci in the infected zone is judiciously divided. It cannot be carried out unless identity booklets and individual medical passports are issued to the natives.

2. *Rational and Complete Treatment of all Patients.* — With this end in view, each zone is provided with a lazaret or principal hospital, in which are kept serious cases, patients in an incurable, demented, feeble-minded or dangerous condition, and also recalcitrant patients. As these only constitute a small percentage, the greater part of the therapeutic work is done in the secondary dispensaries and treatment centres. This name is given to consultations given at one or more places in each district which the doctor or his European or native assistant visits on a fixed day or days each week to see and give injections to patients. The prescription and suspension of treatment are determined by analysis of the cerebro-spinal fluid.

3. *Evacuation of Dangerous Zones.* — A frontier zone 10 kilometers in breadth has been evacuated. Main motor roads have been built with a view to abolishing portage, controlling the movements of the natives and concentrating them all along these routes constructed at a distance from the forest belts.

4. *Deforestation and Sanitation of Infested Villages and River-crossings.* — Taken all round, this organisation is very similar to that which has given excellent results in the Anglo-Egyptian Sudan. Between the Sudan authorities and the Belgian administrative and medical authorities, there exists an agreement which aims at the employment of uniform prophylactic measures and also at preventing the flight and emigration of patients. Apart from its specialised work in connection with sleeping-sickness, the M.M.S.U. also deals with all other endemic or epidemic affections, and even with surgical cases, a fact which has greatly contributed to making the dispensaries and treatment centres popular and has thus facilitated the census work.

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<sup>1</sup> Mission contre la maladie du sommeil dans l'Uele.

Before outlining the results of our inspection in the Upper Uele, we have great pleasure in expressing our gratitude to all those who so generously assisted us in the course of our journey. The Government of the Belgian Congo offered us every facility, and the officials of the Government, as well as the management and agents of the Kilo-Moto Gold-Mines Company, and private individuals, helped us on every occasion with our transport, lodging, recruiting of porters, information, summoning of natives, etc. Our special thanks are due to M. MONTI and M. GOMRÉE, managers of the gold-mines, Baron VAN ZUYLEN, M. MARCOZ, M. VAN DE VELDE, M. CARLIER, Father MOREAU and the Dominican missionaries. Special thanks are due to the doctors of the mines and of the M.M.S.U. and to the medical auxiliary staff, who gave us their personal assistance and from whom we have borrowed a considerable part of our information.

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Having only six weeks to cover an enormous area, we made special arrangements for our transport, and frequently separated in order to divide the work, one of us dealing with biological questions and the other with the examination of the natives, and both of us collecting clinical and epidemiological observations. We investigated in greater detail the districts of Missa, to the north of Aba, the district of Tombo, to the north-west of Faradje, Dungu and its neighbourhood, the mining camps on the Kibali river, Doruma and the neighbouring districts, particularly Likita, Dika, Yapwati and Bendiepwa. Our route from March 10th to April 30th followed the main road passing through Arua-Aru-Watsa-Faradje-Aba; then, while one of us explored the district of Tombo, the other examined Dungu and its neighbourhood. We met again at Dungu to proceed to Doruma and examined independently the neighbouring villages. We returned through the mining district of Mongbwalu, Nizi and Kassenyi, where, thanks to the mines management, we were able to borrow a fishing-boat and visit rapidly the borders of the Kawa forest on Lake Albert. Apart from epidemiological researches proper, our aim was to collect human trypanosomes and cattle and game trypanosomes, to make observations on glossinæ and, possibly, to collect their flagellates with a view to subsequent laboratory work.

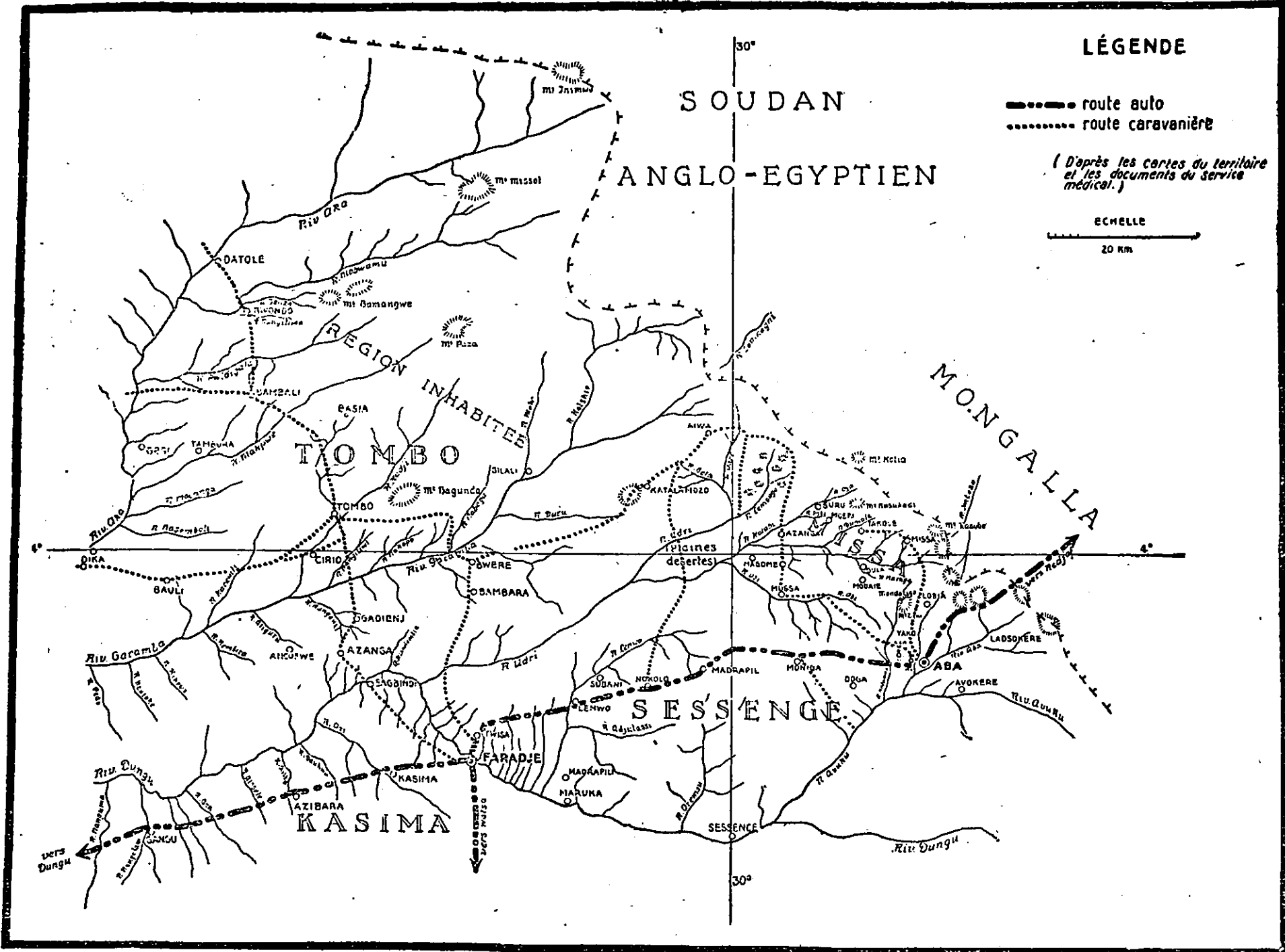
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The portion of the Upper Uele district which we visited consists mainly of large stretches of more or less wooded savanna traversed by innumerable forest belts. Here and there are small open spaces in the savanna where the grass is short and where the outcrops of rock carry a soil which is poor in humus. The elephant grass or big "matete" which grows over large areas in Uganda only appears in a few places and grows sparsely in the area through which we travelled.

Nearly everywhere the soil is rocky and formed of laterite, limonite and other iron ores, which provide excellent material for the good roads which are being rapidly constructed through the whole district.

The whole area is intersected by innumerable watercourses (see maps on pages 349 and 350), ranging from minute streams to fine rivers several meters wide which are tributaries of the Uele, the Kibali and the Dungu. The rivulets run through thick forest throughout, while the wider rivers are often simply lined with a fringe of low scrub of varying density. During the dry season, which lasts from December to March, the smallest affluents of this river system dry up to a considerable distance from the source; but, even near the source, water can be found at a depth of a few feet by digging in the bed of the stream.





SOUDAN  
ANGLO-EGYPTIEN

LÉGENDE

- route auto
- ..... route caravanère

(D'après les cartes du territoire  
et les documents du service  
médical.)

ECHELLE

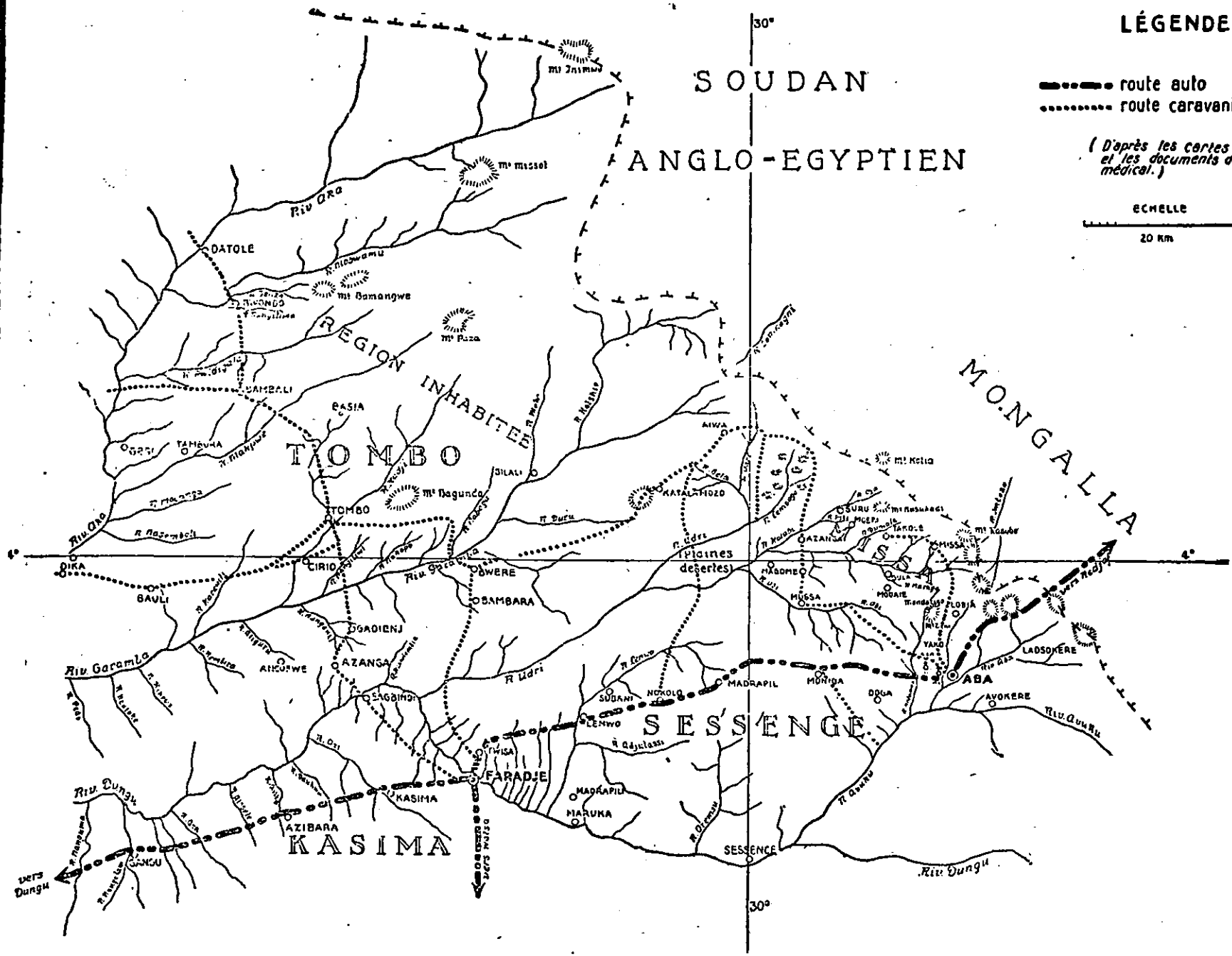
20 km

REGION INHABITEE  
TOMBO

SESENGE

KASIMA

MONGALLA



Generally speaking, the forest is confined to the belts which mark the water-courses, although there are a certain number of patches of forest in the intervals. The forest is usually intersected in all directions by the tracks of elephants and other game ; these tracks are sometimes very numerous.

In general, the area is slightly undulating, with here and there a short range of low hills, where the rocky outcrops are usually granitic. The country gradually rises in the direction of the Anglo-Egyptian Sudan, whose frontier practically coincides with the watersheds of the Congo and Nile basins. A system of wide and excellent roads has been constructed throughout the Uele and permits of an extensive motor traffic through Arua towards the east and towards Buta and Stanleyville, with a branch toward Niangara and to within a few miles of Doruma, connected on the one hand with the section of the royal road towards Aba and Redjaf, and on the other hand with the road system of the Kilo-Moto gold-mines and thence with Irumu and Lake Albert. This road system, serving both the mining and cotton-growing districts in a densely populated country, will play a great part in the campaign against sleeping-sickness.

In the infected zone, the dominant race is the Azande or Niam-Niam race. Their villages, as a rule, consist of groups of a few huts, often only one or two, provided with provision lofts and situated on the border of the forest belt which hides the river whence the inhabitants draw their water. It is rare to find a village more than a hundred meters from its water supply, which is nearly always provided by a forest stream.

On our return journey we crossed the mining district of northern Ituri. Its aspect is very different ; the fertile high plateaux are cut by deep valleys and great stretches of forest. At present the population, apart from the mine labourers, is very small, and sleeping-sickness does not exist. According to the natives, however, the population was formerly decimated by tribal wars and also by sleeping-sickness. Along Lake Albert, again, trypanosomiasis is slightly endemic, increasing towards the Mahagi focus in the north, and the big focus of the Semliki valley and Lake Edward in the south. But the high escarpment which borders on the lake forms a natural barrier against the propagation of the virus towards the Ituri river and the sources of the Uele's tributaries.

#### GLOSSINÆ.

*G. palpalis* is present practically all over the complicated system of the Uele rivers. Our visit took place during the dry season, or, more exactly, at the beginning of the first rains. Several rivulets were dry in their upper courses and everywhere the water level was low. Consequently, the glossinæ were absent from several water-courses ; but it is well known in the country that, during the rainy season, the tsetse go up to the sources of the smallest rivers. On the large rivers, such as the Kibali and the Dungu, the flies were more numerous, all being *G. palpalis*, a predominance of males being found in the experimental catches. On the streams, which were in the condition peculiar to the dry season, glossinæ were more difficult to find, and the percentage of females was higher, in one case reaching 70 per cent.

During this journey, we had the impression that a large part of the population is constantly exposed to *G. palpalis*, and particularly so during the rainy season. In addition to man, the flies have a large choice of other hosts, notably reptiles



and game. In the big rivers crocodiles abound all the year round, but monitors are less numerous. During the floods of the rainy season, the crocodile moves its habitat upstream. The Azande recognise two kinds of crocodile, one of which is reputed readily to attack man. We examined a stuffed specimen of a species which does not exist in Lake Victoria and which is probably *Crocodilus cataphractus*, a species which is common in all the rivers of the Congo basin. It is clear that crocodiles, and also monitors and perhaps the large snakes, constitute a very important item in the diet of the glossinæ. This has been confirmed by the dissection of flies captured in various localities in the course of our journey.

The glossinæ captured on the Uele, the Kibali and the Dungu, where the crocodile abounds all through the year, contained the *T. grayi* in the proportions of 13 and 22 per cent respectively. The dissection of the flies captured on the small watercourses, where at this period there were no crocodiles, revealed no flagellates.

It may thus be concluded that, on the large rivers, the glossinæ find abundant food apart from man. On the small streams, however, at least during the dry season, hosts are rarer, and it is precisely along these rivulets that the majority of the native villages are situated. In these circumstances, the glossinæ depend on man to a considerable degree, even if they are rarely numerous enough to be harassing.

Only one other species of glossina was seen, the *G. fusca* or *G. brevipalpis*, a single specimen of which was captured in a very dense forest on the fresh track of a bongo (*Bocconiscus isaaci*).

During the visit paid to the Kawa forest on the western bank of Lake Albert, we found *G. pallidipes* very abundant in the close vicinity of *G. palpalis*. Twenty-five of these flies were dissected, but no flagellates were found, either in the intestines or in the proboscis.

#### GAME.

We cannot speak of the game authoritatively after the rapid inspection we were able to make during the short time at our disposal. We found, however, that the Azande were experienced and, indeed, born hunters, and in this matter were subject to practically no restriction. They kill and eat any kind of game with equal zest, from the rat to the elephant. This systematic butchery can clearly not go on for long without having its effect, and we had the impression that game was rapidly growing scarcer, particularly the elephant, and that among these the survivors, which are continually being tracked, lead a precarious existence which they only prolong by constant vigilance and thanks to the cover in which they hide. These habits of the Azande, while being seriously detrimental to the preservation of valuable game, considerably increase the opportunities of contact with glossinæ in the forest belts.

The animals whose existence we observed were the elephant, the white rhinoceros, the hippopotamus, the buffalo, the giraffe, the river-hog and the wart-hog, the water-buck, bush-buck, hartebeeste, bongo (*Bocconiscus*), and cob, a large unknown species of forest cephalopoid and the "oribi". Of these the elephant, buffalo, bush-buck, water-buck, hartebeeste, cob and wild pig are widely distributed throughout the whole area. These species certainly play a part, although probably a small one, in the diet of *G. palpalis*. As far as we saw, the principal hosts of the tsetse in the Upper Uele, apart from man, in the circumstances we have described, are crocodiles, wild pigs and the hippopotamus.

TRYPANOSOMIASIS IN THE UPPER UELE.

The following notes are largely based on the documents of the M.M.S.U., as we concentrated our own attention on a few parts of the endemic zone which appeared to us the most interesting. This area is divided into six foci, which, as we said above, are connected by the routes along which the disease is propagated.

1. *Aba-Faradje Focus.* — It is not certain if this is a true focus. Since 1916, cases of trypanosomiasis have been diagnosed; but, apart from a few exceptions, it was easy to prove that they were imported cases. Up to December 31st, 1926, 65 cases of sleeping-sickness were known. The treatment given at first was atoxyl and emetic and, more recently, tryparsamide. Of these 65 cases, there are now 38 survivors, only 7 of whom show an infected cerebro-spinal fluid, and the 31 others, or 50 per cent, may be considered as cured. The proportions by sexes are as follows :

	Per cent
Men . . . . .	82
Women. . . . .	15
Male children. . . . .	3
Female children. . . . .	0

These proportions confirm the hypothesis that the majority of the cases are imported. Moreover, the greater part were found along the main lines of communication, or in the trading centres.

We ourselves visited the Missa district, between Aba and the Sudan frontier, and did not find the slightest trace of sleeping-sickness among the natives.

2. *Dungu focus.* — The wave of infection mounted the course of the Kibali and attacked certain districts situated to the south of the river, at the Nakassa rapids, and spread among the Bakango, who are the specialised fishermen and paddlers of the river. More recently a number of other cases were diagnosed in the districts of the northern Kibali, notably in those of Gilima and Wando. Among these were a certain number of cases imported from the west, *i.e.*, from the Doruma district. Lastly, to the east, there was the big focus of Vankerkhoveville, where sporadic cases still occur in spite of the removal of the population to other sites.

Generally speaking, the progress of the infection from west to east has been very evident.

In 1925, an examination of the population in the vicinity of the Kibali showed 20 cases among 3,825 natives examined, or 0.44 per cent. In 1924, this proportion was only 0.01 per cent. In the course of 1926, the enquiry was extended much further; but among 26,901 persons examined, only 9 new cases were diagnosed. It may therefore almost be said that trypanosomiasis is exceptional. Although the rivers are all infested with glossinæ, and the natives are frequently exposed to their bites, and in spite of the frequent introduction of the virus from Niangara and Doruma, the infection shows no tendency to spread.

The cases diagnosed in this focus since 1915 may be classified as follows :

	Per cent
Men . . . . .	63.2
Women. . . . .	13.2
Male children. . . . .	23.6
Female children. . . . .	0
Advanced cases. . . . .	52.6
Medium cases. . . . .	34.2
Recent cases. . . . .	13.2

Statistics of March 1927 :

Living . . . . .	73.7
Deceased. . . . .	26.3

Apparently cured, having a normal cerebro-spinal fluid after intensive treatment : 27 per cent.

The above figures show that the type of infection prevailing at Dungu is not benign. The following is an additional example :

*Observation 1.* — *Borugba*, boy aged 13. Azande, chief Gilima. Diagnosed on August 19th, 1925, by gland puncture. Treatment from August 19th, 1925, to October 24th, 1925 :

- 3 injections of 0.50 gramme of atoxyl ;
- 7 injections of 0.75 gramme of atoxyl ;
- 9 injections of 0.07 or 0.08 gramme of emetic of potash.

The cerebro-spinal fluid, which was nearly normal at the beginning of the treatment, showed 37 lymphocytes per cubic mm. on April 26th, 1926, and 15 lymphocytes on November 19th, 1926. At this date, there was a relapse with presence of trypanosomes in the glandular lymph and aggravation of the clinical symptoms<sup>1</sup>.

As this observation shows, there are strains of trypanosomes in this focus which offer a strong resistance to therapeutical measures.

The map on page 349 shows the distribution of patients, and indicates the zones which are at present regarded as infectious. It is clear that the river Kibali is the most dangerous point of this territory, owing to the number of glossinæ and the existence of small but definite foci along its banks. Fortunately, however, these are extremely thinly populated, a fact which has so far prevented the appearance of infection on a large scale. On the other hand, the existence of large gold-bearing deposits has raised the question of establishing prospecting camps and, later, mining camps along the banks, which would involve the presence there of large numbers of workmen constantly exposed to infection. We took this opportunity of making a prophylactic experiment with "Bayer 205", and we shall give later the details of this experiment, which is at present still incomplete.

In the course of our visit to the Dungu sector, we were obliged to confine ourselves to the examination of few groups of native working in the mining camps, Bakango of the Uele and Kibali, school-children of the mission, etc., without being able to discover a single new case. Shortly before our visit, the neighbouring districts had been visited by Dr. BERARDI and Father MOREAU with the same result. The last patients observed in 1926-27 deserve to be mentioned on account of the probable place where they were infected and the large proportion of advanced and serious cases.

<sup>1</sup> From the notes of Dr. L. STRADA, former chief of the M.M.S.U.

Name	Sex	Age	District	Infected at	Clinical condition according to spinal puncture.
Bendele . . .	M.	12	Biodi	South of Kibali	Advanced
n'Zolo. . .	M.	10	Wando	River Kibali	Hopeless
Gumbari . .	M.	45	Wando	River Dungu	Middle stage
Missa . . .	M.	32	Biodi	River Kibali	Advanced
Letamisatu	M.	32	(Bakango) <sup>1</sup>	River Kibali	Recent case
Zombo	M.	27	(Bakango)	River Kibali	Recent case
Kambili. . .	M.	25	(Bakango)	River Kibali	Recent case
Wilipanga	F.	22	Wando	River Kibali	Middle stage
Zikundrua	M.	15	Wando	River Kibali	Advanced
Papaye . . .	M.	25	Biodi	River Kibali	Advanced
Mapunga	M.	24	(foreigner)	River Dungu	Advanced *

A small quantity of live-stock has been introduced at Dungu and thence into some of the neighbouring districts. We selected for examination a small herd of cattle belonging to the Catholic Mission and watered daily at a place on the Kibali river, where we found the density of glossinæ to be 12 flies per boy-hour. About 23 per cent of the animals were found at the first examination to be infected by *T. vivax* and *T. congolense-dimorphon*. This cattle came from the district of Lugware and Walogo and, despite this considerable infection, the mortality in the last few years has not been great.

3. *Niangara focus*. — This is formed by the survival of old foci strung out along the Uele from Niangara to Surongo. A few sporadic cases are found, particularly to the south of the river. This is due to the fact that, although the riparian population has been moved and settled at a distance from the banks thickly infected with glossinæ, some of them continue to frequent the former plantations in the forest belts or to carry on illicit fishing in the river. It is interesting to note that, to the south of this focus, the river Bomokandi, although thickly infested with glossinæ, continues to be free from infection.

A large proportion of the patients are Bakango and Barambo, who are professional fishermen.

In 1924, there were 0.43 per cent of cases among the population.

In 1925, out of 25,083 persons visited, there were 77 patients, or about 0.33 per cent.

In 1926, out of 32,959 persons visited, there were 93 patients, or 0.30 per cent.

4. *Amadi focus*. — This has been a focus chiefly since 1925, when 10 new cases were suddenly discovered. Infection was caused by the trade relations of the natives with Dakwa, Doruma and Niangara. It is very probable that all these cases were imported, or the infection contracted elsewhere, particularly in the case of the natives employed

<sup>1</sup> The Bakango seem to be a very different race from the Azande, with whom they live on good terms. Their language is different. Their chief occupations are fishing, paddling and water transport, and pottery. They represent here the Bakenia of Lake Victoria and of the small Kavirondo lakes.

<sup>2</sup> These data are taken from the notes of Dr. BERARDI, of the M.M.S.U. at Dungu.

in the portorage of cotton, among other articles. Since 1926, no new case has been reported. Motor roads are now being completed which will eliminate the dangers involved in portorage and make it easy to control native traffic.

5. *Bangaro focus*. — This focus is the most infected of the Upper Uele, and also that on which the efforts of the medical authorities have been concentrated. In 1924, 3.59 per cent of the population was infected with trypanosomiasis. In 1925, out of 28,082 natives examined, there were only 470 patients, or 1.44 per cent. In 1925, in two districts, Gatanga and Bokoyo, 1,557 patients were undergoing regular treatment with atoxyl or tryparsamide. A frontier zone 10 kilometers wide was evacuated on the Bomu river and on the Bahr-el-Ghazal frontier. The population was evacuated from this zone and other places which were too much infected, and concentrated as much as possible in villages established along the main roads, these being carefully cleared of scrub at the crossings over the innumerable watercourses.

In 1926, the number of patients undergoing treatment was 1,604. During the year, 223 new cases were diagnosed, against 470 the previous year. The total number of patients counted in the most infected districts from 1919 to December 1926 was as follows :

Bokoyo. . . . .	345	out of about	1,000	taxpayers ;
Kiluele. . . . .	1,170	out of about	7,000	taxpayers ;
Yapwati . . . . .	646	out of about	5,000	taxpayers.

The M.M.S.U. reports having followed the progress of the disease from the district of Gatanga towards Doruma, *i.e.*, from west to east. The situation at the end of 1926 was : out of 35,043 persons examined, 223 new cases, or 1.30 per cent.

6. *Doruma focus*. — This focus extends chiefly along the Gurba, Bwere, Nasa, Hoko and Nabaranga rivers and in the districts of Boeli, Adalla, Dika and Bafuka, the latter being on the border of the endemic area of Dungu.

In 1924, the percentage of cases fluctuated in these districts between 0.30 and 0.83 per cent.

In 1925, out of 26,900 natives examined, there were 96 new cases, or 0.35 per cent.

In 1926, out of 33,632 persons examined, there were 104 new cases, giving a proportion of 0.31 per cent.

This focus, though not as important as that of Bangaro, nevertheless constitutes a danger for the Amadi districts and the Dungu territory, with which trade relations are maintained on a scale increasing with the development of the cotton plantations.

During our visit to the Doruma neighbourhood we examined a certain number of natives within the area of this focus and that of Bangaro, particularly in the village of Basangoda, the residents of Doruma, workmen, prisoners, members of the Mission, etc., the labourers on the Amadi road, the village of discharged convicts, and some of the natives of the Yapwati, Bendiepwa, Likita and Dika, etc., districts, giving a total of nearly 2,500 natives.

Only the following three new cases were observed :

*Observation 2.* — *Mayani*, a man aged 25. Chief Yapwati, probably infected on the Gurba river near the confluence of the Nasa. Diagnosed by gland puncture on April 1st, 1927. C.S.F. : nearly normal : 6 lymphocytes per cubic mm.

*Observation 3.* — *Duzungupio*, a child aged 8. District of Bendiepwa. Has already an old infection probably contracted on the Nasa river. Diagnosed on April 8th, 1927, by gland puncture. C.S.F. : affected : 415 lymphocytes per cubic mm, large mononuclears and muriform cells.







*Observation 4.* — *Mabele*, a man aged 30. District of Bendiepwa. Is clinically an advanced case. Diagnosed on April 12th, 1927, by gland puncture. The spinal puncture was not done by us, this patient having been sent to the Doruma lazaret.

\* \* \*

The general situation as regards sleeping-sickness in the Upper Uele may be gathered from the following figures :

In 1924, the proportion of patients to the total number of natives examined in the whole area was 0.50 per cent.

In 1925, out of 108,810 natives examined, there were 680 cases of trypanosomiasis, making only 0.46 per cent, although the census was much more thorough.

In 1926, out of 130,882 natives examined, 432 patients were discovered, which brings the percentage down to 0.30 per cent.

As will be seen, the efforts of the Medical Service have been crowned with success, and the disease is abating.

In the whole zone, the population of which a census was taken by the Administration numbers about 247,598 natives, among whom there were the following cases in December 1926.

Old Cases (1919 to 1925)					New Cases ; 1926					Deaths in 1926	Apparent Cures (tested by spinal puncture)
Men	Women	Boys	Girls	Infants	Men	Women	Boys	Girls	Infants		
989	800	261	142	4	202	137	56	37	0	212 or 8%	522 or 19.8%
Total : 2,196					Total : 432						
Grand Total : 2,628											

The map facing this page shows the extreme dispersion of the cases located by us in the districts of Gatanga, Kiluele, Yapwati and Kinurussa in the sector of Doruma and Bangaro. Except in Gatanga, it may be said that trypanosomiasis has taken the form of an endemic making slow but sure progress, and displaying little tendency towards sudden general outbreaks. In a previous note, one of us<sup>1</sup> drew attention to the high percentage of patients whose spinal fluid was affected whenever the disease was in an attenuated endemic state, and in this case also we found the following proportions<sup>2</sup> in the Doruma neighbourhood :

Out of 57 patients { modified fluid . . . . . 86%  
normal fluid . . . . . 14%

<sup>1</sup> L. VAN HOOF, *Enquêtes épidémiologiques au Budama et au Kavirondo*, 1926.

<sup>2</sup> During the journey to and from Uganda, we observed two other cases of sleeping-sickness in the mining districts. The first had been diagnosed at Watsa by Dr. CALLEWAERT and, from the clinical point of view, was a case of medium gravity. This patient had become infected at the old focus at Vankerkhoveville. The second is a particularly interesting case. Diagnosed in 1926 by Dr. MONCAREY, this patient, a woman, had a relapse after a prolonged and careful treatment with germanine, soamine and stibyl. We injected the blood under the skin and into the peritoneum of a guinea-pig, and the latter displayed a trypanosome which, both by its morphology and its pathogenicity among laboratory animals, must be classed as *T. rhodesiense*. This case will be dealt with elsewhere, and we propose to institute an enquiry as to the origin of this virus.

It is important to note the causes of this improvement. In our opinion, the causes are as follows :

1. *As complete as possible a Census of the Natives.* — In doing this, great help was afforded by the Administration, but a numerous trained medical staff, provided with administrative and judicial powers, was also necessary. The chief medical officer of the Upper Uele zone is a deputy judge, and the European agents under his orders are police officers. Moreover, individual medical passports and, in the case of taxpayers, the identity books containing them are compulsory, and it must be admitted that without this measure the control of the population would be impossible.

2. *Chemical Prophylaxis.* — Formerly, the regulation treatment was the administration of atoxyl and emetic. At present, tryparsamide and Meuricè tryponarsil are being generally introduced. All treatments are tested by analysis of the cerebro-spinal fluid. In 1926, 2,649 spinal punctures were made, of which 57.19 per cent gave a normal fluid, and related either to patients apparently cured after a prolonged treatment or to cases believed to be recent and having less than 3 cells per cubic mm. The 42.81 per cent of modified fluids related to advanced cases, and those whose fluid had not been brought back to a normal cytology by suitable treatment.

Among cases recently diagnosed, however, the example quoted above gives 14 per cent of normal fluids before treatment, and we may conclude that, by the therapeutical processes at present applied, about 43 per cent of advanced cases appear to be cured, and have a normal cerebro-spinal fluid. All these cases are not necessarily permanent cures, but the peripheral blood has been sterilised, and the patient can return to work and social life.

3. *Creation of a Road System,* with a view to the abolition of portage, while permitting the development of cotton-growing and easy control of traffic.

4. *Prohibition of Certain Areas* particularly infected or too difficult to keep under observation, such as the frontiers, and settlement of the population thus removed in villages established along the main roads which follow the heights and keep the natives in the vicinity of the cotton-growing and trading centres.

#### EXPERIMENT WITH PREVENTIVE "BAYER 205".

As stated above, the endemic zone of Dungu comprises a dangerous focus : the Kibali river. This broad and swift river flows between wooded banks infested with *G. palpalis*. The country along its banks is practically uninhabited, with the exception of the villages of Dungu, Nakassa, of a few isolated natives and the Bakango. The latter, having been employed to transport food over long distances in their canoes, became rapidly infected with trypanosomiasis, and this work had to be stopped. At present, they only work within a limited radius and under immediate supervision, and engage in fishing, gathering river oysters and conveying travellers across the river. This has been enough to put a stop to the infection.

At the end of 1925, the management of the Kilo-Moto mines expressed the intention of establishing prospecting camps in the thick of the infected zone on the Kibali and on the banks above the mouth of the Yebu. We advised the Government to authorise this installation, on condition that the workmen, and even the European overseers, should be subjected to an experiment on the preventive action of germanine, the details of which were settled at the beginning of 1926. The governmental medical staff and that of the gold-mining company undertook to carry out this experiment,



and we would take this opportunity of thanking Dr. CALLEWAERT and Dr. BERARDI, whose data we mainly reproduce.

In 1926, four cases of trypanosomiasis were found among the Bakango carrying provisions between Dungu and Yebu. These men, who were natives of non-infected districts, carried medical passports containing records of numerous negative examinations before they had been employed in this work. This fact, taken in conjunction with the known existence of small active foci, justifies the conclusion that the glossina of the Kibali can convey infection.

For the purposes of the experiment in question, the supervision of the natives was particularly easy. Two prospecting camps were to be opened in succession at a short distance from one another. In each camp there were regular workmen engaged for a long period and temporary workmen engaged for periods of from three to six months. The foreign virus could only be introduced by the visits of natives from outside. The camps were situated at a distance from any village and food was only brought in once a week during a period of one hour and under supervision. In a short time, supplies will be brought by motor-lorry.

The experiment was begun on November 15th, 1926. At the preliminary examination, even those whose glands proved negative were segregated, as well as the natives who were not sufficiently robust or who did not bear the first preventive injection of 0.50 gramme of germanine sufficiently well. The preventive cure was concluded at 1.50 grammes per workman. Two Europeans, two children and 33 women and servants were subjected to the same treatment, at about 2 centigrammes of germanine per kilogramme. The temporary workmen, numbering 48 at the outset, received no treatment and were used as controls.

After a month of observation the 12 fresh cases of enlarged glands were found among the controls, but the results of puncture, triple centrifuging of the blood and spinal puncture did not justify a diagnosis of trypanosomiasis.

The observation of these controls has been continued in this way on the arrival or departure of all temporary workmen. After periods varying from three to six months, the preventive injection is repeated, either on individuals already under observation, or on new recruits, which will make it possible to judge the duration of the preventive action of this product. The experiment will shortly be extended to other camps which are to be set up on the Uele, in the endemic zone of Niangara.

So far, the result of the experiment has been entirely negative. No case has been discovered either among those injected or among the controls. Mention must be made, however, of the accidental presence during one day of an infected person from Tora. This native, who had not been treated, was diagnosed a few days later by Dr. CALLEWAERT at Watsa, and we had an opportunity of examining him. He might have introduced the infection into the camp, and this fact must be borne in mind. The second camp has received no suspect visitors.

This experiment may serve to establish two facts :

1. That a fly infected in the salivary glands is capable of infecting man, which seems to us to be the most frequent method of infection in the zones we have visited.
2. How far "Bayer 205" is preventive. At present, the results obtained by VAN DEN BRANDEN<sup>1</sup> and the experiments made by KLEINE, and later by one of ourselves at the Entebbe laboratory<sup>2</sup>, are contradictory and we fear that the duration of the

<sup>1</sup> F. VAN DEN BRANDEN, *Annales de la Société belge de médecine tropicale*, 1926, Vol. I.

<sup>2</sup> Interim Report of the League of Nations International Commission on Human Trypanosomiasis (document C.H.536), page 35. F. K. KLEINE and W. FISCHER, 1922-23, *Deut. Med. Woch.*, Vols. 48 and 49.

preventive action of germanine has been exaggerated. From the experiments in progress, which in our opinion will have to be continued for several years, we hope to obtain conclusive results.

On a first visit to the mining camps on the Kibali, we found that the conditions of labour at the banka-drills, in the workshops and on the plantations frequently and daily exposed practically all the natives to the bites of glossinæ. The policy of the mines management is to isolate these mining camps to an increasing extent from the native settlements and the neighbouring foci. The personnel employed is being increased and, when operations begin, will be very numerous, so that the prospects of success of these experiments will be correspondingly greater.

### BIOLOGICAL ASPECTS OF THE TRYPANOSOMIC ENDEMIC IN THE UPPER UELE.

Short though our tour was, we were able to collect sufficient observations and information to form an opinion on the biological problem of trypanosomiasis in this area.

1. In districts such as Missa and Tombo, in the neighbourhood of Aba and Faradje, there can be no doubt that the number of glossinæ existing near the rivers, and the present density of the population, explain the persistence of an endemic once the virus has been introduced. Despite this fact, and the numerous examples of the introduction of virus by the importation of infected persons from outside, no one can affirm that at the present time it is possible to contract the disease there. As we have said, we did not discover any patients there, and the majority of the patients previously found in these districts had been infected elsewhere.

2. In the sectors where it is really possible to contract sleeping-sickness, and which we regard as actually infected, we observed the extremely sporadic nature of the disease. The map of the Doruma-Bangaro sector (between pages 356 and 357) shows sufficiently clearly the dispersion of the cases throughout a number of adjacent districts. This sporadic character is related to the following facts : (a) the Azande do not live in compact groups ; (b) the watercourses are innumerable and, as a general rule, are all infested with glossinæ.

3. Three facts in connection with the trypanosome allow us to estimate its virulence :

(a) Evolution of the disease. If we read the observations of the doctors of the M.M.S.U., we find that there is no appreciable difference between the evolution of trypanosomiasis in the Upper Uele and on the shores of Lake Victoria ; for instance, as regards the duration of life, the stage at which nervous symptoms appear, and the rarity or non-existence of spontaneous cures.

(b) Efficacy of treatment. We repeat that the use of tryparsamide and tryponarsyl is becoming general. Previously, atoxyl was the usual remedy, applied by series of ten weekly injections of a gramme accompanied or not by emetic of potash. The present tryparsamide treatments are of 20 to 24 grammes by weekly injections of 2 to 3 grammes. The spinal puncture at the end of the course shows whether a resumption of the treatment will be necessary. We have indicated above the results obtained in 1926. We may also refer to the observations published by STRADA AND LOPES<sup>1</sup>. From all the facts available, it appears that, in the case of nearly 43 per cent

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<sup>1</sup> STRADA AND LOPES, *Annales de la Société belge de Médecine tropicale*, 1926, Vol. I.

of the patients, the prognosis was still doubtful at the end of 1926, which hardly suggests an attenuated virus.

(c) Lastly, when we visited the Doruma lazaret, we examined a certain number of chronic and incurable cases. These hospital patients have all undergone a very full treatment. Certain of them have been under treatment for several years. Some of them are blind, demented, lethargic or imbecile. We chose twelve of these patients and made a spinal puncture. An analysis of the fluid quite frequently showed a nearly normal cytology among persons clinically in a desperate state and in whom the treatment brings no improvement but simply prolongs life until pneumonia or some other intercurrent disease carries them off. We gained the impression that, even if the trypanosome and with it the inflammatory reaction of the meninges had disappeared, it had left behind it deep and irreparable nervous lesions.

Without going so far as to say that the trypanosome of the Upper Uele is a strong virus, we may nevertheless conclude that it is comparable with that of Lake Victoria and perhaps even with that of the Semliki valley.

4. Distribution of glossinæ. We have given our observations above. To sum up, *G. palpalis* is perhaps the only carrier. *G. morsitans* has been reported several times in the Uele districts. RODHAIN<sup>1</sup> met it several times in 1914 along the Sudan frontier in the neighbourhood of Doruma, Yakuluku and Aba. Perhaps owing to the season, our exploration of the sources of the Gurba and of the Oya river gave no results. Moreover, the considerable diminution in game may perhaps have already modified in thirteen years the distribution of this glossina.

*G. palpalis* itself does not abound, but, as we have said, it is probably present in small numbers all over the inextricable network of rivers. Certain figures concerning the catches made will give an idea of its prevalence :

- River Oya, nearly dry, first rains, March 19th, 1927, 1 boy, 1 hour : 1 male, 3 females.
- River Aba, March 18th, 1927, 1 boy, 1 hour : 2 males, 4 females.
- River Aba, March 19th, 1927, 1 boy, 1 hour : 2 males, 5 females.
- River Kibali, Dungu Mission, 1 boy, 1 hour : 7 males, 5 females.
- Doruma, river Gurba, outside the scrub-clearing area, April 7th, 1927, 1 boy, 1 hour : 12 males, 4 females.
- Doruma, river Gurba, in the deforestation area, April 8th, 1927, 1 boy, 1 hour : 8 males, 8 females.
- Doruma, river Nasa, April 9th, 1927, 1 boy, 1 hour : 5 males, 4 females.

Other catches were made on the Dungu, the Uele, the Garamba, the Bwere and several lesser streams with results generally in proportion to the volume of water in the rivers.

We have explained above the relations of the flies to the hosts on which they feed.

On the basis of these data, the situation from the biological point of view may be summarised as follows :

1. In the Upper Uele, there is a virus of average strength.
2. This virus is distributed in a very sporadic manner.
3. *G. palpalis* are everywhere, but in small numbers.
4. The natives themselves are everywhere, but never in compact settlements.

If we now consider :

1. The small percentage of flies whose salivary glands become infected ;

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<sup>1</sup> J. RODHAIN, *Bulletin de la Société pathologique exotique*, 1916, page 38.

2. The percentage of natives carrying virus (local maximum about 7 per cent : general maximum 0.3 per cent) ;

3. The percentage of meals made by the glossinæ on man in comparison with other hosts (the percentage of intestinal infections by *T. grayi* gives us some information on this point) ;

4. The probable percentage of strains cyclically not transmissible ; there is no likelihood of sleeping-sickness spreading to any considerable extent, at least in the near future and in existing circumstances.

Moreover, the explanation of the persistence of the present endemic by the accidental occurrence of rare glossinæ infected in the salivary glands is the most satisfactory one ; mechanical transmission could not be reconciled with the sporadic nature and moderate extent of distribution. All this also tallies with the slowness and difficulty with which the endemic spreads to zones hitherto unaffected.

#### CONCLUSIONS.

1. The trypanosomiasis endemic in the Upper Uele is of long standing, and the conditions in which it exists do not allow it to increase to any great extent or give rise to an epidemic extension. It does not tend to spread rapidly in the neighbouring territories. It arises chiefly from one virus, *T. gambiense*, of medium strength, transmitted by *G. palpalis*.

2. Of the prophylactic measures taken against this endemic, two must be recognised as of greater value than all the others, viz., the careful census of patients and their methodical treatment with a view to the sterilisation of the virus reservoir. The settlement of the native communities in villages situated in healthy localities and the supervision of their movements are also of importance. Direct action against the carrier insect is practically impossible in this area. It must be confined to a few judiciously selected places and the work must be thoroughly done and constantly renewed. The evacuation of the neighbourhood of the frontier is in the present case an excellent measure, on condition that, as in this instance, an agreement exists between the medical and administrative services of the two adjacent colonies, making it possible to standardise the methods applied and to prevent the emigration and flight of patients.

3. It is probable that the virus is preserved in this endemic area by glossinæ whose salivary glands are infected. This shows the danger of an individual carrying the virus coming into an uninfected district, and demonstrates the prophylactic importance of controlling the movements of the natives, whether porters, workmen or traders, hunters or isolated travellers.

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## EPIDEMIOLOGY OF SLEEPING-SICKNESS IN BUDAMA AND KAVIRONDO

*Additional Notes and Various Observations regarding Treatment.*

BY DR. L. VAN HOOF.

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During my tour, which lasted a month, I was able rapidly to revisit the areas infected with sleeping-sickness in which an epidemiological enquiry was carried out in 1926. Samia, Kavirondo and the endemic zone of Busitema presented greater interest than the more distant districts of Bunyuli and Bugweri, where the endemic appears to have died out. The principal object of my journey was to revisit the most active foci of trypanosomiasis, to ascertain the effects produced by chemical prophylaxis over a period of one year, to terminate or continue the observation of patients, to record the results obtained by treatment with various medicaments, and finally to check, at a year's interval, the main conclusions of the previous report.

The testing of the therapeutic value of medicaments does not come within the scope of an epidemiological study. It was, however, impossible not to take an interest in the treatment of patients, primarily out of feelings of humanity but also out of a desire to ascertain the action of known or untried remedies on the different strains of human trypanosomes of varying virulence found in Budama and Kavirondo. I had already on a previous occasion employed the action of medicaments as a standard for estimating the virulence of a trypanosome strain.

A number of products were at the Commission's disposal. Apart from tests with tryparsamide, we had to be content with rapid, though adequate, observations in order to discover whether it would be worth while to undertake a full investigation later. Accordingly, tests of the speed of sterilisation and the duration of sterilisation by means of a single dose were generally neglected. A very large number of patients would have been required, and would have had to be accommodated in such a way as to permit of their daily observation, but this was inconsistent with the business of an epidemiological enquiry. Accordingly, the continuance of the treatment was left to other persons, sometimes even to native hospital orderlies, who interrupted or prolonged it unduly, although they had instructions in writing.

### I. MORBIDITY AND MORTALITY DUE TO TRYPANOSOMIASIS.

The shores of Berkeley Bay have been described as an important focus of trypanosomiasis. In the course of the first enquiry, February to October 1926, the population was carefully examined, and all patients diagnosed were either then or previously placed under observation and given treatment.

During this visit of inspection, fifteen new cases were discovered and classified as follows :

Advanced cases . . . . .	10
Cases in the middle stage. . . . .	3
Recent cases . . . . .	2
Examined at first enquiry. . . . .	6
Not examined at first enquiry. . . . .	9

The latter belong chiefly to villages in the Kavirondo district, outside the range of our enquiry. Others are advanced cases whom the natives were afraid to show us on our first trip, and whom they now brought to us in a desperate condition. Among the patients examined and found negative in 1926 are the recent cases and those in the middle stage. It will be seen that the methods of diagnosis employed left only a small margin of error. Moreover, with regard to the 34 middle-stage and recent cases diagnosed in 1926, we found not more than 5 which have undoubtedly become infected since the last censuses. This fact speaks well for chemical prophylaxis. It must be borne in mind, however, that our last tour was very short and that it would have been impossible for us to undertake a second systematic examination of all the natives.

On the basis of the fresh information obtained, the morbidity percentage tables must be modified as follows :

	Per cent
Mjanji . . . . .	3.7
Dadira. . . . .	12.8
Lumino. . . . .	0.7
Jinja. . . . .	1.7
Lunyo . . . . .	0.3

For the whole endemic area of Samia-Uganda, the percentage of infection is at present 2.1 per cent.

This figure should, however, be regarded as a minimum. Certain cases are undoubtedly still escaping our investigations, and there are also patients who, after becoming infected in the Mjanji district, have gone to live in villages in the neighbouring area, which is recognised as immune. A case of this kind was diagnosed at Masaba, and another at Busiko. The natives also brought us a woman whose clinical symptoms showed her to be a very advanced case of sleeping-sickness, microscopic examination and spinal puncture being no longer possible.

The morbidity percentages for Samia-Kavirondo, corrected according to the most recent diagnoses, are :

	Per cent
Odjuanga. . . . .	2.8
Busembe . . . . .	6
Okani . . . . .	0.5
Gugumaz. . . . .	0.55

making for the whole endemic area of Samia-Kavirondo a general percentage of 0.83.

In my previous report I pointed out that endemic trypanosomiasis in Busitema was considerably less grave than in Samia and, generally speaking, in the immediate vicinity of Lake Victoria. During our recent visit to this area, three new patients were placed under observation, one being a recent case and two very advanced. These cases belong to the villages of Buteba and Makima, and, as will be shown later by the observations upon them, it is doubtful whether they all became infected in Busitema.

As compared with the 11 new cases found in 1926, recent cases are rare, if not wholly absent. The morbidity percentages may be estimated as follows :

	Per cent
Buteba. . . . .	1.7
Makima . . . . .	2
Busitema as a whole. . . . .	0.5

The observation of sleeping-sickness cases in Samia and Busitema has thus been prolonged for a period of from nine to fourteen months. In order to give a better idea of the differences between these two areas, a comparative table is set out below, showing the progress of the respective cases during that time.

Progress of Patients	Samia-Uganda and Samia- Kavirondo	Busitema
	%	%
Apparent cure checked by analysis of cerebro-spinal fluid (now normal) and by the absence of clinical symptoms .	31.3	33.3
Improvement checked by analysis of cerebro-spinal fluid (nearly normal), or, failing spinal puncture, by the absence of trypanosomes and the disappearance of clinical symptoms at the end of treatment . . . . .	19.3	61.1
Unchanged . . . . .	2.4	0
Worse, checked clinically and by spinal puncture . . . . .	8.4	5.5
Dead. . . . .	21.7	0
No exact information. . . . .	16.9	0

It should also be borne in mind that the Samia area was much better served as regards the treatment of patients than Busitema, chiefly on account of the proximity of the Lumino dispensary, where tryparsamide treatment has been regularly continued, as in Kavirondo, by Dr. J. ENZER, who personally gave injections during his tours.

Mortality in the Mjanji district was very high, compared with figures in other endemic sleeping-sickness areas (for instance, 10 to 25 per cent in the Semliki valley, 8 per cent in Upper Uele). This high percentage is partly explained by the large proportion of very advanced cases, irregularity of treatment, and the number of relapses after insufficient treatment.

On the other hand, in the Busitema area, although treatment was often manifestly inadequate, it is anticipated that the mortality among all patients will not exceed 6 or 7 per cent in future. Mortality was nil ; there were apparent cures, and considerable

improvements after very irregular treatment, which confirms my opinion that this virus is becoming less powerful. The action of chemical agents on this trypanosome is far stronger than in Samia, as is shown by the following examples :

*Observation 63.* — Relapse after tryparsamide, diagnosed April 25th, 1926 ; approximate weight, 30 kgs., C.S.F. : 660 lymphocytes per cubic mm., large mononuclears and muriform cells.

Treatment :

May 1st, 1926 : 1 gm. germanine intravenously.

Subsequent observation :

June 25th, 1926 : gland puncture negative ; triple centrifuging of blood negative.

May 22nd, 1927 : disappearance of all nervous and mental symptoms ; spinal puncture : 10 lymphocytes per cubic mm.

*Observation 65.* — Diagnosed April 26th, 1926 ; agitated, euphoric, with 695 lymphocytes in the C.S.F., large mononuclears, muriform cells and hyperalbuminosis of 0.85 per mille.

Treatment :

April 26th, 1926 : 1 gm. tryparsamide.

April 29th, 1926 : 0.50 gm. germanine.

May 5th, 1926 : 2 gms. tryparsamide.

May 17th, 1926 : 3 gms. tryparsamide.

June 25th, 1926 : 1 gm. germanine.

Re-examined May 22nd, 1927 ; clinically, his state is perfect ; spinal puncture : 10 lymphocytes per cubic mm., one large mononuclear.

*Observation 77.* — Diagnosed May 6th, 1926 ; C.S.F. deteriorated : 122 lymphocytes per cubic mm., and large mononuclears ; weight approximately 55 kgs.

Treatment :

May 6th, 1926 : 0.60 gm. neosalvarsan (owing to secondary frambœsia).

May 10th, 1926 : 3 gms. tryparsamide.

Re-examined May 24th, 1927, relapse of frambœsia, but all clinical signs of trypanosomiasis had disappeared. Spinal puncture : 3 small lymphocytes per cubic mm.

More especially as regards Observations 63 and 77, it is obvious that, in districts where the virus is powerful or even only fairly powerful, as in Upper Uele, similar results cannot be expected.

These observations likewise confirm Conclusion No. 6 of the first report, with reference to the necessity of ascertaining the activity of a virus before judging of the therapeutic value of any medicament.

## II. OBSERVATIONS ON RECENTLY DIAGNOSED CASES.

The cases diagnosed during this tour were, as usual, questioned as to their occupations, residence, movements, etc., in order to discover the probable place of infection. All these new cases were traced to one or other of the glossina breeding-grounds which were found to be the danger-points of the district at the time of the first enquiry. Generally speaking, the view is now confirmed that the whole endemic zone is split up into secondary foci, few in number, limited in area, and in some cases with few tsetse. Some of these foci can be definitely delimited. Had time permitted, it would have been interesting to make a thorough examination of all the glossinæ in one of these breeding-grounds, where a case has been quite recently infected, in order to trace the fly responsible for the infection.

The village of Busembe (Kavirondo shore of Berkeley Bay) is a good example of these danger-points. It consists of a group of dwellings fairly near the shore, on the edge of a small forest, and with pasture-land adjoining. The forest covers a low hill ending at the shore in a cliff. *G. palpalis* is plentiful at this point ; the numbers caught were mentioned in the *Interim Report* (document C.H.536, page 58). The most frequented breeding-ground is at the boat-moorings. It is at the same point on the bank



that the people of the hamlet draw their water. Owing to its peculiar situation, this group of natives was placed under special observation in April 1926. Among some 50 natives examined, the following cases were discovered : Observations 46, 48, 54, 56, which were advanced cases ; 50 and 55, fairly grave. In September, a seventh case, No. 170, very serious, was found. In May 1927, these natives were collected, and one of the patients, *Mukoma*, Observation 56, brought us a woman he had bought some months previously in an inland village. The observation of this case was as follows :

*Observation 283.* — *Ahuma*, a woman aged 25, a native of Kilima, living at Busembe since December 1926. Occupations: household duties and field work only. She does not go fishing with her husband. She draws water from the neighbouring glossina breeding-ground. First probable symptoms at end of December; fever, cephalalgia, asthenia. Present condition, May 16th, 1927: trypanosomes in the glandular lymph and blood. Spinal puncture: 8.7 lymphocytes per cubic mm. No clinical symptoms.

This is the only recently infected case which has been diagnosed in this very limited area. Only two advanced cases were seen by Dr. J. ENZER in another part of the Busembe shore. These facts suggest that one or more glossinæ with infected salivary glands still remain in this breeding-ground and have accidentally caused this fresh infection.

We give below a few remarks on other cases discovered during this tour :

*Observation 271.* — *Adjambo*, a woman aged 30, a native of Mjanji, probably infected in the prohibited area. A grave case; C.S.F. contains 125 lymphocytes per cubic mm., large mononuclears and muriform cells.

*Observation 277.* — *Atshola*, a woman aged 35, a native of Mjanji, probably infected on the shore of the prohibited area. Clinical symptoms slight. Trypanosomes very plentiful in glandular lymph; C.S.F. contains 50 lymphocytes per cubic mm. and large mononuclears. A year ago, this patient was living in the high, healthy part of Busembe. The disease probably only dates back six or eight months.

*Observation 272.* — *Ekaka*, a woman aged 30. An advanced case, a native of Lumino, probably infected on the river Sio, near Sigalami.

*Observation 273.* — *Blasio Nababa*, a man aged 30. An advanced case, a native of Jinja, a fisherman in Berkeley Bay. C.S.F. contains 275 lymphocytes per cubic mm., large mononuclears and muriform cells.

*Observation 275.* — *Mugivi*, a child aged 6, son of the patient *Adeniy* (Observation 36), a native of Butangasi-Masaba. Recent infection, probably contracted when accompanying his father, who is a fisherman by occupation, on fishing expeditions on the lake. The spinal fluid contains only 22.5 lymphocytes per cubic mm., with large mononuclears. No clinical symptoms.

*Observation 276.* — *Opake*, a boy aged 12, lives at Busiko-Masafu; formerly lived at Sidonge on the river Sio, where he probably became infected.

*Observation 278.* — *Akuku*, a woman aged 30, a native of Dadira. A serious case, probably infected at the Sidonge ford fishing-grounds. Spinal puncture: 166 lymphocytes per cubic mm., large mononuclears and muriform cells.

*Observation 279.* — *Adjambo*, a girl aged 16, a native of Dadira. A desperate case, who was brought to us in a hammock on May 13th, 1927. This patient was treated for frambæsia, and recently received five injections of 0.30 gm. of neosalvarsan. Impossible to detect trypanosomes in the lymph or blood. Spinal puncture showed 290 lymphocytes per cubic mm., large mononuclears and muriform cells. Treatment was started with small doses of tryparsamide, and the patient, when re-examined on June 2nd, 1927, already showed considerable clinical improvement.

*Observation 280.* — *Uhoni*, a man aged 25, a native of Lunyo. Condition fairly serious, infection recent, contracted at Mjanji, where he formerly lived. He has just undergone a course of treatment with tryparsamide.

*Observation 281.* — *Muganda*, a man aged 40, of Busembe. An advanced case, treated with tryparsamide and "Bayer 205".

*Observation 282.* — *Magali*, a man aged 30, of Busembe. An advanced case, treated with tryparsamide and "Bayer 205".

*Observation 284.* — *Nawire*, a woman aged 30, of Odjuanga. A desperate case, turned out by her husband and family; uncompensated heart lesion, cardiac liver and œdema.

*Observation 285.* — *Nondi*, a man aged 30, of Okani. An advanced case, treated with tryparsamide and "Bayer 205".

*Observation 286.* — *Akuonga*, a girl aged 10, a native of Gugumaz. Glands scarcely puncturable, but rich in trypanosomes. The fluid contains 182 lymphocytes per cubic mm., large mononuclears and muriform cells.

In the Busitema endemic area, three fresh cases were diagnosed. Details of these cases are as follows :

*Observation 274.* — *Juliana*, a woman aged 35, of Buteba, but infected at Mjanji, where she formerly lived. Recent infection; C.S.F. contains only 2 lymphocytes per cubic mm.

*Observation 287.* — *Apili*, a young man aged 17, of Buteba. An advanced case, who only returned to Buteba two months ago and probably became infected elsewhere. Previously resided at Jinja. Gland puncture positive. Spinal puncture: 317.5 lymphocytes per cubic mm., large mononuclears and muriform cells.

*Observation 288.* — *Petero Bui*, a man aged 25, of Makima. A very advanced case, probably infected in Uganda, where he has been working for several years on a plantation not far from Jinja. Clinically, he is a heavy sleeper, and fat. Gland puncture positive. Spinal puncture: fluid cloudy, 2,050 lymphocytes per cubic mm., large mononuclears, muriform cells and numerous trypanosomes.

Unfortunately, spinal puncture was not possible in all cases, some having been diagnosed before our arrival, and having already received sufficient tryparsamide treatment to make the results valueless.

### III. OBSERVATIONS ON THE ACTIVITY OF CERTAIN MEDICAMENTS.

As already stated, these observations were made simply as occasion arose, on persons who could not be left without treatment, and who, whenever possible, received the most careful treatment that circumstances permitted.

A. — *Tryparsamide* was used in preference to all other products, especially in advanced cases. Since 1920, this product has proved to VAN DEN BRANDEN at the Léopoldville Laboratory and to me its superiority for the treatment of patients whose cerebro-spinal fluid has greatly deteriorated, and in our first treatise we recorded a higher percentage of apparent cures of such cases in the Léopoldville area than has been obtained with any other product for the same virus<sup>1</sup>.

For comparison with the results of L. PIERCE<sup>2</sup>, CHESTERMAN<sup>3</sup>, JAMOT and his assistants<sup>4</sup>, KELLERSBERGER<sup>5</sup>, VAN DEN BRANDEN<sup>6</sup>, etc., I have summarised below, as briefly as possible, statistics showing the results obtained by me in respect only of the most active virus with which I have had to deal, namely, that of Samia and Kavirondo on the shores of Lake Victoria.

<sup>1</sup> VAN DEN BRANDEN AND VAN HOOF, 1923, *Bull. Soc. Path. Exot.*, No. 8.

<sup>2</sup> L. PIERCE, 1921, *Journ. Exper. Medic.*, December 1st, 1921, Supplement.

<sup>3</sup> C. CHESTERMAN, 1923, *Trans. Royal Soc. Prop. Med. Hyg.*, page 394, and, 1924, page 131.

<sup>4</sup> JAMOT, LE TONTURIER, DE MARQUEISSAC, 1924, *Bull. Soc. Path. Exot.*, page 692, 1926, No. 6.

<sup>5</sup> KELLERSBERGER, 1926, *Trans. Royal Soc. Trop. Medic. Hyg.*

<sup>6</sup> VAN DEN BRANDEN, 1925, *Bull. Soc. Path. Exot.*, page 645, and, 1926, No. 1.

RESULTS OF TRYPARSAMIDE TREATMENT  
(SAMIA AND KAVIRONDO VIRUS).

Observation No.	Age and Sex	Clinical aspect and analysis of cerebro-spinal fluid before treatment	Treatment by weekly intravenous injections of tryparsamide, and other treatment, if any	Clinical aspect and analysis of cerebro-spinal fluid after treatment	Progress, subsequent verification by tests for trypanosomes in the lymph and centrifuged blood. Remarks
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GROUP I. — *First Stage of Disease.*

6	m. 20	1.III.26 : relapse after tryparsamide or incubation of reinfection. C.S.F. : 1 lym. per cubic mm.	Halarsol, 0.20 gm. (for reactivation) Tryparsamide 10 injections of 2 gms.	18.VII.26 : normal. C.S.F. : 1.9 lym. per cubic mm.	Apparent cure. Tests 19. IX.26 and 12.V.27 : negative.
49	m. 30	16.IV.26 : normal. C.S.F. : 1.5 lym. per cubic mm. Massive infection of lym. and peripheral blood.	Neosalvarsan, 0.60 gm. (frambœsia) Tryparsamide 10 inj. of 3 gms.	22.VII.26 : unsatisfactory. C.S.F. : 20 lym. per cubic mm. 16.V.27 : normal. C.S.F. : 2 lym. per cubic mm.	Apparent cure. Had an inflammatory meningeal reaction at the end of the first treatment. The spinal fluid became normal without further treatment.
59	m. 40	19.IV.26 : normal. C.S.F. : 2 lym. per cubic mm.	2 inj. of 1.50 gms. 7 inj. of 3 gms.	22.VIII.26 : normal. C.S.F. : 0.6 lym. per cubic mm.	Apparent cure. Test 16. V.27 : negative.
9	f. 45	1.III.26 : normal. C.S.F. : 2.5 lym. per cubic mm.	1 inj. of 2 gms. 6 inj. of 3 gms.	18.IX.26 : normal. C.S.F. : 0.7 lym. per cubic mm.	Apparent cure. Tests 1. III.27 and 25.IV.27 : negative.

GROUP II. — *Cases in the Middle Stage.*

7	f. 14	1.III.26 : relapse after 2 inj. of 0.30 gm. novarsen. C.S.F. : 6 lym. per cubic mm. ; one large mononuclear.	2 inj. 0.20 gm. Halarsol excitant 2 inj. of 1 gm. trypars. 5 inj. of 2 gms. trypars.	17.VII.26 : normal. C.S.F. : 0.3 lym. per cubic mm.	Apparent cure. Tests 16. III.27 and 12.V.27 : negative.
5	m. 35	1.III.26 : treated since 1922 with neosalv. and trypars. but suspect despite absence of trypan. C.S.F. : 7.5 lym. per cubic mm.	2 inj. 0.20 gm. Halarsol excitant 2 inj. of 3 gr. trypars.	18.IX.26 : normal. C.S.F. : normal.	Apparent cure. Test 12. V.27 : negative.
50	f. 40	17.IV.26 : no definite symptom. C.S.F. : 7.5 lym. per cubic mm.	1 inj. of 2 gms. 9 inj. of 3 gms.	21.VII.26 : normal. C.S.F. : 0.3 lym. per cubic mm.	Apparent cure.
4	m. 50	1.III.26 : normal. C.S.F. : 8.1 lym. per cubic mm.	1 inj. of 2 gms. 11 inj. of 3 gms.	17.VII.26 : normal. C.S.F. : 1.9 lym. per cubic mm.	Apparent cure. Test 12. V.27 : negative.

Observation No.	Age and Sex	Clinical aspect and analysis of cerebro-spinal fluid before treatment	Treatment by weekly intravenous injections of tryparsamide, and other treatment, if any	Clinical aspect and analysis of cerebro-spinal fluid after treatment	Progress, subsequent verification by tests for trypanosomes in the lymph and centrifuged blood. Remarks
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**GROUP II. — Cases in the Middle Stage (continued).**

55	m. 55	18.IV.26 : normal. C.S.F. : 10 lym. per cubic mm.	5 inj. of 3 gms.		Clinical cure, but microscopic tests impracticable.
19	f. 20	5.III.26 : normal. C.S.F. : 10 lym. per cubic mm. and large mononuclears.	2 inj. of 2 gms. 10 inj. of 3 gms.	29.VI.26 : normal. C.S.F. : normal.	Apparent cure. Tests 19.IX.26, 26.II.27, 12.V.27 : negative.
8	m. 15	1.III.26 : no characteristic symptoms. C.S.F. : 11.3 lym. per cubic mm.	6 inj. of 2 gms. 5 inj. of 3 gms.	18.IX.26 : normal. C.S.F. : 3.1 lym. per cubic mm.	Apparent cure. Tests 26.II.27, 12.III.27 and 12.V.27 : negative.
45	m. 30	16.IV.26 : no characteristic symptoms. C.S.F. : 12.5 lym. per cubic mm.	1 inj. of 2 gms. 8 inj. of 3 gms.	17.VII.26 : normal. C.S.F. : 1 lym. per cubic mm.	Apparent cure. Test 12.V.27 : negative.
12	f. 35	4.III.26 : no characteristic symptoms. C.S.F. : 13 lym. per cubic mm.	1 inj. of 2 gms. 8 inj. of 3 gms.	17.VII.26 : normal. C.S.F. : 1.2 lym. per cubic mm.	Apparent cure. Tests 19.IX.26 and 12.V.27 : negative.
52	m. 40	18.IV.26 : no characteristic symptoms. C.S.F. : 25 lym. per cubic mm. and large mononuclears.	1 inj. of 2 gms. 11 inj. of 3 gms.	24.VII.26 : normal. C.S.F. : 1 lym. per cubic mm.	Apparent cure. Test 16.V.27 : negative.
51	m. 30	18.IV.26 : fever and asthenia suspected. C.S.F. : 26.2 lym. per cubic mm.	3 inj. of 2 gms. 3 inj. of 3 gms.	18.V.27 : normal. C.S.F. : 1 lym. per cubic mm.	Apparent cure. Tests 28.VIII.26, 19.IX.26 and 18.V.27 : negative.
22	m. 40	11. III. 26 : very suspicious. C.S.F. : 40.5 lym. per cubic mm., large mononuclears.	2 inj. of 2 gms. 10 inj. of 3 gms.	29.VI.26 : normal. C.S.F. : 1.2 lym. per cubic mm.	Apparent cure. Tests 26.II.27 and 12.V.27 : negative.

**GROUP III. — Serious Cases.**

11	m. 18	2.III.26 : relapse after trypan. C.S.F. : 42.5 lym. per cubic mm., large mononuclears and muriform cells.	2 inj. of 2 gms. 11 inj. of 3 gms.		Disappeared after treatment and died of trypan. in April 1927.
20	m. 20	6.III.26 : typical case treated by several injections of trypars. Not cured. C.S.F. : 50.7 lym. per cubic mm., and large mononuclears.	Second treat. : 1 inj. of 1 gm. 1 inj. of 1.50 gms. 13 inj. of 2 gms. Third treat : 8 inj. of 3 gms.	18.VII.26 : improved after first treat. C.S.F. : 17 lym. per cubic mm. and large mononuclears. 15.V.27 : better after second treat. C.S.F. : 5 lym. per cubic mm.	Improved, but not cured. Treatment continued.

Observation No.	Age and Sex	Clinical aspect and analysis of cerebro-spinal fluid before treatment	Treatment by weekly intravenous injections of tryparsamide, and other treatment, if any	Clinical aspect and analysis of cerebro-spinal fluid after treatment	Progress, subsequent verification by tests for trypanosomes in the lymph and centrifuged blood. Remarks
GROUP III. — <i>Serious Cases</i> (continued).					
15	f. 40	4.III.26 : typical, relapse after trypars. C.S.F. : 152.5 lym. per cubic mm., large mononuclears and muriform cells.	2nd treat. : 1 inj. of 1.50 gms. 4 inj. of 2 gms. 1 inj. of 3 gms. 3rd treat. : 2 inj. of 2 gms. 2 inj. of 3 gms.	17.VII.26 : much improved after first treat. C.S.F. : 15 lym. per cubic mm. and large mononuclears.	Considerable clinical improvement. Tests 26. IV.27 and 12.V.27 : negative. Treatment continued.
10	f. 25	2.III.26 : nervous and mental symptoms. C.S.F. : 198 lym. per cubic mm., large mononuclears and muriform cells.	4 cc. bismuth-tryparsamide. 1 inj. of 1 gm. trypars. 3 inj. of 2 gms. trypars.	29.VI.26 : much improved. C.S.F. : 42.5 lym. per cubic mm. and large mononuclears.	Considerable clinical improvement. Treat. continued.
56	m. 30	19.IV.26 : first signs of nervous disturbance. C.S.F. : 200 lym. per cubic mm., large mononuclears and muriform cells.	11 inj. of 2 gms.	23.VII.26 : much improved. C.S.F. : 10 lym. per cubic mm. 16.V.27 : normal. C.S.F. : 1 lym. per cubic mm.	Apparent cure.
48	m. 37	16.IV.26 : nervous and mental disturbance. C.S.F. : 240 lym. per cubic mm., large mononuclears and muriform cells.	9 inj. of 2 gms.	22.VII.26 : very well, calm, appearance normal. C.S.F. : 3.7 lym. per cubic mm.	Apparent cure (?). Tests 22.VIII.26, 19.IX.26, 16.V.27 : negative.
3	m. 30	1.III.26 : a very advanced case C.S.F. : 410 lym. per cubic mm., large mononuclears and muriform cells.	1 inj. of 0.20 gm. (excitant) 6 inj. of 2 gms.		Disappeared at end of treatment. Died of trypan. November 1926.
46	m. 35	16.IV.26 : fairly advanced. C.S.F. : 450 lym. per cubic mm., large mononuclears and muriform cells.	7 inj. of 2 gms.		Died 21.VII.26 during treatment.
54	m. 40	18.IV.26 : a maniac. C.S.F. : 495 lym. per cubic mm., muriform cells and large mononuclears.	7 inj. of 1 gm. 5 inj. of 2 gms.	22.VIII.26 : very well, calm. C.S.F. : 15 lym. per cubic mm., large mononuclears and muriform cells.	Test 18.V.27 : clinically worse. The patient has not undergone further treatment. Prognosis very bad.
2	f. 30	27.II.26 : very serious, relapse after neosalv. C.S.F. : 550 lym. per cubic mm., large mononuclears and muriform cells.	1 inj. of 1.50 gms. 17 inj. of 2 gms.	18.VII.26 : much improved. C.S.F. : 19 lym. per cubic mm., large mononuclears.	Tests 24.IV.27 and 12.V.27 : negative. Treatment continued.
172	m. 12	22.IX.26 : advanced ; a maniac. C.S.F. : 655 lym. per cubic mm., large mononuclears and muriform cells.	1 inj. of 1.30 gms. 15 inj. of 3 gms.	12.V.27 : appears normal. C.S.F. : 1 lym. per cubic mm.	Apparent cure.

A large number of control examinations were made during my absence by S. A. S. PILLAI, principally by puncture of the enlarged glands or by triple centrifuging of the blood.

The number of cases treated with tryparsamide was much larger than the number mentioned, but the twenty-seven observations given above have been selected from among those which were continued for the longest time and with regard to which a reliable prognosis can be made.

The contents of this table may be summarised by the statement that, given regular treatment, all cases of trypanosomiasis in which serious nervous or mental disturbance has not yet appeared can be cured, provided that the virulence of the trypanosome does not exceed that of Lake Victoria. As regards advanced cases, careful and prolonged treatment usually results in, at all events, a considerable and lasting improvement. These cases can never be left without treatment, and I am convinced that, by perfecting the technique of treatment, it will be possible to sterilise bacteriologically at least three-quarters of them. This sterilisation can be tested by analysis of the cerebro-spinal fluid, although it will not necessarily effect a complete cure from the clinical standpoint; the deterioration of the nervous tissues will remain, and often the corresponding symptoms also.

Among the more than 150 patients treated with tryparsamide, not a single eye accident occurred. I attribute this fact to the preliminary injection of a small dose given to advanced cases in order to test the resistance of the subject, and of moderate doses given to patients whose spinal fluid has greatly deteriorated. In exceptional cases of the latter type, large doses were given, but only under strict supervision and with frequent examination of the eyes. Weekly doses of 3 gms. for recent cases and 2 gms. for advanced cases can be readily tolerated and are sufficient. I think it advisable to begin the treatment by a massive dose, provided the cerebro-spinal fluid does not contain more than 30 to 40 lymphocytes per cubic mm. and there are no muriform cells present, and provided that the specific symptoms do not become serious out of proportion to this degree of deterioration of the fluid — which would occur in the case of a relapse, if the first treatment were inadequate or irregularly followed up.

The necessity, in practice, of restoring the cytology of the cerebro-spinal fluid to normal cannot be too strongly urged. In a previous note<sup>1</sup>, I referred to the very considerable improvements obtained with tryparsamide in the case of monkeys inoculated with trypanosome from Damba Island antelopes. Monkey III received only one dose of the product and soon had a relapse, his trypanosome having already been made arsenic-fast by preliminary treatment with atoxyl. Monkeys 989 and 122, on the other hand, received a series of injections of 0.05 gm. per kg., which, in man, would often be considered a large dose. At the end of the treatment, however, their cerebro-spinal fluid had substantially deteriorated: 27.5 and 15 lymphocytes per cubic mm., large mononuclears and muriform cells. The monkeys received no further treatment. Monkey 989 died on February 9th, 1927, no post-mortem being performed. Monkeys 122 and III died on March 18th and 26th, 1927, respectively. In their case, a post-mortem was carried out by Professor PERUZZI, who found that death was due to unquestionable and very grave trypanosomiasis lesions.

It is difficult to lay down a rule for the total quantity of the medicament to be injected. It will appear from several of the foregoing observations that the patients were blindly given excessive quantities of tryparsamide, and that, on the other hand, advanced cases were apparently cured by a total of 18 to 20 gms. Pending reports on

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<sup>1</sup> *Interim Report of the League of Nations International Commission on Human Trypanosomiasis* (document C.H.536), page 110.

a larger number of experiments, this total of 20 gms. is, I think, the minimum after which the first spinal puncture can be performed for testing purposes, and the treatment can then be suspended, if necessary.

B. — May & Baker's *bismuth-tryparsamide* is presented in suspension in oil or water containing glucose. The ampoules contain 1 c.c. suspension, *i.e.*, according to the manufacturer, 0.20 gm. of bismuth N-phenylglycineamide-p-arsenate.

I will merely mention the two following tests :

*Observation 10 (see Interim Report).*

Treatment :

March 2nd, 1926, 1 c.c. oily bismuth-tryparsamide.

March 5th, 1926, 1 c.c. oily bismuth-tryparsamide.

March 17th, 1926, the treatment was stopped because, 3 days after the second injection, the first symptoms of bismuthic stomatitis appeared, but rapidly yielded. Gland puncture made 12 days after the treatment was started revealed trypanosomes.

*Observation 16 (see Interim Report).*

March 5th, 1926 : 0.20 gm. bismuth-tryparsamide.

March 10th, 1926 : 0.20 gm. bismuth-tryparsamide.

March 15th, 1926 : 0.20 gm. bismuth-tryparsamide.

March 23rd, 1926 : 0.20 gm. bismuth-tryparsamide.

Treatment was stopped owing to stomatitis.

No clinical improvement.

I would add that Professor KLEINE<sup>1</sup> also recognises that this product, after two or three injections of 0.2 gm., has no trypanocidal action.

The trypanocidal action of bismuth in the form of salts which are highly active in frambœsia and syphilis is practically nil. Moreover, the product in question contains only a small quantity of the arsenical base. It was to be expected that it would prove ineffective.

C. — *Sulphoxyl-salvarsan*, a recent preparation of Meister, Lucius & Bruning, is a solution stabilised at 5 per cent of p-arsenophenyldimethylaminopyrazolonsulphoxylate of soda. The special indications for this medicament are due to the slowness of its elimination : so-called latent sero-positive syphilitic conditions, tertiary syphilis, metasyphilitic conditions. The extraordinary slowness of elimination makes it necessary to allow for a considerable interval between injections.

Advanced cases of trypanosomiasis appeared to call for the use of sulphoxyl-salvarsan.

A few brief observations relating to patients who were given this treatment are set out below :

*Observation 141 (see Interim Report).* — An extremely serious case, with the highest degree of deterioration of the spinal fluid which we have observed.

July 19th, 1926 : 10 c.c. sulphoxyl-salvarsan.

Died of trypanosomiasis on September 19th, 1926.

*Observation 142 (see Interim Report).* — A very advanced case ; C.S.F. : 1,200 lymphocytes, muriform cells, etc.

July 19th, 1926 : 10 c.c. sulphoxyl-salvarsan.

August 5th, 1926 : 10 c.c. sulphoxyl-salvarsan.

After this second injection, he complained of intestinal troubles. Liver congested, ascites, trypanosomes still present in the glandular lymph.

Died on August 20th, 1926.

*Observation 143 (see Interim Report).* — Very advanced, sleepy and fat. C.S.F. : 650 lymphocytes, muriform cells, etc.

July 21st, 1926 : 10 c.c. sulphoxyl-salvarsan.

August 9th, 1926 : relapse in the blood. Treated with tryparsamide.

<sup>1</sup> *Interim Report* of the League of Nations International Commission on Human Trypanosomiasis (document C.H.536), page 18.

*Observation 144* (see *Interim Report*). — A middle-stage case.

July 21st, 1926 : 8 c.c. sulphoxyl-salvarsan.

August 23rd, 1926 : Much worse, trypanosomes in the lymph and blood.

*Observation 145* (see *Interim Report*). — A desperate case.

August 21st, 1926 : 10 c.c. sulphoxyl-salvarsan.

Died September 10th, 1926, of trypanosomiasis.

*Observation 146* (see *Interim Report*). — C.S.F. fluid: 85 lymphocytes per cubic mm., no muriform cells.

July 22nd, 1926 : 12 c.c. sulphoxyl-salvarsan.

Relapse in the blood, August 21st, 1926. Treated with tryparsamide and germanine.

*Observation 147* (see *Interim Report*). — Middle-stage case: C.S.F. : 375 lymphocytes per cubic mm. and large mononuclears.

July 22nd, 1926 : 10 c.c. sulphoxyl-salvarsan.

August 23rd, 1926 : centrifuged blood negative, but clinical symptoms worse ; the patient had become thinner and weaker, and we did not dare administer a second dose. Subsequently treated with "Bayer 205" and stibosan.

*Observation 148* (see *Interim Report*). — A very recent case ; C.S.F. normal.

July 22nd, 1926 : 10 c.c. sulphoxyl-salvarsan.

August 21st, 1926, clinical symptoms decidedly worse, trypanosomes in the fresh blood. C.S.F.: 34 lymphocytes per cubic mm. and one large mononuclear. The speed with which these deteriorations occurred and the clinical appearance of the patient at the first examination suggest that his trypanosome is particularly virulent.

Subsequent treatment : tryparsamide : 3 injections of 2 gms. in January and February 1927 (Dr. J. ENZER). Re-examined on May 16th, 1927 : condition desperate.

*Observation 149* (see *Interim Report*). — Serious case, C.S.F. : 202.5 lymphocytes, muriform cells, etc.

July 22nd, 1926 : 10 c.c. sulphoxyl-salvarsan.

July 29th, 1926 : trypanosomes still present in the glandular lymph. Treatment with stibosan started, but after two injections the patient failed to reappear.

Died of trypanosomiasis at end of 1926.

*Observation 153* (see *Interim Report*). — An old and advanced case. As I was already convinced of the ineffectiveness of sulphoxyl-salvarsan, I combined this treatment with stibosan.

July 27th, 1926 : 10 c.c. sulphoxyl-salvarsan.

August 5th, 1926 : 0.20 gm. stibosan.

August 18th, 1926 : 10 cc. sulphoxyl-salvarsan.

January 22nd, 1927 : 0.20 gm. stibosan by Dr. J. ENZER.

January 30th, 1927 : 10 c.c. sulphoxyl-salvarsan by Dr. J. ENZER.

April 6th, 1927 : 0.20 gm. stibosan by Dr. J. ENZER.

This patient was re-examined on May 18th, 1927. Clinical condition much worse, and careful treatment with tryparsamide was started immediately.

*Observation 154* (see *Interim Report*). — A serious case. A test made by means of two cumulative injections of 10 c.c. at ten days' interval did not cause the trypanosomes to disappear from the glandular lymph.

The firm of Meister, Lucius & Bruning claims that sulphoxyl-salvarsan injected intravenously into a mouse infected with trypanosomes (type not indicated — nagana ?) protects it for a time equivalent to the action of other salvarsans injected into the muscles. According to the foregoing observations, it is curious but undeniable that this product is of no value in *T. gambiense* infections in man, whereas the very rapid and often lasting trypanocidal action of salvarsan or neosalvarsan is well known.

D. — "Bayer 205" has been kept for patients for whom it was impossible to provide regular treatment or who were likely to prove intractable.

The results obtained in advanced cases infected with the Léopoldville virus<sup>1</sup> were very disappointing. The inefficacy of "Bayer" on cytological deteriorations of

<sup>1</sup> VAN DEN BRANDEN and VAN HOOF, 1924, *An. Soc. Belge méd. trop.*, page 205.



the spinal fluid has often been noticed, notwithstanding the sterilisation of the peripheral blood. This led us to the conclusion that, in middle-stage and advanced cases, this product did not as a rule arrest the progress of lesions of the nervous system.

On the other hand, I would refer to Observation 63 quoted above, in which the virus was weak, and the deterioration of the cerebro-spinal fluid and also the corresponding mental and nervous symptoms yielded appreciably to a single dose of 1 gm. of germanine.

In recent infections, a single dose, even a small one, may prove to be curative in the strict sense of the word. This dose is, however, extremely variable, as is also the virulence or susceptibility of the trypanosome. The following are examples :

*Observation 33 (see Interim Report).* — Recent infection, Samia virus.

March 17th, 1926 : 0.50 gm. germanine intravenously.

Tested by gland puncture, examination of fresh and centrifuged blood, and spinal puncture. A sensitive monkey was inoculated with the blood.

From March 23rd, 1926, to May 15th, 1927, these various tests remained negative. From the clinical point of view, also, the woman appears to be recovering.

*Observation 36 (see Interim Report).* — C.S.F. : 36.2 lymphocytes per cubic mm., but no nervous disturbance. Samia virus.

March 20th, 1926 : 0.50 gm. germanine intravenously.

Tested by examination of fresh and centrifuged blood in March and April 1926.

Guinea-pig inoculated July 19th, 1926 : negative.

September 19th, 1926 : glands no longer palpable. Blood negative.

May 12th, 1927 : C.S.F. : normal, 1 lymphocyte per cubic mm.

*Observation 38 (see Interim Report, page 54).* — This patient had a relapse four months after an injection of 0.50 gm. germanine.

*Observation 40 (see Interim Report).* — Samia virus, like the previous case. Relapse six months after 0.50 gm. "Bayer 205".

Of these four patients, infected by a virus of approximately the same origin, the efficacy of the medicament was greatest in the most advanced case, whereas an equivalent dose caused a relapse of Case No. 40, which was the least serious, and whose weight was less than that of the others. It is therefore reasonable to deduce (Conclusion 3 of the *Interim Report*) that in a given endemic area the virulence of the trypanosome varies considerably with each case. It might be added that, in similar circumstances to those described in the foregoing observations, "Bayer 205" may be used as a medicament to test the extent of this pathogenicity. This treatment would, however, be dangerous, and, before the reappearance of trypanosomes in the blood, the nervous lesions might become rapidly worse. The following observation is an example :

*Observation 72 (see Interim Report).* — Asthenic patient, but no disturbance of the nervous system as yet. C.S.F. : 51.1 lymphocytes per cubic mm., large mononuclears and one muriform cell. Weak Busitema virus.

April 30th, 1926, 1 gm. germanine.

The result of a series of control examinations, including inoculation of a receptive monkey, was negative. On September 19th, 1926, the patient appeared to be cured. Blood and lymph negative. Spinal puncture not performed.

On May 22nd, 1927, *i.e.*, eight months later, the clinical condition of the patient was worse, but the result of the blood and lymph tests was again negative. C.S.F. : 295 lymphocytes per cubic mm., large mononuclears and muriform cells. Treated with tryparsamide.

*Can "Bayer 205", in conjunction with other active products, help to shorten the duration of the treatment ?* The following observations relate to patients whom it was only possible for members of the Uganda or Kenya Medical Services or myself to see a few times.

*Observation 144* (mentioned above). — Relapse after sulphoxyl-salvarsan treatment.

August 23rd, 1926 : 3 gms. tryparsamide.

August 26th, 1926 : 3 gms. germanine.

January 29th, 1927 : 2 gms. tryparsamide.

February 24th, 1927 : 1 gm. germanine.

April 7th, 1927 : 2 gms. tryparsamide.

May 16th, 1927 : clinically cured. C.S.F. : 1.6 lymphocytes per cubic mm.

*Observation 65* (mentioned above). — Weak Busitema virus.

*Observation 146* (mentioned above). — Relapse after sulphoxyl-salvarsan. Kavirondo virus.

August 21st, 1926 : 3 gms. tryparsamide.

August 28th, 1926 : 1 gm. germanine.

January 29th, 1927 : 2 gms. tryparsamide.

February 24th, 1927 : 1 gm. tryparsamide.

May 16th, 1927 : clinical condition worse.

*Observation 156* (see *Interim Report*). — A serious case, 675 lymphocytes per cubic mm., large mononuclears and muriform cells. Kavirondo virus.

August 9th, 1926 : 1 gm. germanine.

August 16th, 1926 : 0.45 gm. neosalvarsan.

August 18th, 1926 : 1 gm. germanine. A considerable improvement was already apparent.

September 15th, 1926 : gm. tryparsamide.

December 14th, 1926 : 1 gm. germanine.

May 19th, 1927 : obvious clinical improvement. C.S.F. : 90 lymphocytes per cubic mm., large mononuclears and muriform cells.

*Observation 165* (see *Interim Report*). — A very advanced case, which could not be given regular treatment. C.S.F. : 1,565 lymphocytes per cubic mm., eosinophiles, large mononuclears, muriform cells. Kavirondo virus.

September 20th, 1926 : 2 gms. tryparsamide.

September 22nd, 1926 : 1 gm. "Bayer 205".

September 23rd, 1926 : 0.20 gm. stibosan.

The treatment was not continued during my absence, and this patient died of trypanosomiasis in December 1926.

These five observations do not speak well for this method, and it is obvious, if we compare the results with those obtained by an equal number of tryparsamide injections, that it is preferable to use this medicament alone. From the point of view of general prophylaxis, the lengthy duration of sterilisation and the toleration of patients, after a total dose of 3 to 4 gms., which is a fairly large quantity, are favourable to germanine, no account being taken in this case of the interests of the individual. On the other hand, "Bayer 205" is to be preferred in cases of natural or artificial resistance of the trypanosome to arsenical agents, and our observations in the Semliki valley have shown that this accidental resistance is not rare. I would point out that this applies solely to *T. gambiense*, and that these conclusions do not hold good for any other virus, such as *T. rhodesiense*; on the contrary, when *T. gambiense* appears in weak strains, such as those of Busitema and the rivers Mpologoma and Namatala, too much should not be expected of "Bayer 205".

The preventive action of "Bayer 205" is undoubtedly its chief virtue. In the endemic zone of Berkeley Bay, its employment might enable fishing to be carried out on a larger scale, and the proportion of albuminoids in the diet of native workers in all industrial and agricultural areas in the neighbourhood to be greatly increased.

Unfortunately, we have not as yet any exact knowledge of the duration of the preventive action of germanine. This must be sought in endemic zones, and under varying economic conditions. Several experiments are now being carried out. One, which affects Bamanga canoe-builders in the neighbourhood of Stanleyville, has been continued by the local medical service since 1925. Another is being undertaken in the mining camps on the river Kibali, and was mentioned in the epidemiological report

on the Upper Uele. A third has been started in the vicinity of Mjanji, and subsequent observations have been entrusted to Dr. MACLEANE, of Tororo.

This latter test is being applied to the crews of the fishing-boats frequenting the shores of Berkeley Bay and more especially the islands, including Sigulu. Three of the crews received protective injections on August 26th, 1926, and September 9th, 1926, approximately 2 centigrammes per kilogramme being given to the 54 persons tested. The crews of two other boats, consisting of 12 fishermen, who frequent the same glossina breeding-grounds and run the same risks, were not given a preventive injection, and are being used as controls. The last test was made between May 12th and 20th, 1927, no infection being diagnosed. The same subjects are now to receive a second protective injection, the effects of which will be observed until the end of 1927. As in the case of the other two experiments, the entirely negative results do not permit of any conclusion.

E. — *Tryparsamide by ingestion*. This experiment has been discussed in a separate note.

#### GENERAL CONSIDERATIONS, SUMMARY AND CONCLUSIONS.

For the epidemiological survey of a country, especially from the point of view of sleeping-sickness, no factor — economic, pathological, ethnological, or other — is devoid of importance; moreover, factors which appear to have no influence upon the biological conditions of an endemic or epidemic may require to be considered when prophylactic measures are being undertaken. This appears to me a sufficient excuse for any tediousness in these reports, which, I may add, are still very incomplete and will continue to remain inaccurate in many ways until they have been tested by many years' experience. Further, the experiments which appear conclusive in this survey are conclusive only for that particular place and that particular time; investigations of this kind, carried out on a uniform plan, must be repeated wherever the disease exists, and repeated again and again.

The enquiry in the Budama district and Kavirondo was begun at the end of February 1926, and the last visit of inspection was carried out in May 1927. Climatic conditions during these fifteen months were substantially unaltered, and what is lacking in these observations, therefore, is a study of the general aspect of the area during one of the usual droughts and famines. It is, moreover, obvious that in certain parts of this country the population is now rapidly increasing, principally in Busitema, where numerous natives who had left, either of their own accord or by order of the authorities, are returning to their former fields. More especially in the neighbourhood of the Bulugwe hills, which were formerly decimated by sleeping-sickness and where, at the beginning of our enquiry, there was neither population nor fly, natives and even Europeans are now growing crops and establishing enterprises.

This state of affairs may increase the density of the population in other areas from which the fly has not disappeared, as, for instance, on the shores of the lake or the banks of the Mpologoma and Namatala. Very close contact may thus be re-established between man and fly, such as probably existed for many years before the great Uganda epidemic.

Will another epidemic necessarily follow from this new situation? There is nothing to prove that it will be so. In various districts in the Belgian Congo — for instance, on the banks of the river Bomokandi — trypanosomiasis has not yet obtained a footing, although trypanosome-carriers have several times been introduced. It is essential that the virus introduced should be highly contagious, and, as has been

proved by the recent experiments of Dr. H. Lyndhurst DUKE in the case of isolated strains in various parts of Central Africa, that it should be readily transmissible by glossinæ infected in the salivary glands.

Presented in this way, the problem of the spread of trypanosomiasis appears in a new light, and we must deduce therefrom the practical measures to be adopted in the campaign against sleeping-sickness. The first measure will be to watch any fresh virus introduced into an area which, owing to its biological conditions, is likely to develop a further outbreak of an epidemic, whether it is at present immune or in a stationary endemic state.

A fact upon which I have already insisted in these supplementary notes is the limited area of the "danger zones", where it appears certain that natives become infected one by one and from time to time renew the stock of transmissible trypanosomes in the tsetse. As is the case at the edge of the Busembe cliff, it seems to me that these complex reservoirs of trypanosomes are extremely small in area and consequently easy to destroy. Prophylactic measures should therefore take account of this fact and an endeavour be made to discover the reservoirs.

Generally speaking, the conclusions of the first enquiry in Budama and Kavirondo still hold good. A few further deductions can be made with regard to the practical prophylaxis and the therapeutics of sleeping-sickness.

1. As regards prophylaxis :

Necessity for supervising the movements, and consequently for a census and identification, of the natives.

Discovery and destruction of danger-points where the biological conditions necessary for the maintenance of the endemic exist. These places, which are comparatively few in number and very limited in area, can be improved without any great labour or mass removal of the natives.

Efficacy of chemical prophylaxis when combined with the above measures.

2. As regards therapeutics :

Remarkable efficacy of tryparsamide, which should be used systematically in all cases where possible. Sulphoxyl-salvarsan and bismuth-tryparsamide are of no practical value. "Bayer 205" should be reserved for arsenic-fast cases and provisionally as a preventive to be given to natives who are obliged to work under conditions which make them liable to infection.

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4.

GENERAL REMARKS ON SLEEPING-SICKNESS IN THE  
*T. GAMBIENSE* AND *G. PALPALIS* REGIONS BASED UPON  
RESEARCHES CARRIED OUT IN BUDAMA, KAVIRONDO, THE  
UPPER UELE AND THE SEMLIKI VALLEY.

BY DR. L. VAN HOOF.

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Our Commission's programme included a number of "epidemiological investigations" with the object of establishing and justifying the right lines for prophylaxis by properly verified facts in different areas. At the risk of repeating myself, I shall endeavour to condense the results of the investigations made during the last eighteen months in three different endemic areas, bearing in mind the observations I had previously made in the Belgian Congo. To a great extent my conclusions work into the Commission's epidemiological programme. In many cases they agree with facts already known. It is also to be hoped, however, that my work may have the result of securing the recognition of these facts and their practical consequences in the struggle against sleeping-sickness in man.

I. INFECTIOUS BREEDING-GROUNDS OF GLOSSINÆ .

1. These breeding-grounds are very restricted. They are surrounded by sleeping-sickness cases who are chronologically and clinically at various stages in the progress of the disease. The percentage of infected flies in these breeding-grounds ought to be ascertained. As in the case of Mjanji, it is probable that a higher proportion of infected flies will be found in these places than among flies caught at random.

2. Here and there we find "specialised breeding-grounds" where the sole prey of the fly is man; this sometimes occurs in the Semliki valley. In such cases, if a virulent trypanosome is introduced, it spreads rapidly among the people. It can then be inoculated over and over again into the same subject; if an insufficient arsenical treatment is given, the trypanosome may become arsenico-resistant after a number of passages, or may produce reinfections in cases that have already been treated (Semliki).

3. It is in this manner that small independent trypanosomiasis foci are formed. On the other hand, a large but scattered population in contact with flies distributed all over the district (Upper Uele) may escape even if the virus is introduced, or only sporadic cases may occur; in such cases, the degree of transmissibility of the trypanosome is an important factor.

II. CONTAGIOUSNESS, TRANSMISSIBILITY, IMMUNITY.

1. The facility with which the trypanosome spreads is entirely independent of its virulence, of the number of trypanosomes in peripheral circulation, and even of the physique of the native. It would appear to be linked with other factors which it was impossible to ascertain on the spot. The only impression gained is that it varies considerably — even if other things are equal — from one endemic area to the next.

2. The index of transmissibility calculated by Dr. H. L. DUKE at the Entebbe Laboratory gives the degree of contagiousness of the strains observed. The mere impression gained from the facts may be confirmed if this index is ascertained for a large number of areas investigated. Examination of the strains obtained in the endemic areas visited by the Commission has already shown that some of them have lost in the course of nature the power of being transmitted by *G. palpalis*.

3. There is no fact in epidemiology to indicate a human immunity, or even an appreciable human resistance, to the normal virus. Variations in clinical development are to be ascribed to great differences in the virulence of the parasites, and in the last resort the natural resistance of man, however feeble, may — provided it exists at all — be enough to counterbalance the pathogenic action of a greatly attenuated trypanosome (Namatala).

4. In districts in which the endemic is severe, accidental infections produced by a single fly have been observed (Mayombe). This phenomenon conflicts with the idea of a human immunity, and seems to support that of the cyclic transmission of the trypanosome.

### III. VIRULENCE.

1. The practical means of estimating virulence are the gravity of the symptoms, the speed of the clinical evolution, the early occurrence of nervous and mental disturbances and the accompanying alterations in the cerebro-spinal fluid, and the comparative activity of therapeutic agents.

2. Virulence varies greatly in different individuals within the same area, and from one endemic area to the next. The two extremes may be met with together : intense virulence with rapid and fatal development in spite of treatment (Samia and Kavirondo), and attenuation amounting to tolerance and possibly spontaneous cure (Namatala).

3. The activity of chemical agents is in inverse proportion to virulence.

4. Probably the trypanosome has a natural tendency to become attenuated in man, if no new virus is imported and if the conditions of its development are such that contact between the human subject and the tsetse is reduced to a minimum, the percentage of advanced cases is extremely high, and the endemic is progressing very slowly in an area completely isolated from the neighbouring centres of the disease (Namatala). If, on the other hand, new virus is incessantly being imported, virulence remains average, even if the spread of the endemic is limited by other factors (Upper Uele).

5. All investigations into the matter of treatment should be preceded by the determination of the degree of virulence.

### IV. ENDEMIC INDEX.

An endemic index based on the total percentage of cases cannot give an accurate idea of the nature of the infection. On the other hand, an endemic index showing the percentage of recent infections will thereby indicate the tendency of the disease to spread (Budama, Semliki). Thus in certain active centres, even if the number of cases is small, there may be nearly 100 per cent of recent cases, which will show that there is a danger of expansion in an epidemic form (Semliki).

## V. RESERVOIRS OF VIRUS AND MODE OF TRANSMISSION.

1. There is as yet nothing to show that there are any other reservoirs of virus than man and the infected glossina, or any other agent of transmission than the glossina. Domestic animals and game are not to be regarded as habitual vertebrate hosts, nor does any biting insect except the tsetse appear to act as a vector.

2. Prophylaxis directed solely against the human virus-reservoir may fail (Busembe in Kavirondo, Semliki) if the virus subsists in the fly and the fly remains in contact with man. This indicates that an endemic cannot be extinguished by chemical prophylaxis alone (Semliki) unless the preventive action of the therapeutic agent continues for a long period (experiments on the preventive action of "Bayer 205").

3. While it cannot be definitely stated that the mechanical transmission of trypanosomiasis is impossible in nature, epidemiological observations tend to suggest that the normal process of dispersion and conservation of virus is transmission through the salivary glands of infected flies, whether the disease takes the form of a retarded endemic (Upper Uele), or of a severe endemic with a tendency to the development of epidemic foci (Semliki), or of a normal infection (Budama-Kavirondo).

## VI. ENDEMICS AND EPIDEMICS, AND CONDITIONS WHICH FAVOUR THEM.

Sleeping-sickness is favoured by the same conditions as most other diseases, namely, a high degree of contagiousness and frequent opportunities of infecting man — which may be expressed in the present case as transmissibility and intimate contact between the fly and man. The virulence of the strain has nothing to do with the case (*cf.* Budama and Upper Uele). The factor of transmissibility cannot be determined until experimental research work has been done on strains of all origins. The factor of intimate contact depends on a complex of economic and political circumstances which require to be separately studied in each case, the most important being the movement of native virus-carriers.

## VII. PROPHYLAXIS.

The epidemiological researches carried out in these three different areas agree in confirming the value of prophylactic measures which have long been known but have not everywhere been carried out. These are :

(1) The taking of a census of natives and a census of all sleeping-sickness cases ;

(2) The use of the most accurate methods of diagnosis in order to reduce to a minimum the possibility of leaving germ-carriers at large ;

(3) The use of the best methods of treatment in order to sterilise the human virus-reservoir ;

(4) An endeavour to prevent the introduction of extraneous virus by controlling the movements of the natives ;

(5) A scientific campaign against the invertebrate reservoir of trypanosomes. This will require, in every endemic zone, thorough entomological information, on the basis of which breeding-grounds known to be dangerous will be destroyed and natives will be moved and regrouped ; it will thus be possible to avoid needless and costly deforestation and unnecessarily large mass displacements of population.

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5.

THE TREATMENT OF SLEEPING-SICKNESS BY INGESTION  
OF TRYPARSAMIDE.

BY DR. L. VAN HOOF.

In June 1926, the London firm of May & Baker sent to Dr. H. L. DUKE, Chairman of the International Sleeping-Sickness Commission, some samples of tryparsamide in 25- and 50-centigramme tablets for experiments in the prevention and cure of human trypanosomiasis. The consignment was accompanied by a summary of the experiments which had been carried out by the firm's specialists on the curative and preventive action of certain trypanocidal products administered *per os*.

All these experiments had been conducted with *T. equiperdum* on mice, and Dr. LEVADITI, of the Paris Pasteur Institute, had, at the firm's request, undertaken further series of experiments with *T. brucei* from Nagana, the results of which have since been published.<sup>1</sup>

We may summarise the results as follows :

*Preventive and curative action of tryparsamide :*

1. Doses expressed in milligrammes per gramme in mice infected with *T. equiperdum* :  
Intravenous : Maximum tolerated, 2.5 ; curative minimum, 0.7 ; ratio 3.6.  
Subcutaneous : Maximum tolerated, 1.8 ; curative minimum, 0.6 ; ratio 3.  
*Per os* : 14 ; 0.6 and 23 respectively.

*Action of tryparsamide per os :*

2. Rabbits and Nagana trypanosomiasis :  
A dose of 0.025 gm. per kg. administered 24 hours before inoculation is not preventive.  
An additional dose of 0.1 gm. given four days after infection is curative.  
A dose of 0.50 gm. per kg. administered *per os* 24 hours before inoculation retards infection by 11 days. An additional dose of 0.1 gm. per kg. was curative.  
A dose of 0.1 gm. per kg. administered by ingestion 24 hours before inoculation is absolutely preventive.
3. A series of further experiments shows that the preventive action of 0.1 gm. per kg. *per os* does not last more than 24 hours on account of the rapid elimination of the product.
4. In rabbits infected with the Nagana variety, ingested tryparsamide displays a curative action. With a dose of 0.05 gm., followed by a second dose of 0.1 gm., per kg., an animal is not sterilised ; another is given 0.1 gm. two days after infection, and is definitely cured. Others receive repeated doses of 0.2 gm. and are completely cured.

These first results, therefore, obtained before June 1926 by Professor LEVADITI and May & Baker, proved that it would be worth while to make a test with human trypanosomiasis.

Unfortunately, I had so few patients at my disposal that I was unable to ascertain what, in man, was the preventive action of the product, the rapidity and duration of the sterilisation produced by a single estimated curative dose, and the indices of toxicity when extreme doses are given. Of all the patients who underwent this treatment, I propose to report only the four following observations, which will nevertheless enable us to form an opinion as to the value of this therapeutical method.

<sup>1</sup> LEVADITI, *Bull. Soc. Path. Exot.*, 1926.



*Case No. 38.* — *Naweyre*, woman, 40 years of age, of Mjanji. Diagnosed on March 19th, 1926, by gland puncture. Trypanosomes in the peripheral blood; fever. General condition good, does not seem to be in an advanced stage.

March 20th, 1926 : C.S.F. : 15 lymphocytes per cubic mm. and large mononuclears. Weichbrodt's reaction negative. Is given 0.50 gm. germanine intravenously.

March 24th, 1926 : blood test negative.

April 22nd, 1926 : gland puncture negative. Examination of blood after triple centrifuging, negative.

June 29th, 1926 : triple centrifuging of blood negative.

July 17th, 1926 : relapse. Gland puncture positive. Triple centrifuging of blood positive. Weight, 60 kgs.

Treatment — Repeated small doses of tryparsamide *per os*. Total 30 gms. 1 gm. *per diem*, in ten-day series with ten-day intervals.

September 16th, 1926 : relapse. Trypanosomes in the glandular lymph and in fresh blood.

Subsequently this patient was sent to the Lumino dispensary for tryparsamide injections, which she does not seem to have tolerated well.

February 26th, 1927 : trypanosomes in the glands in spite of the treatment. These only disappear when the weekly injections are raised to 3 gms. (S.A.S. PILLAI's observation).

May 13th, 1927 : Clinical improvement ; treatment continued.

*Case No. 40.* — *Naerema Josephine*, a girl aged 15, of Mjanji. Very recent infection diagnosed on March 20th, 1926, by gland puncture. No clinical symptoms.

March 20th, 1926 : given 0.50 gm. germanine intravenously.

March 27th, 1926 : blood negative.

April 22nd, 1926 : glandular lymph and centrifuged blood negative.

June 29th, 1926 : triple centrifuging of blood negative.

Clinical condition good. Primary frambœsic lesion on the left thenar; injected 1 c.c. "Mosurol Bayer".

July 17th, 1926 : trypanosomiasis tests negative. Frambœsic lesion almost cicatrised in a few days, but relapse *in situ*. Patient is given 0.50 gm. *per diem* "Spirocid" Meister Lucius up to 10 gms. in all, and the lesion is definitely healed.

September 16th, 1926 : relapse. Trypanosomes in the glandular lymph. Patient is then given tryparsamide treatment by ingestion — repeated strong doses.

Weight : 45 kgs.

September 16th, 1926 : 2.50 gms. tryparsamide *per os*.

September 17th, 1926 : 5 gms. tryparsamide *per os*.

September 27th, 1926 : 5 gms. tryparsamide *per os*.

October 10th, 1926 : 5 gms. tryparsamide *per os*.

October 20th, 1926 : 5 gms. tryparsamide *per os*.

February 26th, 1927 : General state excellent, no more puncturable glands (S.A.S. PILLAI's observation).

May 12th, 1927 : trypanosomiasis tests negative. Clinical condition very good.

C.S.F. : normal, 2 lymphocytes per cubic mm.

The patient therefore received and tolerated very well doses of 55 and 110 mgms. per kg.

*Case No. 170.* — *Ongwe*, male, 25 years of age, of Busembe (Kavirondo). The patient already displayed symptoms of nervous lesions. Diagnosed on September 19th, 1926, by gland puncture and triple centrifuging of the blood.

September 19th, 1926 : C.S.F. : 330 lymphocytes per cubic mm., large mononuclears and muriform cells.

Weight : 65 kgs.

Treated with tryparsamide *per os*, with average weekly doses :

September 19th, 1926 : 5 gms. tryparsamide.

September 26th, 1926 : 5 gms. tryparsamide.

October 1st, 1926 : 5 gms. tryparsamide.

October 9th, 1926 : 5 gms. tryparsamide.

October 14th, 1926 : 5 gms. tryparsamide.

Not seen again until February 21st, 1927.

February 21st, 1927 : relapse : trypanosomes in the glandular lymph and in the blood, thin film (S.A.S. PILLAI's observation). This patient had tolerated doses of 77 mgms. per kg. His general condition when the relapse was observed was very bad, emaciation, sub-delirium, depression, etc.

*Case No. 171.* — *Adjambo*, girl, 10 years of age, of Dadira (Budama-Samia). Patient feverish, thin and agitated. Diagnosed on September 19th, 1926: glands negative, trypanosomes detected by triple centrifuging of the blood.

September 19th, 1926 : C.S.F. : 75 lymphocytes per cubic mm., large mononuclears and a few muriform cells.

Weight : 20 kgs.

Treated with tryparsamide *per os* in very large doses at intervals of from 7 to 10 days.

September 19th, 1926 : 4 gms. tryparsamide.

September 26th, 1926 : 4 gms. tryparsamide.

October 1st, 1926 : 4 gms. tryparsamide.

October 10th, 1926 : 5 gms. tryparsamide.

October 20th, 1926 : 5 gms. tryparsamide.

This patient was not seen again until February 26th, 1927.

February 26th, 1927 : relapse : trypanosomes in the glands.

The treatment was continued with tryparsamide by injections, which rapidly sterilised the peripheral blood and improved the general condition (S.A.S. PILLAI's observation).

The patient has therefore tolerated doses of 200 and 250 mgms. per kg.

#### CONCLUSION.

It would be overhasty to draw any final conclusion from these four cases. It is enough to note that, in the three patients whose lumbar fluid was more or less definitely impaired, the treatment — even with doses of 25 centigrammes per kilogramme — was entirely ineffective. On the contrary, with the single patient recently infected and with unimpaired fluid, doses of from 5 to 11 centigrammes produced and maintained the apparent cure, an effect which can also be obtained sometimes in a similar case by the intravenous injection of a dose of 5 milligrammes per kilogramme.

We should add that these experiments refer to the *T. gambiense* of Lake Victoria, the virulence of which I have previously estimated at a normal stage in persistent endemics.

It will be necessary later to confirm the therapeutic action of tryparsamide *per os* in the case of patients whose cerebro-spinal fluid is normal, for in such early cases the method may be of practical value.

SUBSIDIARY RESEARCH.

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PREVENTIVE AND CURATIVE ACTION OF "SPIROCID" IN  
AFRICAN RELAPSING FEVER.

BY DR. L. VAN HOOF.

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"Spirocid", manufactured by Meister, Lucius & Bruning, is described as a para-oxy-meta-acetylaminoarsenical acid, and is not unlike Fournau's "Stovarsol". Some of this product had been placed at the disposal of the International Sleeping-Sickness Commission, so that we were able to carry out several experiments with regard to relapsing fever caused by *S. duttoni*.

*O. moubata* has only recently made its appearance in the southern part of the Semliki valley, immediately to the north of Lake Edward. The pacification of this district and of the neighbouring territories has encouraged trade between the northern part of the district of Kivu, the Lubero territory and the Semliki valley. It is clearly from Kivu that the ticks were imported, and they had no difficulty in acclimatising themselves to a country in which geo-botanical conditions are favourable to them. They have already passed beyond the valley, and caravans of porters have spread them throughout the camps, even deep in the Ituri Forest on the road from Beni to Irumu. They are found in the low-lying plains in the direction of Boga and Gety, and I have even noticed them at Bogoro, in the house of a colonist who employed labour from those districts. But it is in the southern part of the Semliki valley that they have multiplied most prolifically, and have become such a scourge that the porters are obliged at every halt to construct temporary shelters outside the camps or other over-frequented places.

Professor LAVIER accompanied me to this district when engaged in a trypanosomiasis enquiry, and was good enough to assist me in these experiments. I wish to offer him my most sincere thanks for his assistance.

When we left Bungulu (New Beni), our caravan consisted of about a hundred porters, two soldiers, four hospital orderlies and nine "boys".

On January 10th, 1927, we stayed at the sleeping-sickness lazaret near Old Beni. All the brick-walled and corrugated-iron-roofed buildings are infested with ticks. The porters constructed for themselves shelters outside the enclosure, and we and a few boys passed the night in the lazaret. Two orderlies, one woman and three boys slept in the buildings, but even before midnight the three boys, in spite of the fact that they had copiously swilled the floor with creoline and water, were forced to take to the open after being stung in a large number of places. The same occurred in the case of one of the hospital orderlies and the woman.

The next morning each of the boys received 1 gm. of spirocid. The hospital orderlies and the woman were not treated and were kept as controls.

One hospital orderly, an apprentice fly-boy and a native of Bungulu, where the ticks have not yet penetrated into the native villages, showed spirochaetes in the blood on January 18th, 1927, *i.e.*, after eight days' incubation. The woman, who came from a village on the Beni Penge road in a non-infested forest, was positive on January 19th, 1927, *i.e.*, after nine days' incubation. The other hospital orderly, who was

thoroughly accustomed to travelling in this country and was probably immune, did not become infected throughout the whole of the journey. The patients were treated with novarsenobillon and were cured by a single injection.

The three boys who had been given preventive doses of spirocid did not become infected. They were under observation for at least five months.

On January 17th and 18th, 1927, we stayed at Amakoma, on the shore of Lake Edward. There is no camping-ground in this wretched village, in which there are not more than a score of males, and where about 30 per cent of the population has been treated for trypanosomiasis. The natives brought a rush hut intended to be used as a kitchen, set it up on the site of a former native hut, and during the night a fly-boy and a little boy took refuge there. On the morning of January 19th, 1927, they brought me some specimens of the ornithodoros which had bitten them; several of the insects had made a good meal.

The fly-boy, who, like the other boys, comes from Uganda or has recently spent at least one year there, was treated preventively with spirocid and, like the rest, after taking a dose of 1 gm., he did not contract spirochætosis. The little boy, on the contrary, had spirochætes in his blood on January 25th, 1927, after at least six days' incubation.

It should be observed that in reality, since January 10th, in all the camps along the road the natives had been exposed to infection. It is quite possible, therefore, that in this last case spirochætosis may have been contracted several days previously.

The porters had been recruited in the immediate neighbourhood of Bungulu, in villages as yet uninfected. They knew the ticks quite well, calling them "kimputu" (as in Kivu), and feared the consequences of their bites. The form taken by this disease is particularly serious in the Beni and Lubero territories, much more serious — at least so it seemed to me — than in Ruanda or Urundi and particularly about Léopoldville. There is even a fairly high death rate; Europeans who have been attacked can recall nervous and cardiac disorders of extreme gravity.

In spite of that and in spite of our supervision, the porters gradually came to neglect precautions against the ticks, so that towards January 20th, 1927, the first cases occurred. On January 24th, 1927, there were 17 patients duly diagnosed by blood examination. From then onwards until our return to Bungulu on February 3rd, 1927, fresh cases were diagnosed daily. During this time a personal servant was the last person subjected to the preventive action of spirocid after four consecutive days (January 20th to 24th) passed in a thickly infested hut in the Kasindi camp. On the 24th and 25th, in the morning, he ingested 1 gm. of the drug and escaped infection.

By this time, the number of able-bodied porters had been so reduced that at every station, even when the number of packages was reduced to a minimum, about twenty-five additional porters had to be engaged, although during the five days at Kasindi a large number had recovered from their first attack, had not come forward for treatment and were continuing work on the return journey, pending a relapse. Several, on the other hand, had been cured by an injection of 0.60 gm. of novarsenobillon and had no relapse. Among the hammock-bearers there was a 37 per cent loss through relapsing fever.

From a clinical point of view, the disease was in most cases severe. The first attack was sometimes accompanied by delirium, and lasted with high temperatures for four consecutive days. In three cases at least there was pronounced icterus and signs of myocardial intoxication, feeble and irregular pulse and great weakness. On the whole, it was clear that the virus was particularly active. We must, however, not forget that the porters were not of a very robust race and were tired out by a difficult journey in rough country where the tracks were hardly practicable, a country which

sleeping-sickness had almost entirely cleared of its inhabitants and in which food was often very difficult to obtain.

In these circumstances, a curative therapeutical result from spirocid would confirm our experiments with regard to its preventive action. Here are our observations regarding certain experimental cases :

*Observation 1. — Kalipili, man, 25 years of age, Banande.*

January 24th, 1927 : first attack occurred in the morning, microscopic diagnosis in the afternoon. The patient received 1 gm. of spirocid.

January 25th, 1927 : temperature had not diminished, no improvement ; 1 gm. of spirocid.

January 26th, 1927 : temperature fell in the night ; patient declared he was much better. He was given another gramme of spirocid.

It was impossible to observe this patient again until February 4th, 1927, that is to say, nine days later, which often represents one of the shorter intervals between the relapses. At Bungulu, where he had been discharged, he refused to come forward again, and up to February 10th, 1927, he was, according to the messengers' information, well.

*Observation 2. — Kilolo, man, 30 years of age ; robust porter of Banande race.*

January 25th, 1927 : first attack in the morning ; diagnosis by blood test in the afternoon. 1 gm. of spirocid at 3 p.m.

1 gm. at 4.30 p.m.

January 26th, 1927 : no improvement. Sub-icterus ; saline purge and 1 gm. spirocid.

January 27th, 1927 : general state serious. Icterus, high temperature, sub-delirium ; agitated, feeble, irregular pulse. Injection of " hexetone " and stimulants and 1 gm. of spirocid.

January 28th, 1927 : patient at his last gasp. Intense icterus, enormous liver. In the morning the patient spat blood and suddenly died at about midday.

In a camp at the foot of the Ruwenzori, amid a distrustful and scarcely pacified population, it was impossible for us to carry out an autopsy. The only thing we could do was to puncture the liver there and then. The pulp did not show any spirochætes, and although it is difficult to admit that a total of 4 gms. of spirocid administered in three days could have produced such toxic effects, that was our first impression. The extreme virulence of the spirochæte and the frequent complication of icterus being known, we thought it logical to continue treatment with spirocid. The death of this patient surprised us, and if we had not been aware that a rapid and fatal evolution of the disease is not uncommon in this part of the country, we should have been forced to believe that the individual was particularly susceptible to this medicament.

*Observation 3. — Manieka, man, 25 years of age, weakly and underfed Banande.*

January 20th, 1927 : the attack began in the morning on the march, the patient dragged himself to camp, and on his arrival spirochætes were discovered.

He was given 2 gms. of spirocid.

January 21st, 1927 : fever completely disappeared. The patient says he is cured. Another 2 gms. of spirocid administered.

The patient was observed every day up to February 3rd, 1927, and was seen for the last time on February 10th, 1927, *i.e.*, 20 days after the first attack. He had no relapse.

*Observation 4. — Malimingi, man, 25 years of age, forest Bambuba.*

January 27th, 1927 : first attack in the night of the 26th-27th. Spirochætes not discovered till January 29th, 1927.

January 29th, 1927 : 1 gm. of spirocid.

January 30th, 1927 : 1 gm. of spirocid. On this date the temperature became normal again, but the patient was very weak and depressed.

February 1st to February 4th, 1927 : influenza-bronchitis. No spirochætes.

This patient was seen for the last time on February 10th, 1927, *i.e.*, 14 days after the first attack. He was well, and had had no relapse.

*Observation 5. — Kule, man, 20 years of age, Banande from Bungulu.*

January 20th, 1927 : attack began in the morning with fever. At 2 p.m., spirochætes in the fresh and stained blood.

1 gm. spirocid.

January 21st, 1927 : general improvement, but fever not yet entirely disappeared.

1 gm. spirocid.

On the following days the patient was well, and rested until the 25th.

January 25th to February 1st, 1927 : fatiguing march with baggage on the slopes of Ruwenzori.

February 1st, 1927 : fever, bronchitis, influenza. The blood-tests made on February 1st, 2nd and 3rd, 1927, were negative.

This patient was seen from time to time up to February 9th, 1927, *i.e.*, 20 days after the first attack ; he had no relapse.

It should be noted that spirocid, even in 2-gm. doses taken at once, caused no gastric or intestinal troubles.

To sum up :

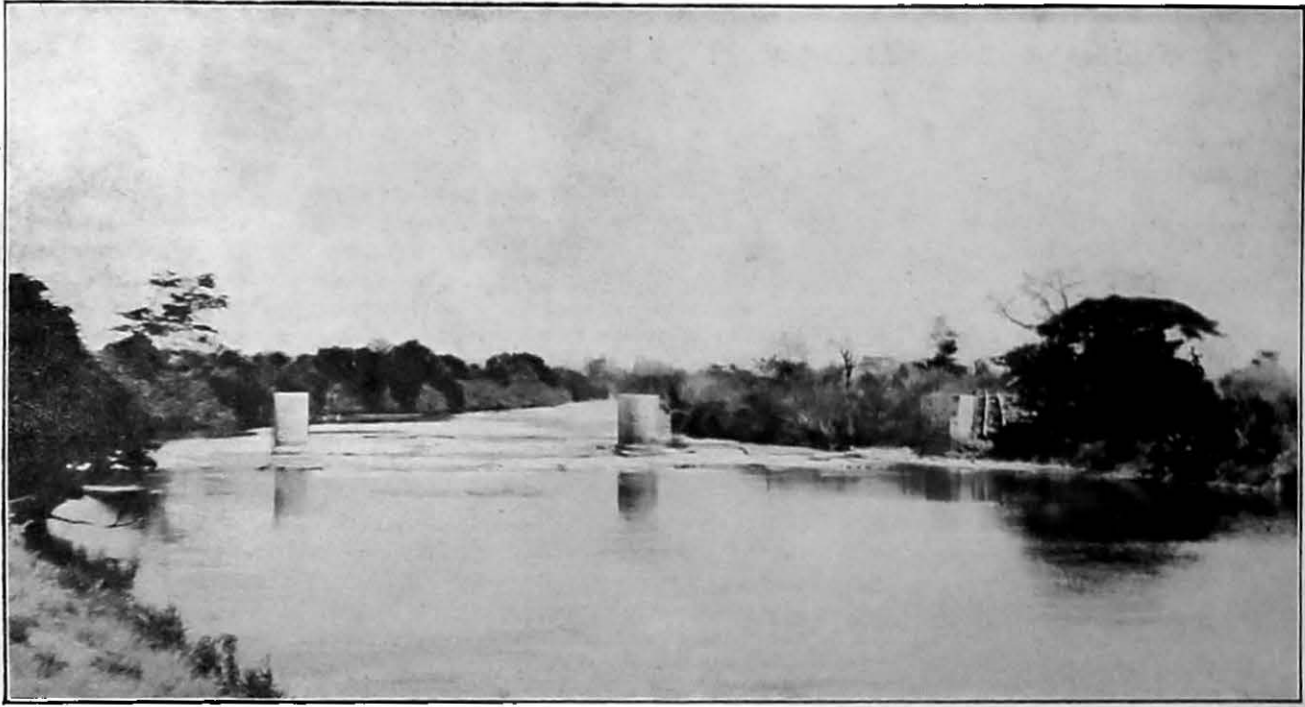
1. *Preventive Action.* — Nine persons were bitten by infecting ticks. Of these, five who had been treated with 1 gm. of spirocid escaped infection. Of the four controls, three were infected, while the fourth, who had probably become immunised, escaped.

2. *Curative Action.* — Five undoubtedly infected persons were treated with spirocid during or soon after the first attack. One of them died — the cause of death was unknown, but it was probably the result of a particularly serious infection — but the four others had no relapse after 9, 20, 14 and 20 days' observation.

#### CONCLUSIONS.

Spirocid has an undoubted preventive and curative action on African relapsing fever. Its preventive effect is in practice the more important, for it may help travellers in infected areas to avoid serious accident to their native assistants and to themselves. It would be of the highest interest to repeat these preventive experiments with "Stovarsol" and other products of the same arsenical series ; moreover, it is important to ascertain what is the minimum preventive dose and the maximum time which can be allowed to elapse between the infecting bite and the administration of the effective dose. The curative action of spirocid is undeniable, but it seems obviously weaker and slower than that of the arsenobenzols. Far from medical aid, however, this product, which can be administered without difficulty and is ordinarily well tolerated, will often make it possible to arrest the disease at the first attack.

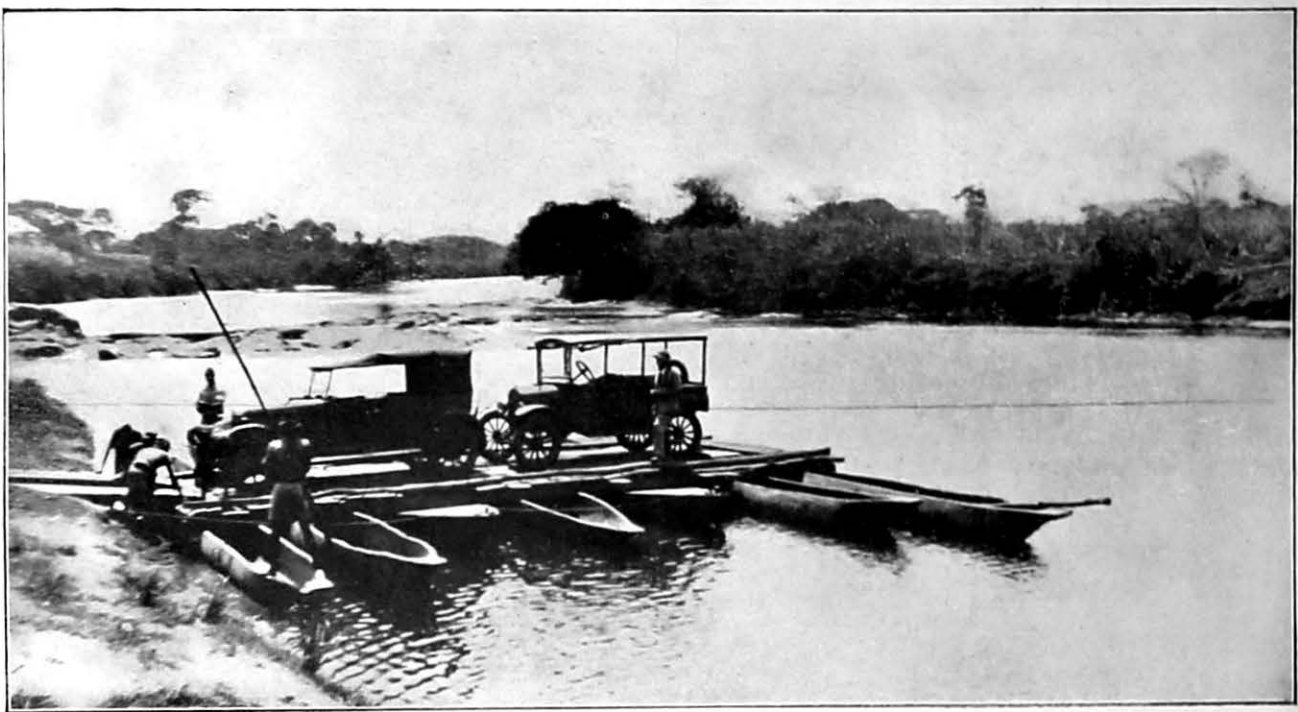
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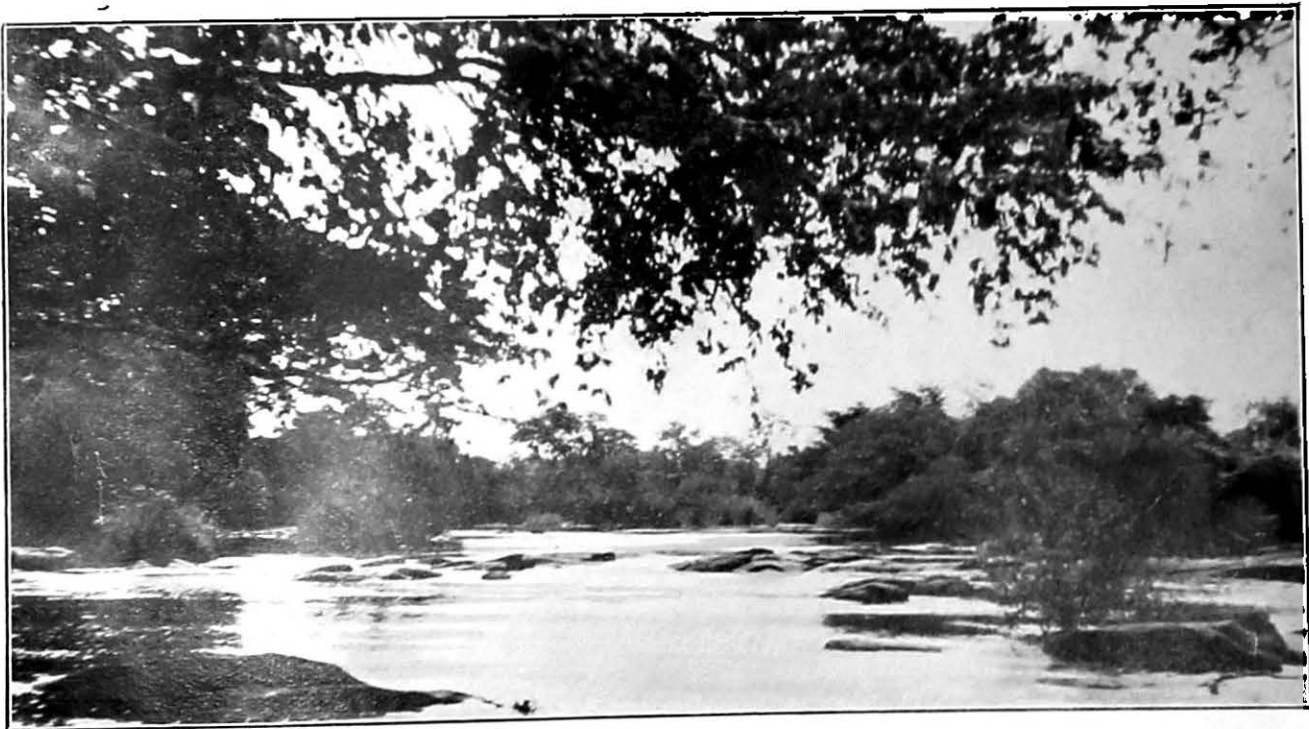
1. Bridge in construction over the River n'Zoro.



2. The River Dingu at Faradje.

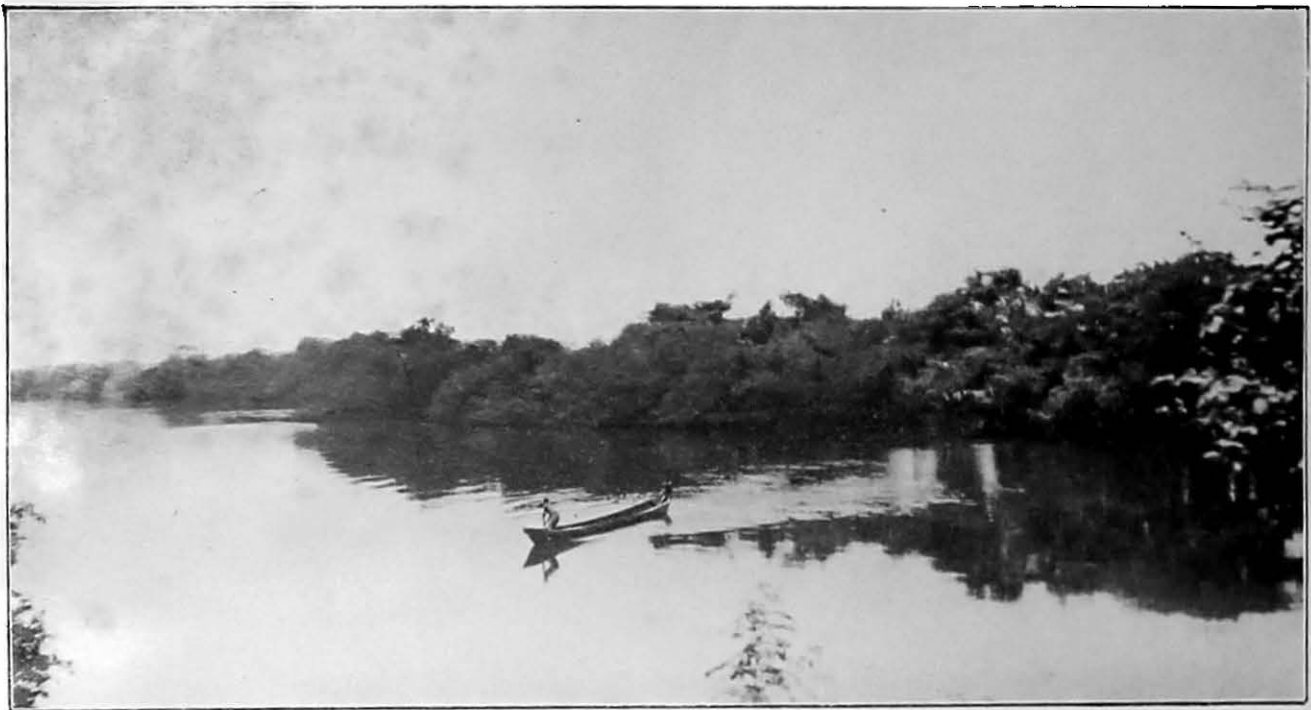


3. Pontoon ferry on the River n'Zoro.

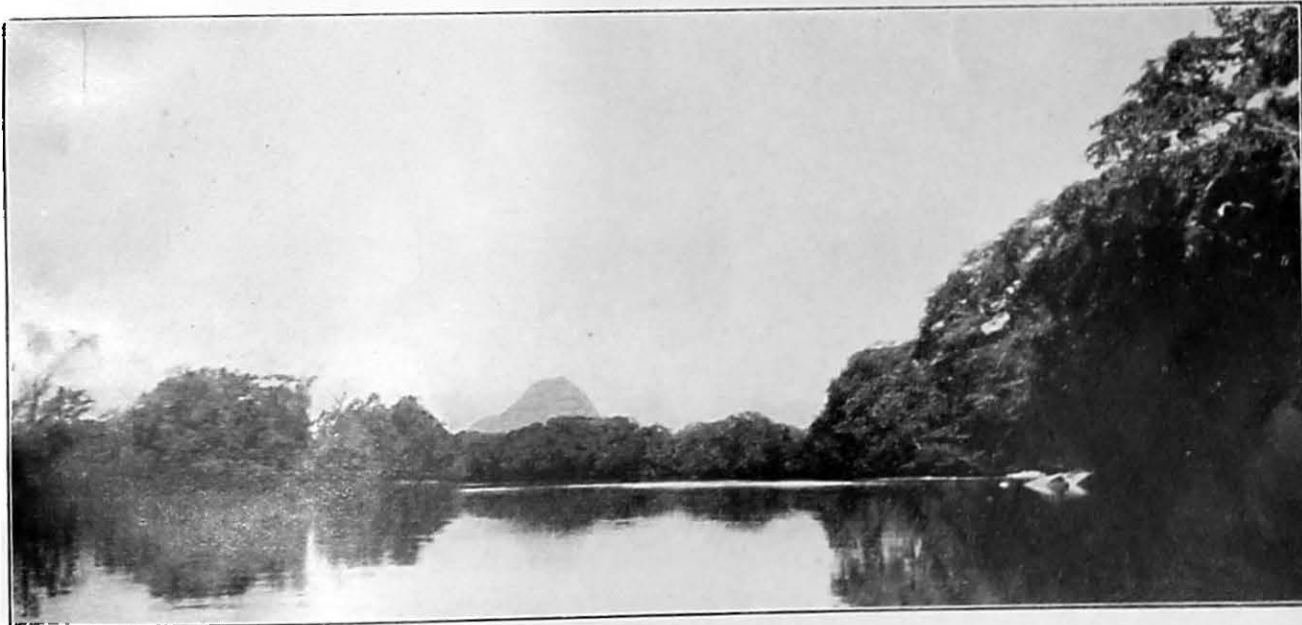


4. The River Dingu on the road from Faradje to Tombo.

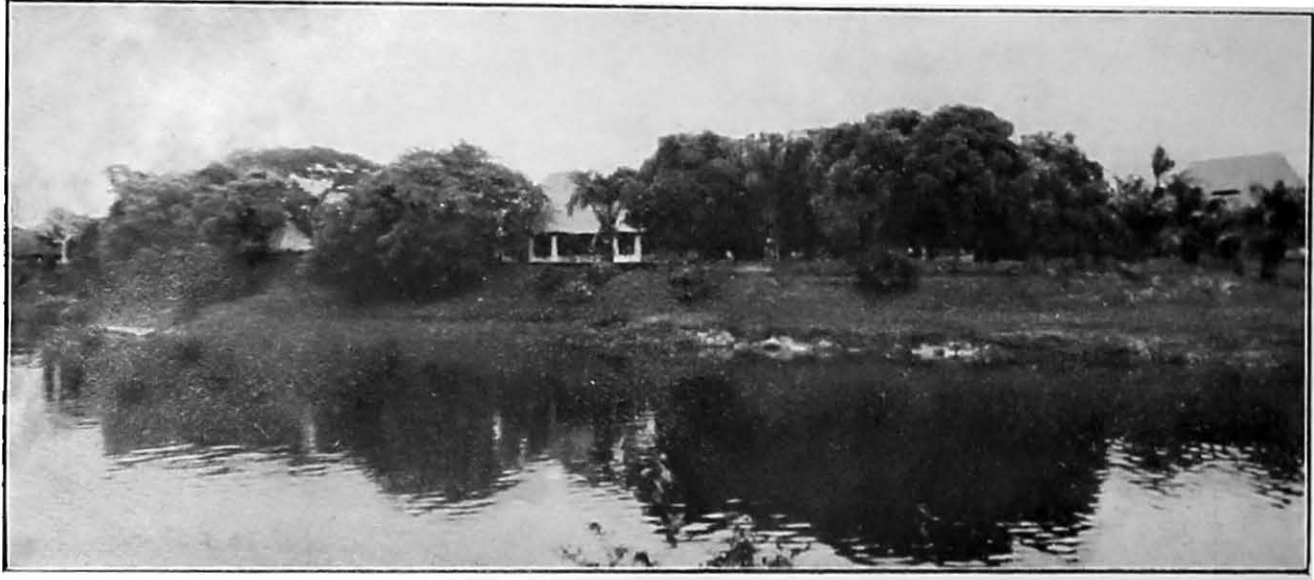




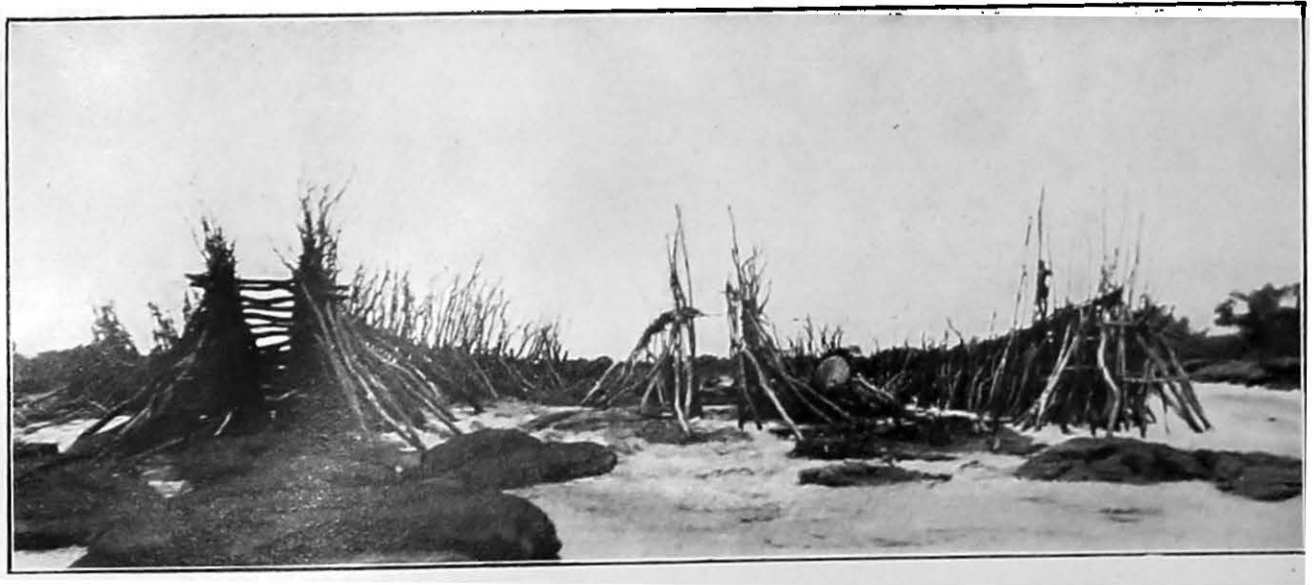
5. The River Kibali, near the mining camps, where a trial of "Bayer 205" as a prophylactic is being carried out.



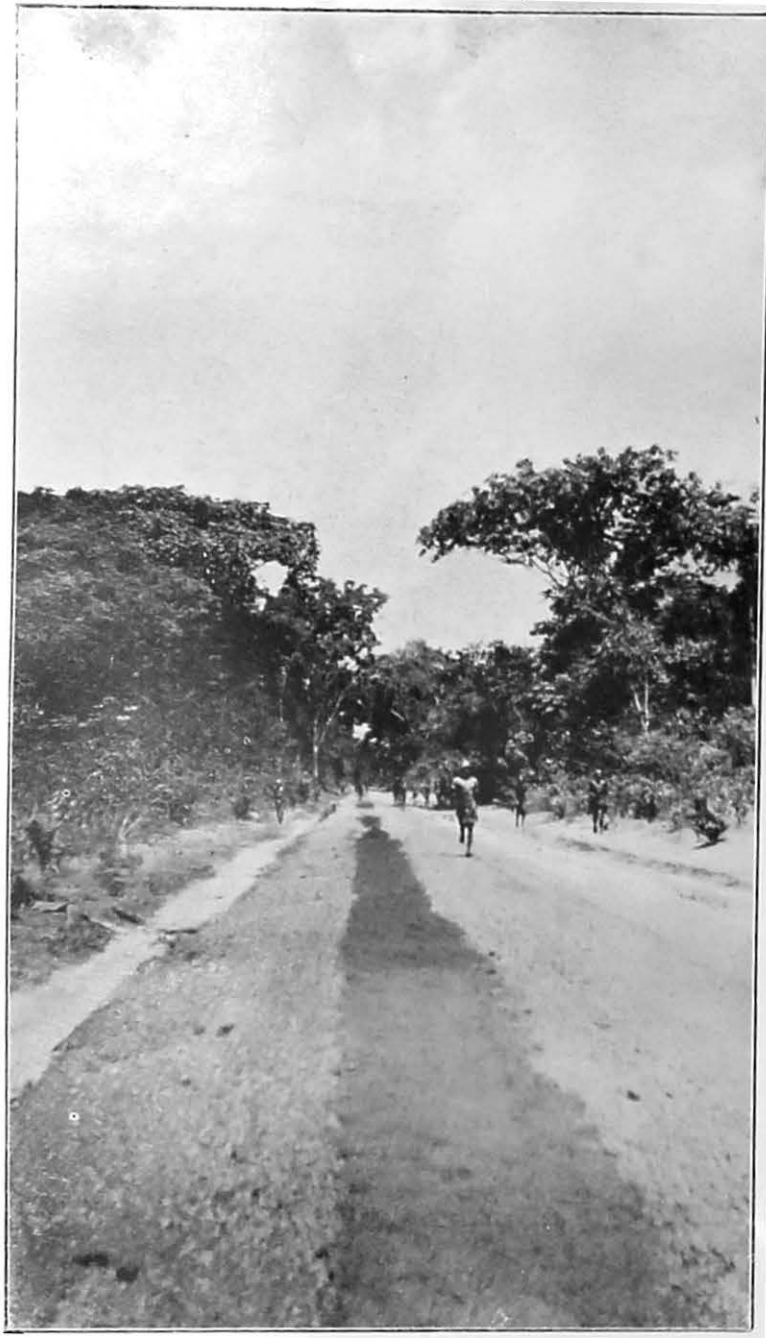
6. The River Kibali and Mount Arama.



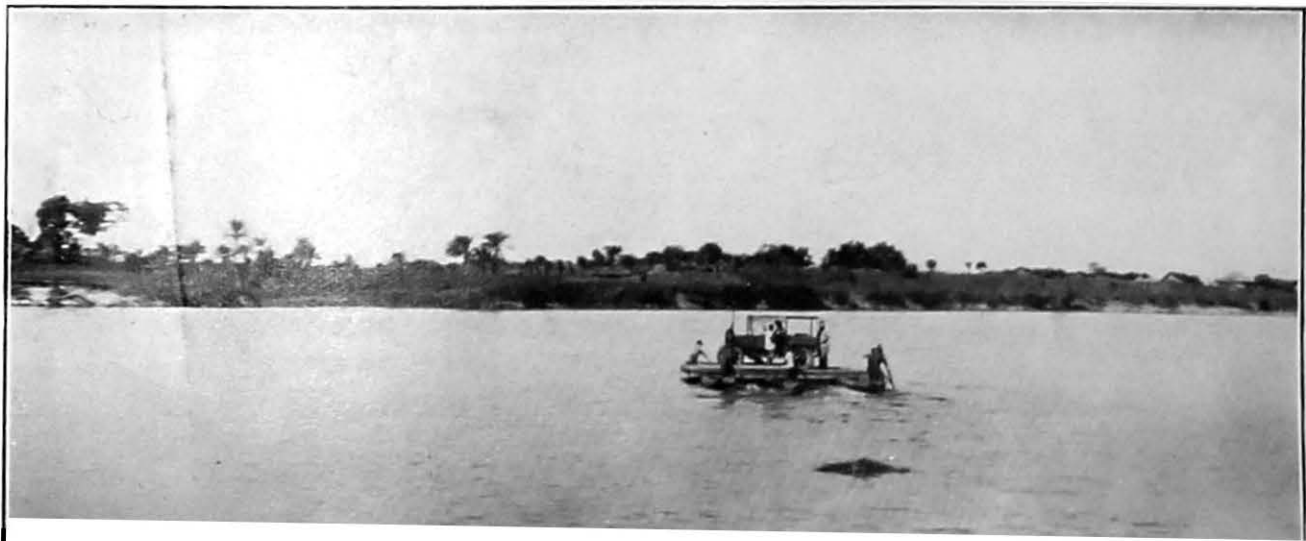
7. Administrative Post of Dungu, at the junction of the Rivers Dungu and Kibali.



8. Fishing-ground in the River Kibali at Dungu.



9. Road under repair between Dungu and Niangara.



10. Pontoon crossing the River Uele at Niangara



11. Bridge over the River Kibali, near Watsa.





13. Bridge over the River Kibali.



14. The River Kibali, near Adranga.



15. Ford across the River Kibali,  
near Adranga.



16. Bamboos on the road from  
Adranga to Tapala.



17. Tree ferns in the Mongwalu Forest

**VII.**

**GENERAL RECOMMENDATIONS FOR THE CONTROL  
OF SLEEPING-SICKNESS IN AFRICAN DEPENDENCIES**

By Professor F. K. KLEINE, Dr. L. VAN HOOF and Dr. H. Lynchurst DUKE.



## GENERAL RECOMMENDATIONS FOR THE CONTROL OF SLEEPING-SICKNESS IN AFRICAN DEPENDENCIES.

By Professor F. K. KLEINE, Dr. L. VAN HOOFF and Dr. H. Lyndhurst DUKE.

In the course of studies undertaken in the field during a number of years past, including the epidemiological work done on this Commission, Professor KLEINE, Dr. VAN HOOFF and Dr. H. L. DUKE have collected a number of observations and facts about human trypanosomiasis — some already published in diverse journals — from which may be drawn certain essentially practical conclusions about the control of the disease both from the point of view of the administrator and of the medical authorities. The following is a brief résumé of the recommendations.

I. The movement of natives ought to be controlled.

This implies :

- (a) A census, carried out by the Administration, of all natives.
- (b) The institution and employment of an identity book or card for each native, in which can be included a medical passport, to which reference will be made later on.
- (c) Delimitation of areas, entry into and departure from which are contingent on the possession by the native of a visa from the medical authorities stating that he is free from trypanosomiasis.
- (d) The formulation and promulgation of a law or laws giving force to all these regulations.  
It is desirable, and in French and Belgian territory has been proved practicable and efficacious, that the medical authorities should, in certain circumstances, be endowed with judiciary powers, as far as concerns the enforcing of this legislation.
- (e) An international agreement, between the Administrations of all countries in which human trypanosomiasis occurs, about the control of the disease on frontiers<sup>1</sup>; an agreement similar to those adopted to control epidemic infectious diseases such as plague and smallpox.
- (f) The establishment of observation-posts where the examination and control of visas can be carried out<sup>2</sup>.

<sup>1</sup> Actually an agreement of this kind already exists on the frontier between the Anglo-Egyptian Sudan and the Belgian Congo, where the administrative authorities hold mixed tribunals, composed of English and Belgian officials, to judge infractions of the local rules that govern native movements across this frontier.

<sup>2</sup> These observation-posts will be, evidently, all administrative or commercial centres where native traffic lines converge — the departure and arrival points of caravans, centres for labour-recruiting activities, ports, Customs posts, etc. The most difficult problem is perhaps that presented by fishermen on the lakes and rivers. Experience acquired on Lake Victoria suggests the establishment of special markets where fish must be brought for sale, and where medical examination of the fishermen can be carried out. This measure would allow the Administration to concentrate the supply of fish in places where it is most in demand for increasing populations or collections of native labour, to regulate prices and at the same time facilitate medical control.

2. The census of infected natives ought to be thorough and complete,  
To ensure this aim there must be :

- (a) An adequate control over the movements of natives, as already indicated above.
- (b) An adequate medical personnel employing the accepted methods of diagnosis.
- (c) A medical passport for every native, on which can be periodically recorded the results of examination for human trypanosomiasis.
- (d) Legal means of compelling natives to submit to medical examination and treatment.

3. Natives infected with trypanosomiasis ought to be treated.

Without insisting on the method of treatment, which will vary according to local circumstances, infected natives ought to be compelled to submit to treatment

This implies :

- (a) If necessary, confinement in a hospital.
- (b) Withdrawal of travel permit and medical passport, *pro tem*.
- (c) After-treatment, careful record of visits, treatment received, etc. This is especially important in the case of natives who come up at intervals for treatment, but who are not confined to hospital.

4. The evacuation of heavily infected zones.

This is an urgency measure, and only to be applied in special cases ; such are epidemic extension in an endemic focus ; impossibility of breaking contact between fly and man ; insufficiency of medical and administrative personnel to carry out prophylactic measures, or of natives to set on foot and maintain sanitary measures.

Before resorting to this measure, organised concentration of population should be tried in carefully chosen areas, where contact between fly and man can be eliminated or adequately controlled and reduced.

5. Clearing measures are generally very costly, and, unless carried out under the control of trained entomologists, are of little permanent benefit. In *palpalis* regions, such measures should be restricted to much-frequented places, and they must be thorough and well maintained. Otherwise they are useless.

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