STUDIES IN TRYPANOSOMIASIS,

WITH SPECIAL REFERENCE TO THE ADHESION TEST.

THESIS

submitted by

MATTHEW JACKSON, M.A., M.B., Ch.B., D.T.M. and H.

PRESENTED FOR DEGREE OF M.D.

TABLE OF CONTENTS

I. HISTORICAL

In his book "The Navy Surgeon" (1734), John Atkins gave the earliest account of Sleeping Sickness on the West Coast of Africa, and, in a few simple, expressive words, records, "The sleepy Distemper (common among the Negroes) gives no other previous notice than a want of appetite two or three days before. Their sleeps are sound and sense of feeling very little: for pulling, drubbing or whipping will scarce stir up sense and power enough to move..... the judgment generally pronounced is Death, this Prognostick seldom failing." Even as late as 1900, the prognosis was equally gloomy.

In 1803 Thomas Winterbottom wrote a short description of the disease in a paper entitled "An account of Native Africans in the neighbourhood of Sierra Leone", and in 1840 there appeared in the London Medical Gazette "Observations on the Disease Lethargus, with Cases and Pathology", by Robert Clarke, Colonial Surgeon, Sierra Leone. In his account of the symptomatology Clarke recorded, "The patient is first observed to become fat, then emaciated. There is an uncontrollable desire to sleep, the patient sometimes falling asleep in the act of conveying food to his mouth: sometimes squinting and convulsions. The glands of the neck become tumefied. This is not always present. Negroes call this

disease sleepy dropsy and regard it as being always fatal." Whereas Winterbottom noted that slaves from the Bight of Benin were most subject to the disease, Clarke observed it prevailing equally among several tribes which inhabited the Continent far inland.

Between 1860 and 1900 French authors provided numerous descriptive accounts of sleeping sickness. Guérin (1869) submitted a Thesis on sleeping sickness to the University of Paris. All his cases occurred amongst negroes who had been transported from West Africa to the West Indies, and in twelve years at Martinique he collected 148 cases of sleeping sickness. In 1876 Corré, who worked amongst the natives of Senegambia, published a very complete account of the clinical manifestations of the disease.

In an interesting account published in the British Medical Journal, Gore (1875) observed that in Portuguese Senegambia the enlargement of the glands of the neck was considered amongst the West Coast Africans to be a premonitory symptom of "African Lethargus", and he gave statistics of the cases encountered at the Colonial Hospital, Sierra Leone. For the four years ending March 31st, 1850, 112 cases of sleeping sickness were treated, and 67 for the seven years ending 31st December, 1866. Of the 179 cases admitted during the eleven year period under survey, 132 died and 47

recovered. Gore further mentioned that on the Gold Coast he had recently treated a private of the 2nd West India Regiment who suffered from lethargic prodromata.

Patrick Manson (1898) gave a clinical lecture on sleeping sickness, illustrated by two actual cases which had been sent from a village on the Lower Congo to Charing Cross Hospital, London, for observation and treatment. Discussing the etiology of sleeping sickness he concluded, with logical and almost prophetic precision, "I think therefore, that the germ of sleeping sickness in this respect resembles that of malaria and that of elephantiasis, that is to say, that at one stage of its existence it must necessarily live in some living host other than man; some animal or plant found only in the Equatorial regions of West Africa." He suggested Filaria perstans as a cause, on the ground that the geographical distribution of F. perstans coincided with that of sleeping sickness. On the other hand he noted that in the Congo, and in other parts of West Africa, fully fifty per cent. of the "healthy" population harboured this parasite, and further, that sleeping sickness was limited to certain endemic foci in Equatorial West Africa, whilst F. perstans was much more widespread in its incidence. In the treatment of these two patients chief reliance was placed on purgatives and tonics, and one, the younger, was put on arsenic with an apparent

amelioration of symptoms. On the death of these two patients, the pathology of the disease was carefully studied by Mott (1899) who shewed that the lesion was essentially a meningo-encephalitis characterised by a perivascular small-celled infiltration.

In May 1901 the European master of a Government boat plying weekly up the River Gambia was admitted to hospital suffering from fever. On May 10th R. M. Forde (1902) examined fresh preparations of this patient's blood microscopically and found therein many actively-moving, worm-like bodies, the nature of which he was unable to ascertain. On 18th December, on Forde's invitation, Dutton, (1902) who was then in the Colony, examined the blood of the patient and found a flagellated protozoon which he at once identified as belonging to the genus Trypanosoma, and in his published description named it "Trypanosoma Gambiense".

Early in 1902, whilst endeavouring to estimate the prevalence of endemic malaria in the Gambia, Dutton found trypanosomes in the blood of an apparently healthy child aged 3 years in a native village about seven miles from Bathurst. In a blood-smear preparation three parasites were counted, the morphology of which was identical with that of the trypanosomes found previously in the blood of the European.

In a letter to the British Medical Journal, Sambon (1902) drew attention to a paper entitled "Sur un trypanosome dans

le sang de l'homme" read by Dr. G. Nepveu in Paris on 24th December, 1898, which contained the statement "In 1890, in consequence of researches made in Algeria on the malarial parasite, I found in the blood of a patient, besides Laverania, a flagellate which seemed rather common. This trypanosome presents all the characters of the genus." The description and diagrams of Nepveu, however, are so lacking in precision, that the accuracy of his diagnosis is doubtful in the extreme. The dubiety becomes almost a certainty by the fact that the blood of a large number of individuals has since been examined throughout Algeria and trypanosomes have never been found.

During 1902 Dutton and Todd (1903) continued to find new cases in a series of investigations in the Gambia on the subject of trypanosomiasis, carried out under the auspices of the Liverpool University School of Tropical Medicine. They examined the blood from 1043 individuals and found trypanosomes in six natives and in one white man (a quadroon), all of whom presented neither definite symptoms of illness, nor anything clinically abnormal, save an occasional rise in temperature and an acceleration of the pulse rate.

Manson (1903) and Manson and Daniels (1903) published the case histories of two European ladies each of whom had contracted "trypanosoma fever" in the Congo. In one of the

cases trypanosomes were not actually discovered by Manson, although his clinical diagnosis was confirmed by Broden of Leopoldville who found the parasite in the patient's blood after she had returned to the Congo from England. Brumpt (1903) at Boumba in the Congo, found T. gambiense in the blood of a ship's officer who had been suffering from an irregular fever of five months' duration, which fever had not been amenable to treatment by quinine. In May 1903 Baker (1903) drew attention to three cases of human trypanosomiasis in African natives at Entebbe in Uganda. This was the first time trypanosomes had been found in human blood in East Africa. Up till this time no suspicion had been entertained of the relationship between "trypanosoma fever" and sleeping sickness.

Bettencourt, Kopke, and other workers (1903), belonging to the Portuguese mission investigating the etiology and treatment of sleeping sickness in Portuguese West Africa, were of the opinion that the malady was due to a diplo-streptococcus discovered by them in the sub-arachnoid fluid. They believed that this organism was identical with a streptococcus which Castellani (1902) had independently described as the cause of sleeping sickness. Broden (1901), of the Leopoldville Bacteriological Laboratory described a slightly motile bacillus which he believed to be the causal agent.

Low (1903) attacked the theory that F. perstans was the cause of sleeping sickness on the two main grounds (1) in British Guiana he found F. perstans in the blood of at least fifty per cent. of the aboriginal Indians of that territory, whilst sleeping sickness was entirely unknown there, and (2) on arriving in Uganda he found no cases of F. perstans in Kavirondo, and yet sleeping sickness was spreading there with great rapidity. These points at once suggested that the nematode had nothing to do with the disease on the point of geographical distribution alone.

In a letter to the Royal Society dated from Entebbe, Uganda, 5th April 1903, Dr. Aldo Castellani, bacteriologist to the Royal Society's Sleeping Sickness Commission, stated: "On 12th November 1902, when examining a specimen of cerebro-spinal fluid taken by lumbar puncture during life from a well-marked case of sleeping sickness, I was surprised to observe a living trypanosome." The parasites were not numerous; about 10 cc. of cerebro-spinal fluid had to be centrifuged for 15 minutes, the trypanosomes being found in the sediment. In 70 per cent. (sic) of 34 cases of sleeping sickness, Castellani found trypanosomes in the cerebro-spinal fluid. In the blood he found the trypanosome once with certainty, but only a few cases were thus tested. In patients with other diseases the cerebro-spinal fluid taken during

life in no case contained trypanosomes, and three of these controls (i.e. with no evidence of sleeping sickness) were cases of the usual "trypanosoma fever". While the trypanosome found in the cerebro-spinal fluid of sleeping sickness cases did not, as far as he could make out, differ materially in size and shape from the species found in the blood of cases of "trypanosoma fever", (i.e. T. gambiense), yet on the other hand there appeared to be certain differences in the position of the micronucleus and in the size of the vacuole, and movements were not apparently so active. Lest it should prove to be a new species Castellani named it T. ugandense.

Bruce and Nabarro (1903), also members of the Sleeping Sickness Commission, soon confirmed this important discovery. Practically every case of sleeping sickness examined by them proved to have trypanosomes in the blood as well as in the cerebro-spinal fluid. In their series, all of 38 cases of sleeping sickness examined had trypanosomes in the cerebro-spinal fluid, and 12 cases out of 13 had trypanosomes in the blood. In the Congo, in a first series of examinations, Brumpt (1903) found trypanosomes in the cerebro-spinal fluid of 78 per cent. of cases of sleeping sickness. In the Congo Free State, Dutton, Todd and Christy (1904) found trypanosomes in the cerebro-spinal fluid of a large number of negroes suffering from sleeping sickness.

Bruce, Nabarro and Greig (1903) in Uganda now attempted to discover whether the trypanosome of sleeping sickness and that of "trypanosoma fever" were identical. They examined the blood of 80 natives in areas where sleeping sickness was endemic and found trypanosomes in 23 cases, while in 117 natives in areas where sleeping sickness did not exist the blood was entirely negative. They shewed in addition, that T. gambiense and the trypanosome of sleeping sickness were morphologically indistinguishable.

Manson (1903) published the notes of a case which, if properly appreciated, would have put the matter beyond all doubt. He traced the clinical history of a case of "trypanosoma fever" through the varied chain of symptoms typical of sleeping sickness ending in the death of the patient from the latter malady. The case was that of a lady who contracted "trypanosoma fever" from the bite of an infected tsetse fly in the Congo on 14th August, 1901. She was seen on 3rd October, 1902 by Manson, whose clinical diagnosis of trypanosomiasis was confirmed subsequently by Daniels finding trypanosomes in her blood. All treatment having proved of no avail she was discharged from hospital on 27th March, 1903. In October she developed a well marked drowsiness and died in a coma on 26th November 1903. At the necropsy and from subsequent histological examination of

the brain by Drs. Low and Mott (1904), unequivocal evidence of sleeping sickness was obtained.

Dutton, Todd and Christy (1904), of the Trypanosomiasis Expedition to the Congo Free State in 1903-1904, set out to settle the same question. They found trypanosomes in the peripheral blood not only of cases in which the diagnosis of sleeping sickness was certain, and of those in whom the case picture was atypical, but also in apparently healthy individuals. In all they examined the blood of 1172 individuals and found trypanosomes in 103 cases. The examination of trypanosomes found in blood from cases of trypanosomiasis, in blood and cerebro-spinal fluid from cases of sleeping sickness (typical or doubtful), and in the blood of experimental animals, led them to the following conclusions:- (1) The trypanosomes seen in the blood of man, whether symptoms of sleeping sickness were present or not, were always the same. The number examined of trypanosomes obtained from the cerebro-spinal fluid was too small to permit of definite conclusions, but the forms observed were similar to those seen in finger blood from the same cases and to those described by Bruce and by Castellani. (2) Organisms found in the blood of rats inoculated with trypanosomes from any of the three indicated sources shewed no difference from those observed in animals infected with T. gambiense. Dutton and his colleagues therefore believed that the organisms seen

by them in the Congo were specimens of T. gambiense.

Thomas and Linton (1904) also studied experimentally in Liverpool this question of identity with the following strains of trypanosomes:-

(a) From Senegambia, three strains of T. gambiense brought by Dutton and Todd.

(b) From Uganda, (1) a strain of trypanosomes obtained by Bruce from the cerebro-spinal fluid of a case of sleeping sickness.

(2) a strain of trypanosomes obtained by Bruce from the blood of a case of "trypanosoma fever".

(c) From the Congo Free State, (1) a strain of trypanosomes from the cerebro-spinal fluid of a case of sleeping sickness.

(2) a strain of trypanosomes from the blood of a case of "trypanosoma fever".

(3) and (4), strains of trypanosomes from the blood of two natives then under observation in Liverpool.

They conducted numerous experiments on rats, mice, guinea pigs, rabbits, cats, puppies, one goat, one donkey, two monkeys, one chimpanzee and one horse. They reached the conclusion that the trypanosomes found in (a) cerebro-spinal fluid of Uganda sleeping sickness cases, (b) cerebro-spinal fluid of Congo Free State sleeping sickness cases, (c) blood

of Uganda "trypanosoma fever" cases and (d) blood of Congo Free State "trypanosoma fever" cases, were all identical in morphology and in animal reactions with T. gambiense, and that the specific name "gambiense" (Dutton) must include all trypanosomes from the above mentioned sources. Laveran (1906) reached the same conclusions from his researches on T. gambiense and T. ugandense.

It was now realised that "trypanosoma fever" and sleeping sickness were both pathogenic manifestations of the one parasite in the human organism; and as neither name of itself was sufficiently comprehensive, the term "Human Trypanosomiasis" came into use.

In 1910 Stephens and Fantham described and named the T. rhodesiense, a new species differing in several respects from T. gambiense. (1) T. rhodesiense was more virulent to laboratory animals, (2) it was resistant to atoxyl, and (3) it differed morphologically in that among the stumpy forms seen in laboratory animals, some individuals had the nucleus situated at the posterior or non-flagellar end of the protozoon. These posterior nucleated forms had never been described previously in films from laboratory animals infected with T. gambiense nor from the blood of patients infected with T. gambiense. Moreover the patient whom they investigated, and who had contracted the sickness in Rhodesia,

had never been in an area infected with Glossina palpalis, but had undoubtedly passed through several areas infected with G. morsitans which they presumed to be the carrier of T. rhodesiense.

Yorke (1910) investigating the morphological characteristics of the new parasite found in the patient of Stephens and Fantham, confirmed their findings regarding the posterior nucleated forms, which he found in experimentally infected rats, guinea-pigs, rabbits, monkeys, dogs, mice, horse and donkey, although never in the blood of the patient himself.

In 1912 Bruce, Harvey, Hamerton, Davey and Lady Bruce, studied the morphology of the organism found in human trypanosomiasis in Nyasaland and concluded (1) that the trypanosome concerned was T. rhodesiense (Stephens and Fantham) and (2) that this was a distinct species, nearly related both to T. brucei and to T. gambiense, but more closely resembling the former than the latter. From this it followed that the human trypanosome disease of North-east Rhodesia and Nyasaland was not the disease known as sleeping sickness in Uganda and on the West Coast of Africa. Gill (1930) proved that Glossina tachinoides was the carrier of T. gambiense in cases of human trypanosomiasis in Northern Nigeria.

II. THE EPIDEMIOLOGY OF TRYPANOSOMIASIS

Geographical Distribution.

Prior to 1901, the known geographical distribution of sleeping sickness was limited to an area of intertropical West Africa of some 1500 miles of latitude, stretching from Senegambia in the North to Loanda in the South, including the islands of Fernando Po, Princes Island and St. Thomas in the Gulf of Guinea. Cases, however, had been encountered as far inland as Stanley Pool on the Congo, almost in the centre of equatorial Africa. It had also been observed in the West Indies, but only amongst negroes who had been brought over as slaves from the West Coast of Africa.

In 1901, several cases of sleeping sickness were discovered in Uganda by Drs. A. R. and J. H. Cook of the Church Missionary Society's Hospital at Mengo, Uganda, into which province in all probability, it had been imported by negroes migrating from the basin of the Congo. The endemic, or possibly epidemic, locus in East Africa was primarily confined to a strip of territory two hundred and fifty miles long by ten miles deep, bordering the northern shores of Lake Victoria Nyanza, and the neighbouring islands. It spread northwards along the Nile, and south eastwards towards German East Africa, the latter territory having been affected by

September 1902.

In 1910 the Mongolla province, the southernmost part of the Soudan was infected, and by September 1911, 208 cases of sleeping sickness had been found and segregated. The epidemic died out in 1914 and the district has since remained free. In March 1918 the Bahr-el-Ghazal province of the Soudan was implicated in an outbreak of sleeping sickness. This lasted till 1928, and 3,596 cases were recorded in the eleven years. At the present time only sporadic cases are found in the Soudan. Whereas in West Africa the disease was endemic and of a chronic nature, it assumed the form of an alarming epidemic in East Africa and it is stated that in Uganda alone, between 1905 and 1909, over 24,000 natives perished of sleeping sickness.

It is to be feared that the disease has not even yet ceased to spread and that ultimately its distribution will become co-incident with that of the appropriate tsetse fly.

The Role of the Tsetse Fly in the Transmission of Sleeping Sickness.

Once it was clearly established by Castellani and his colleagues, Bruce and Nabarro, in Uganda that the trypanosome was the causal agent in sleeping sickness, it became apparent to all skilled in this field of work that, on the analogy of nagana, the etiology of which had been so brilliantly elucidated by Bruce in 1896 in Zululand, a biting fly must

be directly concerned in the spread of the parasite and that the tsetse fly, if present, would be incriminated. Until the time of Bruce's arrival in Entebbe it had been believed that the tsetse fly did not exist in that part of Uganda. Immediately, however, on search being made, the fly proved to be quite common. Specimens were caught in the Botanic gardens at Entebbe and forwarded to Austen at the British Museum for identification, who quite unexpectedly found it to be the Glossina palpalis, a West African fly. Chiefs and missionaries in Uganda were at once circularised and requested to capture and forward to Entebbe as many biting flies as possible for identification. The results proved that the distribution of G. palpalis coincided most strikingly with the distribution of sleeping sickness.

It remained now to experiment with wild G. palpalis fed on laboratory animals and again Bruce and Nabarro were successful. Freshly caught wild flies from the neighbourhood of Entebbe were fed on a healthy monkey, whose blood on 13th May, 1903, contained no trypanosomes. Flies were fed daily on the monkey, and the blood of the latter was examined daily until May 27th, when T. gambiense were found in the monkey's blood. Other experiments were carried out with the same result, establishing the fact that G. palpalis was responsible for the spread of human trypanosomiasis of the T. gambiense type.

Until the beginning of 1909 it was believed that the trypanosomes causing sleeping sickness and nagana were mechanically transmitted by the bite of the tsetse, the theory being that after the fly had fed upon an infected individual the trypanosomes remained in the proboscis of the fly and were injected into a fresh animal at subsequent feeds. Experiments seemed to prove that the fly retained its infectivity for not longer than 48 hours. At the end of 1908, however, Kleine (1909) had begun his classical experiments in East Africa. G. palpalis fed on animals infected with T. brucei were set to feed on fresh animals at various intervals. The flies remained non-infective for a period of eighteen days, after which they remained infective up to the forty-seventh day, when the experiment concluded.

In 1910 Bruce and his co-workers, Hamerton, Bateman and Mackie, (1910), in a series of experiments designed to find out if mechanical transmission could actually take place in nature, proved that (1) mechanical transmission of sleeping sickness by G. palpalis could take place if the transference of the flies from the infected to the healthy animal were instantaneous, i.e., by interrupted feeding: (2) this mechanical transmission did not take place if an interval of time elapsed between the feedings: and (3) mechanical transmission played a much smaller part, if any, in the spread of sleeping sickness, than had been supposed. As late as 1919, however,

Duke (1919), advanced the hypothesis that mechanical transmission by G. palpalis of a virulent strain of T. gambiense played a most important part in the production of a recent epidemic in Uganda.

Yorke and Kinghorn (1912), definitely proved that T. rhodesiense is transmitted by G. morsitans.

According to Hope Gill (1930), Glossina tachinoides carries human trypanosomiasis in Northern Nigeria. This fly has been found infected in nature, and experimental transmission has been effected with T. brucei, T. gambiense, T. vivax and T. congolense. G. tachinoides is believed to be mainly responsible for epidemic sleeping sickness in Nigeria in the northern provinces, but Marshall (1927), is of the opinion that in the Northern Territories of the Gold Coast around Bole, Glossina submorsitans is the carrier.

Kleine (1928), working at Ikoma, Tanganyika Territory, studied an outbreak of sleeping sickness, 56 cases of human trypanosomiasis being discovered. In this instance the parasite was T. rhodesiense, and the vector proved to be Glossina swynnertoni.

It has been ascertained chiefly through the work of Miss M. Robertson, of the Sleeping Sickness Commission, that in the tsetse fly the trypanosomes undergo a cycle of exogenous development. First of all they pass into the gut of the fly,

where they do not attach themselves in any way to the gut wall, but begin to divide into trypanosomes of varying sizes. About the tenth day slender forms begin to predominate. These make their way forward into the proventriculus, thence into the base of the proboscis, and so to the hypopharynx, from which they invade the salivary glands. Here they attach themselves to the walls of the glands and assume crithidial forms in which they continue to multiply. Finally small forms of trypanosomes are produced which resemble closely the type found in the blood. The development in the salivary glands takes from two to five days, after which the fly becomes infective. In the blood of the vertebrate the trypanosomes undergo an endogenous cycle of development; apparently the multiplication takes place only in the circulating blood, intra-cellular dividing forms never having been observed in the lungs, liver or spleen of experimental monkeys. No sexual forms have been observed. There is an enormous fluctuation in the number of trypanosomes to be seen in the blood, due, it is believed, to the trypanolytic action of the host's blood.

It is estimated that 0.03-0.34 per cent. of wild G. palpalis in Uganda are infective; Bruce and his co-workers (1914) have found that in Nyasaland 1.35 per cent. of all wild tsetse flies are infected with some species of disease-producing trypanosomes, 0.02 per cent. being infected with

T. rhodesiense vel Brucei, whilst in the Northern territories of the Gold Coast in 1929, of 569 G. tachinoides caught and examined, T. vivax were present in 14 flies (2.5%), T. Congolense in 14 (2.5%) and T. Grayi in 30 (5.3%).

The Insect Vectors of Trypanosomiasis.

The tsetse flies are of sombre colour, and measure from 6 to 13 millimetres long. When resting, the fly's wings are folded back one over the other, like the blades of a pair of scissors, the abdomen being completely hidden from view. Both sexes suck blood, and after feeding, which takes place about once in three days, the fly hides in the shade of bushes during the process of digestion. The female hatches its eggs within the abdomen, and extrudes single larvae at intervals of eleven days, choosing a shady spot where the larva will find cover under loose dry sand or vegetable debris. A female will produce about twelve larvae in a season. The imago emerges from the puparium in from four to nine weeks.

Geographically the genus Glossina occurs only in the Ethiopian region, viz., in Africa and in Southern Arabia. In Africa, tsetse flies have a wide distribution in the tropical and sub-tropical zones.

The Glossinae have been classified into four groups by Austen (1911), his classification being founded upon external characters. The members of each group may be tabulated thus:-

I. Glossina Fusca Group.

G. tabaniformis
G. nigrofusca.
G. fusca.
G. fuscipleuris.
G. haningtoni.

II. Glossina Brevipalpis Group.

G. longipennis.
G. brevipalpis.
G. medicorum.

III. Glossina Morsitans Group.

G. pallidipes.
G. longipalpis.
G. morsitans.
G. swynnertoni.

IV. Glossina Palpalis Group.

G. caliginea.
G. palpalis.
G. pallicera.
G. tachinoides.
G. austeni.
G. ziemanni.
G. newsteadi.

Several varieties of Glossina morsitans have been described. Newstead (1910) described a form which he termed Glossina submorsitans, but Austen regards G. submorsitans as merely a variety or race of G. morsitans, and states that all West African specimens of G. morsitans really belong to the form "submorsitans".

In the light of present knowledge only members of the Morsitans and Palpalis groups are incriminated in the spread of human and of cattle trypanosomiasis, but it may be affirmed

that all species of Glossinae are suspected of carrying trypanosomes. The habitat of the tsetse fly is of epidemiological importance both as regards diagnosis and prophylaxis, and while later, (vide p. 38) I shall discuss the bionomics of the tsetse fly in the Gold Coast, a brief survey of the main species will indicate the essential features upon which territorial distribution depends.

G. palpalis is essentially a riverine species, haunting the edges of streams where the heavy growth of timber has been cut down, although it may establish itself in the shade surrounding permanent pools of water. Its choice of waterholes as a habitat ensures a constant supply of food from the local villagers and it is thus not dependent on game for its existence. In unpopulated areas it feeds largely upon reptiles such as crocodiles and monitors (varanus).

G. morsitans lives in dry thorny scrub, and is the most widely distributed of tsetse flies. It is not found along the banks of rivers or lakes. It attacks game or man with great voracity and is more likely to be attracted by a moving object than a stationary one.

G. submorsitans is essentially dependent upon the presence of large antelopes for its food supply. Its typical habitat is a country consisting of open grassy glades with scattered clumps of dense shade trees. It does not appear to be dependent on surface water.

G. tachinoides is essentially a riverine species inhabiting a savannah type of country, and is found breeding on the banks of rivers and small streams.

G. longipalpis appears to favour dense thick secondary bush, an intermediate type of vegetation between thick forest and savannah country. It is associated with the smaller antelope.

III. THE SYMPTOMATOLOGY AND DIAGNOSIS OF HUMAN TRYPANOSOMIASIS

It is generally stated that the bite of an infected fly produces an intense local irritation subsiding within a few days. My own experiences in Yeji indicate that a local reaction does not always obtain, and indeed was not met with in any of my patients there or at Accra. After a varying interval of time there follow recurring attacks of fever of an irregular type, several or many days of pyrexia being followed by an afebrile period. The fever may be mild or severe and the evening temperature is always higher than that of the morning. Again, the fever may be continuous over quite a long period, or on the other hand there may be months of remission. The patient usually complains of headaches and of a gradually increasing feeling of weakness. The pulse rate is often accelerated, and the slightest exertion increases the heart rate out of all proportion to the effort involved.

The superficial lymph-glands become enlarged, the posterior cervical and, to a lesser extent, the axillary and inguinal groups being chiefly involved. At first the glands are soft and may be tender, but later they become indurated and may vary in size from that of a pea to that of a pigeon's egg. There is usually a slight degree of anaemia. There may be an annular erythema chiefly seen on the chest, and

occasionally, as in several of my Yeji cases, an itchy papular eruption. The spleen is often enlarged, not a characteristic sign, as splenic enlargement is very common in Africans, quite independently of trypanosomiasis.

After months, or it may be years, signs arise which point to involvement of the central nervous system. The patient develops a dull and listless expression, assumes a shuffling gait, and shows a general disinclination for any exertion; he complains of dull headache, and shirks his day's work. In some instances the victim develops an acute form of mania, which may be either suicidal or homicidal.

A tendency to drowsiness develops and the patient may fall asleep whenever he sits down, or even in the act of mastication. It is no uncommon experience to find a patient drop off to sleep whilst being medically examined. There may be tremors of the tongue or of the hands, and bodily weakness increases.

In the final stage the patient lies down on the floor of his hut and appears to sleep continuously. All power of locomotion is lost and his relatives carry him out of the hut during the day to lie in the shade of a tree, carrying him in again at night. There may be slight fever at this stage, but more usually the temperature and pulse rate are sub-normal. Sometimes there appears to be a fulness of the face in marked

contrast to the condition of the body, which gradually becomes more emaciated. The skin becomes harsh and dry, bedsores may form, and control of the sphincters is lost. Finally the patient dies in coma, or from asthenia.

The duration of the illness is very variable. The disease associated with T. gambiense tends to be much more chronic than that due to T. rhodesiense but in both types of disease, untreated cases usually die. No recovery has been recorded in cases caused by T. rhodesiense, and when the lethargic stage has been reached even in T. gambiense cases, death is inevitable.

From the clinical point of view human trypanosomiasis may be divided into several stages, concerning which unfortunately, there is a considerable lack of unanimity in definition. Clinicians may be divided into two schools, both of which employ a combined clinical and pathological basis of differentiation. The one, the French school, base their definition of stages upon the locus of the parasite, the other lay emphasis primarily upon the state of the cerebro-spinal fluid. The French authors describe two stages, (1) when the parasite is confined to the bloodstream and to the lymphatic glands, with symptoms of loss of power, irregular pyrexia, acceleration of the pulse rate, cutaneous eruptions and hypertrophy of the lymphatic glands: (2) when the parasite has invaded the cerebro-spinal

fluid, a stage characterised by emaciation, headaches and general weakness, accompanied by nervous symptoms with a tendency to somnolence. Koch apparently adopted this classification, dividing his cases into "Leichtkranken" and "Schwerkranken".

On the other hand many physicians describe three stages, (1) where the cerebro-spinal fluid is normal, (2) where there are slight changes in the cerebro-spinal fluid but no nervous symptoms, and (3) where gross changes in the cerebro-spinal fluid are present, accompanied by marked nervous symptoms. British authors adopt the latter classification, the third stage of which is actually that of "sleeping sickness".

Diagnosis:

It will be remembered that in the first cases of human trypanosomasis recorded by Dutton in Gambia, diagnosis was effected by finding the parasites in fresh coverslip preparations or in stained films of finger blood. Castellani found trypanosomes by examining the sediment obtained after centrifuging the cerebro spinal fluid for ten minutes. Bruce and Nabarro (1903) in examining the blood of sleeping sickness patients expected that by centrifuging 10 cc. of blood the trypanosomes would be easily found in the sediment. This proved abortive, however, and so these two workers evolved another technique. 10 cc. of blood were withdrawn from the

arm into a tube containing a small quantity of potassium citrate solution, as an anti-coagulant. This blood was then centrifuged for 10 minutes and the sediment examined. The supernatant fluid was again centrifuged for ten minutes. This process was repeated four times, before the trypanosomes were thrown down, these being usually found in the fourth sediment.

Greig and Gray, (1905), influenced by a suggestion of Mott's, attempted to diagnose cases of sleeping sickness by the aspiration of lymphatic fluid from the enlarged cervical glands and met with instant success. In 62 cases of sleeping sickness, trypanosomes were found by gland puncture in 59 cases, parasites not being found in 3. In 11 cases only, trypanosomes were found in the blood. Of the 3 cases where the gland juice was negative, none showed trypanosomes in the blood.

Koch in 1906-07, in East Africa, found that gland puncture was the most reliable method of diagnosis. Out of 356 punctures he found trypanosomes in 347.

Thomas and Breinl, (1905), recommend that if blood and gland juice examination be negative, and if the examination of centrifuged blood be also negative, then recourse should be had to animal inoculation. Rats are in many ways unsatisfactory, as the incubation period may be prolonged and the parasites present only in small numbers. Monkeys are most

suitable since they are most easily infected. Next in order come young puppies, kittens, rabbits and adult dogs. Kittens can receive 2 cc. of blood intraperitoneally. The parasites appear early in the blood and generally persist in large numbers until death eventuates; periodicity of the parasites is not remarked. The symptoms of the disease in kittens are severe anaemia, loss of weight and stoppage of growth. Purulent conjunctivitis is common. The duration of the disease is from 3 to 7 weeks. Cats are also susceptible and in them the disease runs a course of from three to ten weeks. Puppies are very susceptible and can receive 2 to 4 cc. of blood intraperitoneally. Dogs may survive many months. Emaciation becomes marked after a time, and there is a profuse discharge from the eyes, the conjunctivae becoming inflamed and infiltrated.

Working in the Belgian Congo from 1909 to 1911 Broden (1920), set out to determine the value of different methods of diagnosis in human trypanosomiasis. Three methods were compared, (1) gland puncture, (2) blood examination, direct and after centrifuging 10 ccs. and (3) spinal puncture. The method of centrifuging adopted was as follows:- One cc. of 6% citrate solution was taken into a ten cc. syringe after which the latter was filled with blood from a vein. The citrated blood was centrifuged for three minutes at 900-1000

revolutions per minute. The supernatant fluid was removed and centrifuged again for ten minutes at a speed of 1500 revolutions per minute. In the deposit were found leucocytes, some red blood corpuscles and platelets, almost all the microfilariae, and if trypanosomes were plentiful, a few might be found. Finally the supernatant fluid from the second centrifuging was again centrifuged at 1800 to 2000 revolutions per minute for twenty mimutes, and the deposit examined. In it were to be found a few leucocytes, and red cells, microfilariae and the trypanosomes, if present.

336 patients, examined by the three methods indicated, gave the following results:-

Gland puncture showed trypanosomes in 87.7% of cases.

Centrifuged blood (3rd deposit) showed trypanosomes in 80.7%.

Lumbar puncture showed trypanosomes in 4.5% of cases. Gland puncture was the easiest and most certain method. The triple-centrifuged blood method was indispensable in all cases where the glands were not yet enlarged, or where the enlargement had subsided. Lumbar puncture proved of little diagnostic use.

Jamot (1926) tabulated a series of cases examined by him in the French Cameroons. The trypanosomes were first sought by gland puncture and, when this proved negative or when there were no puncturable glands, the blood was examined

by the thick drop method.

Of 11,482 suspected cases, 7,944 had puncturable glands, and of these 1,507 (18%) were found to contain parasites. Subsequent blood examination revealed parasites in 406 cases. Thus, of 1,913 cases microscopically diagnosed, in 78% parasites were discovered in the gland juice, and in 21% in the blood. These figures show that blood examination is a necessary complement to gland puncture, as the latter alone will allow a definite number of cases to escape detection. It is also very important to re-examine suspects in four or five days, as by that means Jamot found 91 additional infections.

As regards the state of glands in human trypanosomiasis, in a series of 935 positive cases, 36 (3.8%) had normal glands, 38 (4%) had glands which were not puncturable, 137 (14.6%) had small puncturable glands, 273 (29.1%) had glands which were atypical as regards number, form, size, or consistency, and 451 (48.2%) had glands in all respects typical of the disease (Jamot, 1926).

It is interesting to compare the results obtained at other laboratories. Van den Branden and Van Hoof (1923) in their report of work done at the laboratory at Léopoldville state that, in 1922, 9,381 individuals were examined for the presence of trypanosomes. Gland punctures were made in 2,569

individuals, and triple-centrifugalisation of blood in 1,016 cases. By these means 196 cases of human trypanosomiasis were diagnosed. According to Georgelin (1923), at Libreville Laboratory in French Gaboon, in the year 1921-22, 2,529 natives were examined for the presence of trypanosomes, and 99 were found to be infected.

The blood of patients suffering from trypanosomiasis exhibits the phenomenon of auto-agglutination to a notable degree. Martin, Leboeuf and Roubaud (1906-08), of the French Sleeping Sickness Mission working in the French Congo, found that auto-agglutination of the red blood cells was always present in the large number of cases of human trypanosomiasis examined by them. They studied this phenomenon in coverslip preparations of fresh blood. Todd (1910), classified 1406 cases examined by Dutton and himself in the Congo Free State. Auto-agglutination was present in 395 cases, and trypanosomes were found only in 183 of these patients. He admits, however, that probably many cases were missed as these individuals were seen and examined upon one occasion only. Warrington Yorke, in a critical survey of the question, published in 1911, says "in the light of information available, a well-marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence apart from infection with trypanosomes."

IV. CLINICAL AND LABORATORY STUDIES AT YEJI

Most of my work on trypanosomiasis was performed at the field laboratory of the Medical Research Institute at Yeji in the Northern Territories of the Gold Coast during 1930. The staff of the laboratory consisted of the Pathologist (the author) and an African laboratory attendant, together with an animal boy, and several labourers. The laboratory itself was a mud hut (unglazed) with a grass roof, of native construction, and water had to be carried from the River Volta, over a mile distant.

The experimental animals, namely rats, puppies and dogs, kittens and cats, and monkeys, were kept in fly-proof cages in a fly-proof animal house.

In addition to his other duties, the Pathologist acted as Medical Officer, treating a large number of cases of yaws and leprosy, both of which diseases were very prevalent. It was felt that by doing so the laboratory would become a rendezvous for the sick of the district and in this way more cases of trypanosomiasis could be selected than by any other method. Numerous expeditions were made on foot, by car, and by canoe along the river Volta, and into the surrounding Bush in the search for cases of human trypanosomiasis. The Africans themselves recognized advanced cases of sleeping

sickness, and also co-operated in conveying to the laboratory for examination friends who presented enlarged cervical glands.

The main road from the French territory north of the Gold Coast to Kumasi, the Capital of Ashanti, and the rail head of the Gold Coast Railway, runs through Yeji, which is a village situated on the south bank of the River Volta, 140 miles north of Kumasi. By the ferry at Yeji, all traffic entering the Colony from the North and East must cross the river.

Yeji was chosen for the site of the field laboratory in the first place because it is an endemic focus of sleeping sickness, being situated in a fly-belt infested with G. palpalis, G. tachinoides and G. medicorum, and provided the necessary population of fishermen, petty traders and farmers to supply the human reservoir and food supply for the fly. In the second place, the Medical Research Institute had undertaken to examine cattle entering the Gold Coast Colony for the presence of trypanosomes. These animals could not be reared in the Colony because of the deadly and ubiquitous tsetse fly and were consequently imported from the fly-free French territory to the North (Haute Volta). For one year previously the cattle had been examined at the frontier, and it was now decided to examine them after their passage through the fly-infested zone, in order to determine the percentage

of new infections. The two main roads to the south from the French border united north of Yeji, which thus afforded an ideal point for the examination of cattle.

Cattle Trypanosomiasis.

As it was impossible, on account of the amount of work involved, to examine the blood of every animal, one in every three was taken. The cattle were driven into a rude enclosure about fifty yards long by thirty yards wide, and there roped and thrown, after which the Pathologist or his assistant cut the animal's ear with a pair of scissors, and took therefrom a thick drop of blood on a glass slide, this being afterwards stained and examined under the oil immersion lens for the presence of trypanosomes. Two kinds of trypanosomes were observed, T. vivax and T. congolense, of which the former largely predominated.

One third of all cattle had been examined at the frontier during the year 1928, and at Yeji from June 1928 onwards. The highest infection rate at the frontier had been in August 1928, when the percentage of cattle infected amounted to 25%. At Yeji in September 1928 the percentage infected had risen to 75%. Bush clearing was therefore instituted in the Northern Territories along the cattle route at all points where the latter crossed the tsetse "fly-belts". In order to estimate the efficiency of this bush clearing we investigated

the cattle infection rate during 1930 at Yeji.

A table is given below, showing the Yeji cattle figures from March to September 1930.

YEJI FIGURES. CATTLE TRYPANOSOMIASIS

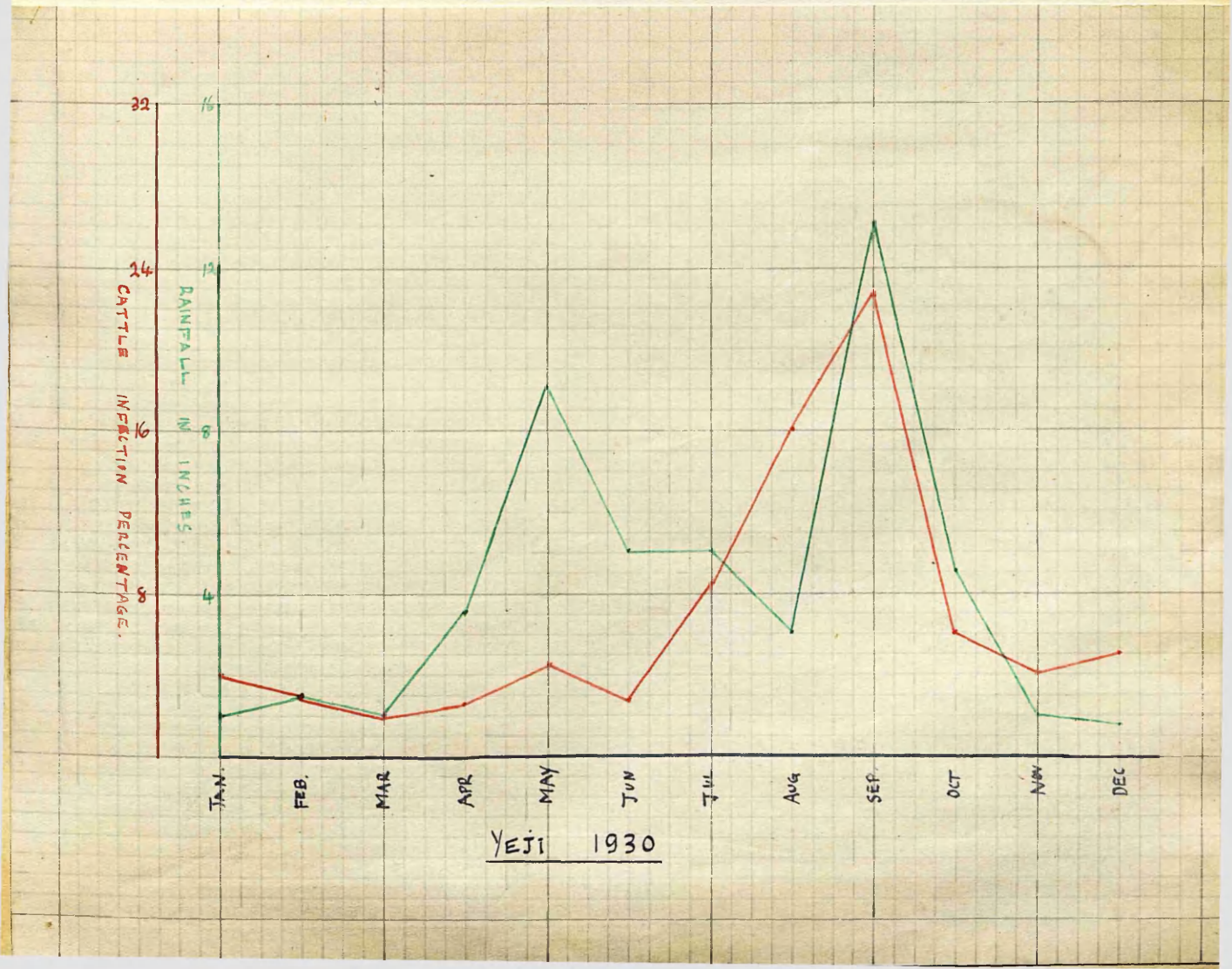
Month	Total Cattle Through	Cattle Examined	Total Infected	Percentage Infected	Type of Tryp.
March 1930	3,319	1,051	15	1.4%	T. vivax
April 1930	3,345	1,051	24	2.3%	T. vivax
May 1930	2,514	792	39	4.9%	(38 T. Vivax (I.T.Congolense
June 1930	2,105	687	20	2.9%	T. vivax
July 1930	2,061	648	55	8.5%	T. vivax
Aug. 1930	1,694	550	89	16.2%	(88 T. vivax (I.T.Congolense
Sep. 1930	749	250	57	22.8%	T. vivax

The blood of 5,009 animals was examined for trypanosomes during seven months, and 299 were found to be infected with trypanosomes, a total infection rate of 5.9%. T. congolense were found only on two occasions, the parasite in every other case being T. vivax.

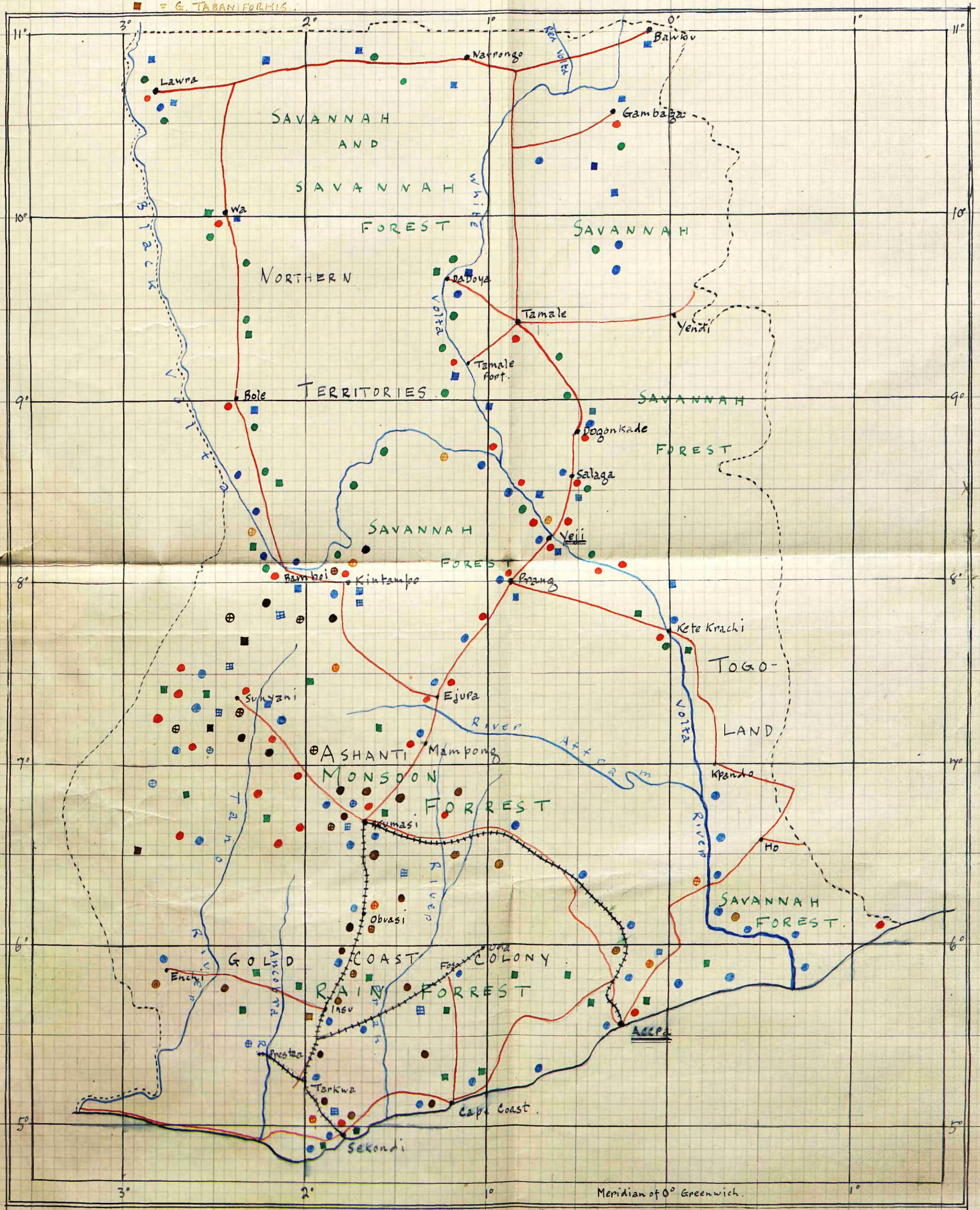
It will be noticed that the percentage infection rate rises to a sharp peak at Yeji in September, rising from 2.9% in June to 22.8% in September. In October it fell sharply

to 6.5%. This peak is due to entomological factors, and is closely associated with the heavy rains of September, which bring about an enormous increase in the numbers of G. palpalis, the carrier, according to Bruce and others, of T. vivax. The accompanying graph shows the relationship between rainfall and cattle trypanosome infection very clearly.

GRAPH SHEWING RELATIONSHIP BETWEEN RAINFALL AND CATTLE TRYPANOSOME INFECTION



● = CASES OF SLEEPING SICKNESS.
⊕ = G. MEDICORUM.
⊕ = G. CALIGINEA.
● = G. PALPALIS
■ = G. TACHINOIDES
⊞ = G. PALLICERA.
● = G. MORSITANS
■ = G. LONGIPALPIS
● = G. FUSCA
⊕ = G. NIGROFUSCA
■ = G. TABANIFORMIS.



The Bionomics of the Tsetse Fly in the Gold Coast Colony.

My personal observation of the territorial distribution in the Gold Coast Colony of the various species of tsetse fly accord with the views of Simpson (1914-15) (1917-18), Pomeroy (1931), and Morris (1931).

A. The Palpalis Group.

(1) G. palpalis is widely distributed in the Colony from the coast to the Northern border. Essentially a riverine species, it is closely associated with all the river systems of the Colony and of Ashanti, whilst in the Northern Territories it is found chiefly in the vicinity of the White and Black Volta rivers.

(2) G. tachinoides is the predominant species of this group in the Northern Territories. It thrives in savannah forest type of country, but is not found in the forest belt nor in the coastal area of the Eastern Province of the Gold Coast Colony.

(3) G. caliginea is found in the heavily forested parts of Western Ashanti.

(4) G. pallicera is distinctly rare, and is confined to the moist forest of Western and Southern Ashanti.

B. The Morsitans Group.

(1) G. longipalpis is found in the dense, thick, secondary

bush country intermediate between the forest and the savannah types. It is common in many parts of the Northern Territories, and on the Eastern coastal plains.

(2) G. morsitans is met with in the western parts of the Northern Territories, around Bole and Kintampo, and near the northern border in the vicinity of Navorongo.

C. The Fusca Group.

(1) G. fusca is essentially a forest species, occurring fairly abundantly along the forest paths and the edges of clearings in the forest belt.

(2) G. nigrofusca, of the same habitats as G. fusca, has been recorded from Sunyani. It is not common.

(3) G. tabaniformis is very rare. It requires abundant shade.

D. The Brevipalpis Group.

Only G. medicorum is found, a large species which has been caught in the dense shade of the thickets and the tall trees fringing the river Volta. It has been recorded from Obuasi in Ashanti and from Sekondi on the coast.

Apparently the carrier of human trypanosomiasis in the Northern Territories and in Ashanti is G. palpalis, although G. tachinoides may also carry here as it has been proved to be a vector in Nigeria. In the Gold Coast we have to deal

with T. gambiense infections only, as is fully borne out by the following evidence. (1) The type of infection produced in experimentally infected laboratory animals is essentially a T. gambiense infection. (2) There are no posterior-nucleated forms of trypanosomes found in the blood of experimental animals. (3) The human cases are all examples of the chronic type of illness produced by T. gambiense and (4) the therapeutic response of the patients to Bayer 205, to tryparsamide and to atoxyl in our hands fully confirms this.

The human material investigated at Yeji was drawn from a wide area, embracing all the Eastern part of the Northern Territories and the adjacent portions of Northern Ashanti. Many patients came from Ejura, over 100 miles distant on the Kumasi road, some from Dogonkade, 40 miles to the north, and some from the confluence of the Black and White Volta rivers, fifty miles upstream.

Trypanosomiasis Surveys.

Apart from the diagnosis of cases at the laboratory, expeditions were made into the neighbouring districts in order (1) to discover new cases of trypanosomiasis and (2) by treating other diseases, to create confidence in the Africans so that they would bring their sick to Yeji where a more thorough investigation could be made. The Africans disliked intensely the procedure of gland puncture and so on these surveys only

direct examination of the blood was made. Individuals with enlarged glands were urged to go to the laboratory at a future date. Such surveys were without immediate results in the diagnosis of new cases as can be seen from the following list.

Village	Number Examined	Positive
Kotokunji	48	-
Kafaba	52	-
Gulibi	78	-
Dogonkade	25	-
Wiase	35	-
Basa	40	-
Jato	38	-
Kawlaw	20	-

Diagnosis.

The routine procedure followed for the examination of patients who presented themselves for treatment at the laboratory was as follows:-

(1) Direct examination of finger blood in a fresh coverslip preparation. Auto-agglutination of the red blood cells although constantly present, was not recorded, as microscopical diagnosis was aimed at. Finger blood examination was repeated at every attendance of the patient.

(2) The patient was examined for palpable glands, especially in the posterior triangle of the neck. When such glands were present, a gland puncture was made, and the gland juice examined as a fresh coverslip preparation. The gland puncture was repeated at subsequent attendances.

(3) If these two methods were negative, examination was

made of triple-centrifugalised blood. 9 cc. of the patient's blood were withdrawn from a vein, 1 cc. of citrated saline added, and the mixture centrifuged slowly for ten mimutes. The supernatant fluid was pipetted off and put aside. The leucocyte layer and the sedimented red corpuscles were each examined microscopically for trypanosomes. If no parasites were found the supernatant fluid was again centrifuged, slowly for five mimutes and rapidly for the same length of time. The supernatant fluid was again pipetted off, and the second sediment examined for trypanosomes. If still negative, the supernatant fluid was once more centrifuged, this time rapidly for twenty mimutes, and the final sediment examined for parasites. It was in this last deposit that trypanosomes were most frequently found in positive cases.

(4) In cases negative to the above tests, but in which, in my opinion, there were grounds for believing that trypanosomiasis was present, I had recourse to animal inoculation.

Results of Direct Examination of the Peripheral Blood.

In all 350 individuals were examined at Yeji, many of them on numerous occasions, and only in one case (Case No. LXX), were trypanosomes found by direct examination of peripheral blood. The trypanosomes in this single instance were comparatively numerous, being present to the extent of

3 to 5 in one field. The parasites were also found in this case by gland puncture, the cervical glands being much enlarged and soft.

Results of Examination by Gland Puncture.

Of 350 individuals examined at Yeji, enlarged and puncturable glands were found in 123, in each of whom gland puncture was performed. Trypanosomes were discovered in 7 cases, the parasites having already been discovered in the finger blood in one case (vide supra).

Results of Examination by Triple-Centrifugalised Blood.

In 101 patients, examination of triple centrifugalised blood led to the diagnosis of 24 new cases. In testing for cure at the end of a course of treatment this procedure was carried out in 15 cases and trypanosomes were found in 4.

The following table shows the deposit in which the trypanosomes were found:-

Leucocyte layer	-	0 times
1st sediment	-	2 "
2nd sediment	-	4 "
3rd sediment	-	18 "

In the tests for cure trypanosomes were found only in the 3rd sediment (4 times).

Lumbar Puncture.

No attempt was made at Yeji, in the absence of hospital facilities, to perform lumbar puncture, it being felt that this procedure was not justifiable, and that it would only result in the Africans refusing to come to the Laboratory at all. Later, at the Gold Coast Hospital in Accra, some experience was gained in this subject, the discussion of which is deferred meantime (vide p. 90).

Results of Inoculation of Blood into an Animal.

Eight cases of human trypanosomiasis were diagnosed by inoculation of the blood of patients into laboratory animals.

Summary of Diagnostic Results at Yeji.

Trypanosomes in Fresh Blood	1 Case
Trypanosomes in Gland Juice	6 Cases
Trypanosomes in Triple Centrifugalised Blood	24 Cases
Inoculation of Blood into Animal	8 Cases

In addition to those microscopically diagnosed, there were several cases which appeared clinically to be sleeping sickness, but in which, despite the most careful investigation, trypanosomes could not be demonstrated. In one of these, seen in Salaga hospital, a lumbar puncture was made, and the cerebro-spinal fluid showed an increased cell count, but no trypanosomes. Triple-centrifugalization of the blood, gland puncture, and animal inoculation all likewise proved

negative. The adhesion test, on the other hand was positive, (+ + - - -) and this, in my opinion, established the diagnosis. The Adhesion Test is discussed in full (p. 101).

Animal Inoculation and the Maintenance of Strains of Trypanosomes

For reasons which will be apparent later, it was necessary to maintain a series of strains of trypanosomes. These were obtained primarily from the inoculation of human infected blood either (a) in the course of attempted diagnosis or (b) from cases of known infected individuals before treatment commenced. All were strains of T. gambiense, which had been isolated from patients seen at Yeji.

The animals at my disposal were young and adult dogs, and cats, monkeys and rats. There were two types of rats, one small and brown, slightly smaller than the common English rat, light brown on the head and back, and white underneath; the other was a larger black animal, Cricetomys Gambianus (the African pouched rat), about the size of an adult rabbit. In my experience and in that of my predecessor Dr. G. F. T. Saunders, it was found that direct inoculation from man to rat was rarely, if ever, successful. This being the case, in the diagnosis of human trypanosomiasis the first inoculation from a patient was never made into a rat.

Method of Inoculation.

Kligler and Weitzman, (1924) working on the subject of trypanosomiasis in Palestine, had found it possible by the injection of olive oil, to break down artificially the resistance of rabbits and guineapigs to trypanosomes. They found that in rabbits and guineapigs, in which trypanosomes, without treatment, had temporarily disappeared from the blood-stream, the intra-peritoneal injection of 4 cc. of olive oil caused the reappearance of trypanosomes in the peripheral circulation in from 24 to 48 hours. In a few animals previously "cured" from infection by a dose of Bayer 205, similar injection of olive oil succeeded in bringing to light an infection which had apparently remained latent.

Before inoculation the animal's blood was examined for trypanosomes. 2.5 cc. of human blood, diluted with an equal quantity of citrated saline, were injected intraperitoneally into the experimental animal. Following the method of Kligler and Weitzman, two days after the animal received the injection of human blood, 2 cc. of olive oil were given intraperitoneally. Thereafter examinations were made daily, or at intervals of two or three days, for trypanosomes in the peripheral circulation.

Experiment to show the effect of the intraperitoneal injection of olive oil on rats previously inoculated with T. gambiense.

Day of Experiment	Rat 81	Rat 82	Rat 83	Rat 84	Rat 85	Rat 86
1.	All inoculated with T. gambiense.					
3.	3 cc. olive oil	3 cc. olive oil	3 cc. olive oil			
5.	Tryps. numerous	Tryps. 4 to a field.	Died	Negative	Negative	Negative
9.	Killed	Numerous		3 cc. olive oil	3 cc. olive oil	3 cc. olive oil
10.		Numerous		Numerous	Tryps. 4 to a field	Numerous

The injection of olive oil two days before rather than two days after inoculation may be preferable, but as one could not always foresee when infected cases would arrive at the laboratory, olive oil was injected after the inoculation with trypanosome infected blood.

Several illustrative examples of animal inoculation experiments follow:-

Source of human blood: Dogo Gariba. 17/4/30.

Clinical Record: Glands enlarged, gland puncture negative, blood negative, triple centrifuged blood negative. Adhesion test + - - - -

4 cc. citrated whole blood injected intraperitoneally to Monkey J.I.

Date	Blood Examination	Trypanosomes found in blood.	Remarks
17.4.30	negative	nil	4 cc. citrated whole blood injected intraperitoneally from Dogo Gariba.
19.4.30	do.	do.	2 cc. olive oil injected intraperitoneally.
22.4.30	do.	do.	
25.4.30	do.	do.	
28.4.30	do.	do.	
1.5.30	do.	do.	
4.5.30	do.	do.	
7.5.30	do.	do.	
10.5.30	do.	do.	
14.5.30	do.	do.	
19.5.30	positive	very few	
21.5.30	positive	3-5 in field	
23.5.30	positive	3-5 in field	
26.5.30	positive	3-5 in field	
30.5.30	negative	nil	
3.6.30	do.	do.	
11.6.30	do.	do.	
15.6.30	do.	do.	
23.6.30	do.	do.	
29.6.30	do.	do.	
9.7.30	do.	do.	
16.7.30	do.	do.	
18.7.30	do.	do.	
23.7.30	do.	do.	
5.8.30	do.	do.	
13.8.30	do.	do.	

In this case the incubation period was thirty-two days, after which trypanosomes were in the blood only for a short period and thereafter the blood remained negative for two and one half months when the experiment concluded.

Source of human blood: Iddi. 23/4/30.

Clinical Record: Glands enlarged, gland puncture negative, blood negative, triple centrifuged blood negative. Adhesion Test with Rat 301 (strain XV.) + + - - -

4 cc. citrated whole blood injected intraperitoneally to Monkey J.2.

Date	Blood Examination	Trypanosomes found in blood.	Remarks
23.4.30	negative	nil	4 cc. citrated whole blood injected intraperitoneally from Iddi.
25.4.30	do.	do.	2 cc. olive oil injected intraperitoneally.
28.4.30	do.	do.	
1.5.30	do.	do.	
4.5.30	do.	do.	
7.5.30	do.	do.	
10.5.30	do.	do.	
14.5.30	do.	do.	
19.5.30	do.	do.	
21.5.30	do.	do.	
23.5.30	do.	do.	
26.5.30	do.	do.	
30.5.30	do.	do.	
3.6.30	do.	do.	
11.6.30	do.	do.	
15.6.30	positive	3-5 in field.	
16.6.30	positive	numerous	
18.6.30	positive	swarming	blood to Cat 41 and to Rats 433, 434 and 435. Monkey moribund, killed with chloroform.

In this case the incubation period was fifty-three days. The infection proved to be very severe, however, and proceeded to a fatal issue in four days.

Source of human blood: Sodengi. 23/4/30.

Clinical Record. Glands enlarged, gland puncture negative, blood negative, triple centrifuged blood negative. Adhesion Test + - - - - .

4 cc. citrated whole blood injected intraperitoneally to Monkey J.3.

Date	Blood Examination	Trypanosomes found in blood.	Remarks
23.4.30	negative	nil	4 cc. citrated whole blood injected intraperitoneally from Sodengi.
25.4.30	do.	do.	2 cc. olive oil injected intraperitoneally.
28.4.30	do.	do.	
1.5.30	do.	do.	
4.5.30	do.	do.	
7.5.30	do.	do.	
10.5.30	do.	do.	
14.5.30	do.	do.	
17.5.30	do.	do.	Monkey died. No trypanosomes in blood, post-mortem.

Experiment negative.

Cats.

With regard to cats, the incubation period varied from six days in one case (the shortest of all primary inoculations), to one hundred and fourteen days (the longest of all the incubation periods), in another case. Several illustrative inoculation experiments are given.

Source of human blood: Yao Maja. 17/4/30.

Clinical Record: Glands enlarged, gland puncture negative, blood negative, triple centrifuged blood negative. Adhesion Test, + + - - - - .

4 cc. citrated whole blood injected intraperitoneally to Cat 20.

Date	Blood Examination	Trypanosomes found in blood	Remarks
17.4.30	negative	Nil	4 cc. citrated whole blood injected intraperitoneally from Yao Maja.
19.4.30	do.	do.	2 cc. olive oil injected intraperitoneally.
21.4.30	do.	do.	
23.4.30	positive	numerous	
25.4.30	positive	numerous	
29.4.30	positive	swarming	1 cc. citrated whole blood to Rats 317, 318. Left eye of cat inflamed. Purulent conjunctivitis. Cat thin and emaciated.
4.5.30	positive	swarming	
5.5.30			Cat died.

Incubation period six days. Duration of disease, fourteen days.

Source of human blood: Bavia (of Zongo Zerigi) 21/4/30.

Clinical Record: Sleeps whenever he lies down. Symptoms of 2 months' duration. Glands enlarged, gland puncture negative, fresh blood negative, 24/6/30. Adhesion Test + + - - - , triple centrifuged blood, third sediment, trypanosomes present.

2 cc. citrated whole blood into Cat 38, intraperitoneally.

Date	Blood Examination	Trypanosomes found in blood.	Remarks
21.4.30	negative	Nil	2 cc. citrated whole blood injected intraperitoneally from Bavia.
23.4.30	negative	Nil	2 cc. olive oil injected intraperitoneally.
Examined till			
5.8.30	negative	Nil	
13.8.30	positive	1-3 in field	
18.8.30	positive	numerous	
23.8.30	positive	swarming	Blood to Rats 560, 561.
2.9.30	positive	swarming	Blood to Dog J.9.
4.9.30			Cat died. No trypanosomes in blood P.M.

Incubation period One hundred and twelve days; duration of disease twenty-three days.

Source of human blood: Mamah Kanjarga. 18/6/30.

Clinical Record: Glands enlarged, gland puncture negative, fresh blood negative, triple centrifuged blood negative. Adhesion Test + + - - - .

4 cc. citrated whole blood injected intraperitoneally into Cat 34.

Date	Blood Examination	Trypanosomes found in blood.	Remarks
26.5.30	negative	Nil	4 cc. citrated whole blood from Mamah Kanjarga injected intraperitoneally.
28.5.30	do.	do.	2 cc. olive oil injected intraperitoneally.
30.5.30	do.	do.	
3.6.30	do.	do.	
7.6.30	do.	do.	
11.6.30	do.	do.	
15.6.30	do.	do.	
16.6.30	do.	do.	
18.6.30	positive	1-3 in field	
20.6.30	positive	numerous	
22.6.30	positive	Swarming	Blood to Rats 439, 440. Cat died in evening.

Incubation period twenty-three days, duration of disease four days.

Source of human blood: Kwamie. 11/8/30.

Clinical Record: Glands much enlarged, gland puncture negative, fresh blood negative, triple centrifuged blood, second sediment, positive. Adhesion Test + + - - - .

3 cc. citrated whole blood injected intraperitoneally to Cat 54.

Date	Blood Examination	Trypanosomes found in blood.	Remarks
11/8/30	negative	Nil	3 cc. citrated whole blood injected intraperitoneally from Kwamie.
13/8/30	do.	do.	2 cc. olive oil injected intraperitoneally.
15/8/30	do.	do.	
23/8/30	do.	do.	
29/8/30	do.	do.	
2/9/30	do.	do.	
4/9/30	do.	do.	
6/9/30	do.	do.	
8/9/30	positive	3-5 in field	
10/9/30	positive	numerous	
12/9/30	positive	numerous	
14/9/30	positive	swarming	Blood to Rats 608, 609
15/9/30	positive	swarming	Cat died.

Incubation period twenty-eight days, duration of disease seven days.

After the trypanosome infection became well established in the blood of the experimental cat, dog, or monkey, (i.e. when each field as seen by the microscope contained a large number of trypanosomes), 1 cc. of blood taken from the animal's ear was diluted with an equal quantity of citrated saline, and inoculated intraperitoneally into a rat. Thereafter the strain was maintained by passage through rats. In the latter the incubation period averaged 4 days, the shortest being one day, and the longest, in strong strains, six days. The length of life of rats after successful inoculation averaged eight days; sometimes in a virulent infection the rats died in two days, and on the other hand they might survive for sixteen days. It was part of the difficulties with which one had to contend, that on returning from trek one would find that a strain had been allowed to die out completely through the death of all the infected animals. For this reason it was always necessary to keep a number of strains in existence.

With regard to the incubation period in rats, I made the interesting observation that the presence of sepsis in an animal delayed the appearance of trypanosomes in the peripheral blood. The following table shows an illustrative example:-

Date	Rat 509	Rat 510
	septic tail	normal tail
26.7.30	1 cc. blood from Cat 43 (*T. gambiense*)	
29.7.30	negative	swarming
10.8.30	swarming	

This phenomenon was noticed on several occasions. I also found occasionally that although two rats each received the same dose of infected blood, the one became infected and the other remained free from trypanosomes.

In inoculating fresh rats from infected rats the general procedure was to add two drops of blood from the tail of the latter to a small quantity of citrated saline and divide the dose between two fresh rats. When adhesion tests were being performed, however, the infected animal was killed, the heart's blood withdrawn by means of a syringe containing citrated saline, and 1 cc. of this mixture inoculated into each of the fresh animals.

When a rat once became infected it never on any occasion recovered naturally from the infection. The disease was always progressive and invariably proved fatal, the great majority dying in convulsions. The only symptoms observed during life were lessened activity and a refusal to take food.

V. THE THERAPEUTICS OF HUMAN TRYPANOSOMIASIS

The earlier writers on the subject of sleeping sickness were unanimous in recording the fact that treatment was of no avail. Braid (1858) suggested that arsenic in small doses, such as ½ grain or more, might be given to oxen bitten by the tsetse fly. Balfour (1858) recommended larger doses, not less than ten or twenty grains, to be given in solution as arsenite of potash. In May 1858 the great David Livingstone recorded that the idea of employing arsenic in the disease which follows the bite of the tsetse fly had occurred to him in 1847 or 1848. He gave two grains of arsenic daily for a week to a mare which had fallen sick after prolonged exposure to the bite of the tsetse. The mare seemed to recover and he thought that the disease was cured. Two months later, however, the malady returned and the animal, refusing the arsenic, ultimately died six months after it had been bitten. Strangely enough, Livingstone stated that the bite of the tsetse fly was harmless to man, and to wild animals, and even to calves as long as they continued to suck the cows.

In 1893 Lingard, in his researches upon Surra, found that of all the drugs which he used in the treatment of horses, only arsenic was of any use in modifying the course of the disease. In 1896 Bruce working in Zululand, found arsenic

to be of value in the treatment of nagana, the drug prolonging the animal's life, and causing the disappearance, for a time at least, of trypanosomes from the peripheral circulation. A complete cure was not usually obtained.

In 1902 Laveran and Mesnil published the results of their work on animals experimentally infected with T. brucei. They found that by subcutaneous injection of sodium arseniate in infected mice, rats, and dogs, they could cause the parasites to disappear temporarily from the peripheral circulation. The treatment had its disadvantages as the curative dose was little removed from the toxic dose, and in the end, a treated animal died of nagana if the treatment were suspended, or from the toxic effects of arsenic if the treatment were persisted in.

In 1904 Ehrlich and Shiga published the results of their experiments with a new dye called by them "Trypan red" by which they were able to cure mice experimentally infected with "mal de caderas" (T. equinum). In rats the dye caused merely a temporary disappearance of the parasites from the circulation. Various workers took up this line of research. Thomas and Breinl (1905) tried a combination of trypan red and arsenic in the treatment of experimental trypanosomiasis. Trypan red they found to cause a nephritis, whilst arsenic, injected subcutaneously, caused a local necrosis. They

determined therefore, to seek a less toxic preparation of arsenic, and one which would entail less danger of necrosis if injected into the tissues. This they found in atoxyl, the anilide of met-arsenious acid. This was later shown by Fourneau not to be a new preparation, it having been synthesised as early as 1863 by Béchamp in the early days of the synthesis of aniline dyes. Ehrlich, in 1907, stated that atoxyl was the sodium salt of p-amido-phenyl-arsenic acid (i.e. sodium arsanilate).

Atoxyl in Trypanosomiasis.

Thomas (1905), and later Thomas and Breinl (1905), experimented very fully upon the use of atoxyl at the Runcorn Research Laboratories, Liverpool, trying it against five different strains of T. gambiense and against T. brucei, T. evansi, T. equinum, T. equiperdum and T. dimorphon in numerous laboratory animals, including monkeys, dogs, kittens, rabbits, guinea-pigs, rats and mice. These authors, in reviewing their experience, concluded, "Of the various preparations tried, atoxyl has proved the most satisfactory, but it is not ideal. It is not non-toxic, but it is not so toxic as sodium arseniate. It does not produce the sloughing which so often follows the subcutaneous inoculation of sodium arseniate, it causes no pain, and its administration can be continued over a period of many months. It is the

only remedy at present giving any prospects of a cure." They therefore recommend the treatment of human trypanosomiasis with atoxyl.

Breinl and Todd (1907), also of the Liverpool School of Tropical Medicine, wrote a brief article on atoxyl in the treatment of trypanosomiasis, in which they stated that since October 1905 the Liverpool School of Tropical Medicine had sent 2,800 grammes of atoxyl to doctors and missionaries in East and West Africa, with instructions as to its use. In Europe in the interim, the drug had been used in the treatment of trypanosomiasis both of Europeans and of Africans with varying degrees of success. Daniels of the London School of Tropical Medicine informed the authors that he had treated two cases over a period of 10 and 14 months respectively, with good results. There were no parasites in the patients' blood and they exhibited no symptoms of the disease. Van Campenhout (1907) obtained good results in three Europeans in the third stage of trypanosomiasis by means of atoxyl, in conjunction with strychnine and cold baths. Both Daniels and Van Campenhout used a five or ten per cent. solution of atoxyl injected intramuscularly. The treatment recommended by Breinl and Todd is the injection of a 20% solution in saline, commencing with 0.6 cc. and increasing to 1.0 cc., daily injections of the latter dose being given for a fortnight, and then maintained by giving twice weekly doses until

the blood is negative and all symptoms have disappeared. Thereafter a weekly dose of 1.0 cc. of this 20% solution of atoxyl is given for as long a period as possible.

Robert Koch, in an expedition to East Africa in 1906-07, treated a large number of cases of sleeping sickness with atoxyl. He started very cautiously, commencing with a dosage of 0.06 G. given subcutaneously in the back. By stages he increased the amount of atoxyl given in a single dose, until he had reached 0.5 G. On giving this dose on each of two successive days he sometimes obtained symptoms of arsenical poisoning. By means of repeated examinations of aspirated gland juice he found that in patients who had received full doses of atoxyl, the trypanosomes disappeared from the lymphatic glands eight hours after the injection had been given. In most cases the sterilization was lasting.

Writing from his headquarters on Sese Island in Lake Victoria Nyanza on November 5th, 1906, Koch was most enthusiastic about his results with atoxyl. He believed that the drug was as specific for sleeping sickness as was quinine for malaria. He said "no doubt can any longer exist as to the specific action of the drug." In 1907, however, he had cause to modify his views. Finding that advanced cases ("Schwerkranken") tended to relapse, he increased the dose of atoxyl to 1.0 G. at intervals of 7 or 10 days, an amount which caused many cases of blindness from which the patients

did not recover, while the results were no better than before. He therefore returned to the earlier dosage of 0.5 G. The course of treatment finally adopted by Koch was a subcutaneous dose of 0.5 G. atoxyl on each of two successive days, repeating the "double dose" over a long period with an interval of ten days between each "double dose".

Early cases of sleeping sickness (Leichtkranken) could be cured by such a course of atoxyl lasting from four to six months. In advanced cases it was difficult to drive out the parasites. Up to September 5th, 1907, Koch had treated 1633 cases of sleeping sickness of whom 131 (8%) had died in a period of ten months, and as a result of treatment with atoxyl 22 cases had become permanently blind.

Broden and Rodhain (1906) treated with atoxyl four Europeans suffering from trypanosomiasis, with good results in three cases. In the fourth case the drug was not well tolerated, and no improvement was obtained.

In 1908 Manson published records of ten Europeans suffering from trypanosomiasis treated by himself. He concluded that "atoxyl has a marked effect in checking the clinical manifestations of the infection and in causing the parasites to disappear from the peripheral circulation," and that "Trypanroth, mercury and parafuchsin seem ineffective in human trypanosomiasis." He regarded the action of atoxyl

in trypanosomiasis as being almost on a par with mercury in syphilis and quinine in malaria.

Gamble (1913), in the Portuguese Congo, had 41 natives under treatment for trypanosomiasis between December 1908 and August 1909, the parasites being found by gland puncture in each case. After giving 100 grains of atoxyl subcutaneously into the forearm in doses of 4 grains daily, 19 of the 41 patients were alive and well, three years and seven months after treatment.

Clapier (1921), gave the results of the re-examination of a large number of cases of sleeping sickness treated by Ouzilleau in the French Congo from December 1913 to July 1914. These patients had received one, two or three injections of atoxyl, in doses of 1.0 G. for an adult, and 1.5 to 2.0 Cg. per kilo of body-weight for a child. Six years after atoxylisation one-third of them were re-examined and of these about 80 per cent. were free from infection, and almost all were leading a normal life under ordinary conditions.

Broden and Rodhain (1921), discussed successively the effect of atoxyl (a) in doses of 0.5 G. every 10th and 11th day as recommended by Koch, (b) in weekly doses of 1.0 G. and (c) in doses of 0.5 G. every fifth day. They concluded that one cannot hope to obtain a cure by atoxyl alone in the second stage of the disease. A certain number of patients

in the first stage can be definitely cured, but in the second stage the administration of atoxyl ought never to be discontinued, but only interrupted from time to time. In the first stage the course of treatment should last for from four to six months, and should be followed at regular intervals by shorter periods of treatment. The optimum results are obtained from weekly doses of 1.0 G.

Boyé (1927) stated that as a result of their work at Brazzaville from 1922 to 1924, Blanchard and Laigret found only 6 per cent of relapses within an observation period of one year, following a course of six injections of atoxyl at ten days' interval. Boyé, therefore, in 1924, decided to put this method into operation in the two districts, Middle Congo and Oubangin. In the former, 192 cases were treated. 34 (17 dead and 17 absent) were not re-examined and in the remaining 158 cases, examination of the blood was negative one year after treatment. In the Oubangin district the patients were re-examined fourteen months after treatment. Of 60 in the first stage, 2 showed parasites in the blood, and of 40 in the second stage, 3 had parasites in the blood. Boyé therefore decided to adopt this method of treatment exclusively.

Bayer 205 and Fourneau 309.

In 1920 the German firm Bayer marketed a new preparation

Bayer 205 (Germanin), for the treatment of trypanosomiasis, its composition being undisclosed. The French workers, Fourneau, Tréfouël and Vallée, (1924) obtained quantities of Bayer 205, and after analysis, synthesised a product (Fourneau 309), which is now recognised as being identical with Bayer 205, and which chemically is the symmetric urea of acid sodium m-amino-benzoyl-m-amino-p-methylbenzoylnaphthyl-amino-trisulphonate. Kleine (1924), stated that "Bayer 205 is a complex organic body which contains neither arsenic, mercury or any other metal. Chemo-therapeutically it is standardised to act in such a manner that a mouse infected 24 hours before with nagana trypanosomes can be permanently cured of trypanosomes by a single injection of 0.1 Mg. per 20 grammes of body weight."

In 1920 Haendel and Joetten showed that in mice infected with nagana, a dose of 0.5 Mg. of Bayer 205 caused complete disappearance of trypanosomes from the blood. The fatal dose was 30 Mg., and 10 Mg. and 20 Mg. were well borne. Equally good results were obtained in infected rats, guinea-pigs and rabbits.

Mayer and Zeiss (1920) tried Bayer 205 against T. brucei, T. equiperdum, T. gambiense (various strains), T. rhodesiense and Schizotrypanum Cruzi, and found it to be active against all of these except Schizotrypanum Cruzi. Cured animals

were protected for a lengthy period against reinfection with the same trypanosome although in vitro the drug exhibited no harmful effect upon the parasites. They believed that the drug was a great advance upon all the other known trypanocidal preparations.

In this country, Wenyon (1921) experimented with Bayer 205 on mice infected with a very virulent strain of T. equiperdum which killed the animals in 3 or 4 days. He concluded that the drug was a powerful trypanocide. In every instance a single injection of a suitable dose apparently brought about a "therapia sterilisans magna" and this quantity was considerably lower than the minimum lethal dose.

The first case of human trypanosomiasis treated with Bayer 205 came from Fernando Po. The patient was infected with T. gambiense in October 1919 and admitted to the Hamburg School of Tropical Medicine in March 1920. Two intravenous doses of Bayer 205, each consisting of 0.2 G., were given at an interval of seven days with no effect. The trypanosomes increased and tartar emetic was given. Subsequent experience, however, showed that the dose given had been too small.

The second case was a patient of Yorke's (1921), infected with T. rhodesiense in North-east Rhodesia in September 1920 and treated unsuccessfully in Africa with tartar emetic, antimony oxide and soamin (a proprietary brand of atoxyl).

He was admitted to the School of Tropical Medicine in Liverpool in March 1921 where he received further injections of tartar emetic and atoxyl. As his condition became steadily worse, he was sent to Hamburg where he received on July 9th, 0.5 G. Bayer 205 intravenously, followed by 1.0 G. on July 10th, 1.0 G. on July 11th, and 1.0 G. on July 18th. Because of albuminuria and slight haematuria the treatment was stopped. The patient's blood became negative within a few hours of the first injection, he rapidly improved, and was still alive and well in May 1925, three years after treatment with Bayer 205.

Manson-Bahr and Low (1922) published a preliminary note on the treatment with Bayer 205, of 9 cases of human trypanosomiasis in Europeans. 8 were infected with T. gambiense and 1 with T. rhodesiense. Following treatment 1 died of cerebral trypanosomiasis, 7 were in good health and apparently cured, whilst the Rhodesian case, although greatly improved, did not appear to be cured.

Kleine and Fischer (1922) (1923), made a special trip to Africa to test the drug in cases of sleeping sickness. In Northern Rhodesia in the vicinity of Ndombo, they treated 35 T. rhodesiense cases. As about half of these had to be carried into camp, the proportion of advanced cases was presumably very high. They injected 1.0 G. Bayer 205

subcutaneously into the back, high up in the scapular region, on each of the first, tenth and eighteenth days. Those cases with trypanosomes in the cerebrospinal fluid, and those who relapsed with trypanosomes in the blood each received two further injections. One patient died before they left Rhodesia and within six months other three had died. In a letter to Yorke dated 15th September 1924, Dr. May, Principal Medical Officer, Northern Rhodesia, stated that Kinghorn had traced all Kleine's Rhodesian cases, and that of the 35 cases treated, 29 had died, mostly of sleeping sickness. In a further letter dated 9th October 1924, Kinghorn stated that only 5 of these 35 patients were alive, and that all the others had died of sleeping sickness. The 5 survivors showed no trypanosomes in the peripheral circulation, and were quite well.

In the Congo Kleine and Fischer treated 150 T. gambiense cases with intravenous doses of 1.0 G. of Bayer 205, on the first, third and fifteenth days. Of 96 cases who were carefully observed, and in whom the blood was examined at least once weekly for five months after the third injection, only 2 relapsed with trypanosomes in the blood, these being two young children. Two maniacal patients made a remarkable recovery. 7 patients who showed advanced nervous symptoms made no clinical improvement, but their blood appeared to be permanently sterilised. The only drawback to the administration

of Bayer 205 which these two observers noticed was a tendency to albuminuria.

Fontana (1924), stated that of Kleine's 150 Congo cases he had been able to trace 83. Of 65 in the first stage, 51 were probably cured, 8 had relapsed and 6 had died. Of 18 in the second stage, 2 were cured, 8 had relapsed and 8 had died.

Walravens (1924) writing of cases treated by him in the Belgian Congo in 1923 by Bayer 205, stated that the results of treatment were especially apparent in advanced cases, who quickly improved physically. The improvement was not so noticeable, however, in patients suffering from mental symptoms. The trypanosomes disappeared from the blood and from the gland juice in less than forty-eight hours in all cases. He believed that the drug resulted in cure in probably eighty to ninety per cent. of cases, but noted, however, that patients after treatment with Bayer 205, suffered from albuminuria, which often persisted for more than six months.

Tanon and Jamot (1924) reported on 39 cases treated by them in the Cameroons, with Bayer 205. There were 13 first stage cases (trypanosomes in the blood), 18 second stage cases, (trypanosomes in the cerebro-spinal fluid), and 6 in the third or sleeping sickness stage. Each received 2.0 G. of Bayer 205. There were no relapses in 30 cases after an observation period of nine months. 7 cases showed relapses,

and 2 cases who received intra-thecal injections of the drug died as a result of the injection. Both these observers state that the condition of the cerebro-spinal fluid is in no way altered by Bayer 205.

Low (1924), published a second series of cases of human trypanosomiasis treated with Bayer 205. All did well, but all developed albuminuria. Of the 9 cases previously reported by Manson-Bahr and himself, 6 were still well, 1 had relapsed and 2 were dead.

Van den Branden and Van Hoof (1924) tested the drug in 79 cases of human trypanosomiasis at Léopoldville in the Belgian Congo. Some of these escaped observation. Of 19 cases in the first stage, with trypanosomes in the blood, 12 had not relapsed in the observation period, (5-16 months). Of the other 7, 1 died, 1 disappeared, and 5 relapsed. Of 25 in the second stage with profound meningeal reaction, 13 received intravenous treatment and of these 6 died, 1 relapsed, and 6 remained negative but in bad condition. Of 9 treated intravenously and intrathecally, 1 improved, 4 died and 4 made no progress.

Hanington (1923), treated 114 cases of sleeping sickness at Sherifuri in Northern Nigeria, giving 1.0 G. intravenously on the 1st, 3rd, 5th, 12th, and 19th days, with 11 deaths. In March 1925 Lloyd reported that of these cases treated at Sherifuri, 15 had died.

Kellersberger (1926) reported on a series of 150 cases of sleeping sickness treated by him with Bayer 205 at Katanga in the Belgian Congo. These he grouped as follows:- first stage, with normal cerebro-spinal fluid, second stage with slight pathological changes in the cerebro-spinal fluid, and third stage with gross changes in the cerebro-spinal fluid and marked clinical signs. Of 89 cases in the first stage, 30 remained well for over one year, 20 remained well for nine months and 35 for shorter periods.

Of 46 cases in the second stage, 40 relapsed after two to six months, and 4 died. None were cured.

Of 15 cases in the third stage, 6 died and the others relapsed.

The usual treatment given was a course of from eight to ten intravenous injections of one gramme of Bayer 205, given over a period of eight or nine weeks.

Illustrating the dangers of treatment by Bayer 205, Stones (1924), reports the death of an African in Kenya from acute nephritis following a total dosage of 3.5 G. of Bayer 205, given intravenously. Of 17 advanced cases of sleeping sickness treated with Bayer 205, Chesterman (1924) records that 2 died of dysentery, 2 of acute nephritis, and 2 who had amblyopia from previous treatment with soamin (atoxyl) became completely blind. 8 returned worse than when treatment had

been suspended, 1 remained in statu quo, and 2 improved.

The general consensus of opinion is that in early cases, with unaltered cerebro-spinal fluid, Bayer 205 may be confidently expected to effect a cure. The improvement, however, in chronic cases with changes in the cerebro-spinal fluid is but transient.

Fourneau 309.

Fourneau 309 is a French preparation which is believed to be identical with Bayer 205. It was used by Maclean in Tanganyika Territory in 1927 in a limited number of cases with results similar to those obtained when using Bayer 205.

Tryparsamide.

Tryparsamide, a new preparation for the treatment of trypanosomiasis, was synthesised in America by Jacobs and Heidelberger, and its biological action studied by Brown and Pearce in 1915, but the first publication did not appear until 1919, and no systematic clinical study could be undertaken until 1919-20, when its action in trypanosomiasis and in syphilis was investigated. Tryparsamide, a pentavalent arsenical, containing 24.57 per cent. of arsenic, is the sodium salt of N-phenylglycineamide-p-arsonic acid, a white crystalline salt, soluble in water.

Studying the action of tryparsamide in experimentally

infected animals, Brown and Pearce (1919) found its trypanocidal value to be much greater in T. gambiense infections than in T. rhodesiense infections. In 24-hour infections in mice and rats, one injection could cure the animals, although a much larger dose was required in the case of T. rhodesiense than in the case of T. gambiense.

In May 1920 a mission was sent by the Rockefeller Institute to the Belgian Congo to study the effect of tryparsamide upon cases of human trypanosomiasis. At Léopoldville 77 cases were treated representing all grades of infection and including cases previously untreated as well as cases which had received treatment with various drugs for varying periods. The parasites were demonstrated by gland puncture in 67 cases, in centrifugalised blood in 1 case, and at the time of treatment no trypanosomes were found in 8 cases. In most instances a 20% solution of the drug was injected intravenously, although in a few cases the drug was given intramuscularly. The observations were made on the effects of (1) single doses in early cases, (2) repeated doses in early cases and (3) repeated doses in advanced cases, all in T. gambiense infections.

It was found that tryparsamide was an active trypanocidal agent. Its action was consistently followed by the disappearance of trypanosomes from the superficial lymph glands and from the blood within twenty-four hours. This sterilisation

of the peripheral blood and superficial lymph glands lasted from 17 to 58 days in patients who received single injections varying from 0.5 G. to 5.0 G. The general physical reaction of the patient in early cases of the disease was very satisfactory. Symptoms disappeared within two or three days and pulse rate and temperature became normal. Within a month the blood count had improved and the cervical lymph glands had become small and indurated.

In advanced cases repeated dosage was necessary, several doses of from 1.0 to 7.0 grammes being given at intervals of 3 to 14 days. In these cases also the physical condition of the patient was bettered, while the nervous and mental symptoms present were either greatly improved or completely disappeared, except in two very advanced cases. There was also marked diminution in the number of cells in the cerebrospinal fluid after treatment.

The only untoward effect, noted in 9 advanced cases, was a dimness of vision which in the majority proved transitory, and in the remainder improved in varying degrees.

Van den Branden and Van Hoof (1923), published information regarding the fate of Pearce's 77 cases treated in December 1921 with tryparsamide. 20 of the 21 early cases were traced, 3 of whom had relapsed after Miss Pearce had left the Congo. The others were all apparently cured, and

the relapsed cases were all successfully retreated with tryparsamide. In the same year these two authors gave details of the fate of 35 advanced cases treated by Pearce. 3 died; in 16 the cerebro-spinal fluid became normal; in 11 the lymphocytosis decreased appreciably, in 2 it remained stationary, and in 3 it increased.

Chesterman (1923), investigated the action of tryparsamide in a series of 40 cases of trypanosomiasis treated at Yakusu in the Belgian Congo, paying special attention to the results obtained by the repeated intravenous administration of large doses at weekly intervals, the effects being controlled by observation of the cell count in the cerebro-spinal fluid in advanced cases. He used a 30% solution of the drug in boiled rain water; the course consisted of eight weekly injections, although this had to be modified when visual disturbances arose. His conclusions were:- (1) the maximum tolerated dose (not more than 4 G. per week in a full-sized adult), if given regularly for eight weeks, completely removed trypanosomes from the cerebro-spinal fluid and brought the cell count within the normal limits, in even the most advanced cases. (2) This change was accompanied by a very marked clinical improvement in the patient's condition. (3) Improvement was hardly less marked in cases which had received previous treatment by other arsenical preparations.

Chesterman (1924) in a further report on the condition of the 40 cases mentioned above, sub-divided them as follows:-

(a) Of 24 cases which had received no previous treatment, a single course of tryparsamide had resulted after two years' observation in 9 apparent cures, 5 non-cures (of whom 2 were dead), 9 relapses (3 dead), and 1 untraced.

(b) Of 10 cases which had received previous treatment with atoxyl and tartar emetic, 7 had relapsed (4 dead), and the other 3 seemed cured after tryparsamide.

(c) Of 6 cases who had previously been treated by the intra-thecal injection of atoxylised serum (4 cases), or tryparsamised serum (2 cases), all had had relapses, and 2 had died before further treatment could be instituted. Of 4 who survived and were thereafter treated by tryparsamide, 3 were in good health and 1 was still under treatment.

In brief, out of 37 patients, 15 had remained well and without relapse for periods averaging over 2 years from the end of a single course of treatment. Chesterman was convinced that the curative power of tryparsamide in nervous infections was superior to that of Bayer 205, and that the amblyopia caused by the former was less dangerous than the nephritis caused by the latter.

Letonturier, de Marqueissac and Jamot (1924), gave the results of 14 cases of sleeping sickness treated by tryparsamide in the Cameroons. 1 patient in the first stage improved greatly to begin with, but later relapsed. 9 patients in the second stage and 4 in the third stage showed great

improvement.

According to Laigret (1925), as a result of the treatment of human trypanosomiasis with tryparsamide, the mortality in the Hospital at Brazzaville, which had previously ranged from 4% to 12% per month, fell to between 2% and 3% and in certain months to nil.

Kellersberger (1926), obtained encouraging results in a series of 100 cases of sleeping sickness in all stages, treated by tryparsamide. All were alive and well except one who had died of a hemiplegia resulting from the disease.

Ledentu and Vaucel (1927), after reviewing a large number of cases concluded that tryparsamide in the early stages was not entirely satisfactory: relapses followed and invasion of the cerebro-spinal fluid was not prevented. On the other hand later cases, with involvement of the central nervous system were much benefited by the drug. Of 129 second stage cases treated with tryparsamide Vaucel (1929), found 30% cured, 6% improved, 3% failures, 6% dead, 54% in whom the prospect of a cure appeared hopeful, and 0.7% who gave indications of failure.

Lauterburg (1929) stated that tryparsamide had proved of the greatest value in 29 cases in the second and third stages of sleeping sickness in French East Africa. His results indicated that (1) a cure might be expected in at

least 60 per cent of cases, (2) in about half of the patients treated severe disturbances of vision may ensue, and (3) a relatively small total quantity of tryparsamide (7.0 G.) may produce a cure even in severe cases.

The accumulated researches of nine years suggested that whilst Bayer 205 is efficacious in the treatment of early cases of trypanosomiasis, tryparsamide is of greater value in those cases which shew nervous manifestations. In 1925 Chesterman (1925) had given the opinion that sterilisation was effected most quickly by tartar emetic, less so by atoxyl, and least quickly by Bayer 205. But the reverse order held good for the maintenance of sterilisation when once effected. None of these drugs, however, could be relied upon to cure a case which had shown signs of involvement of the central nervous system. Unfortunately there were a number of cases that resisted the action of tryparsamide and therefore this drug could not be claimed to cure all cases.

Maclean (1929) recommended the use of a combination of Bayer 205 and tryparsamide, a method which he had used in 25 cases of human trypanosomiasis in Tanganyika Territory between November 1924 and December 1925. The course consisted of at least two injections of Bayer 205, each of 1.0 G., followed after an interval of one month by twelve weekly doses of tryparsamide, with intervals of one month between the fourth

and fifth, and the eighth and ninth injections. Of 6 cases which received the full course of combined treatment, 3 were dead and 3 were well in 1928. Of 19 cases which received an incomplete course of Bayer 205 followed by tryparsamide, 3 were dead, 3 relapsed, and 13 were well in 1928.

Fourneau 270.

Among other drugs used in the treatment of human trypanosomiasis is Fourneau 270, one of the series of phenyl-arsenic acids studied by Tréfouël and Trévise: it is the sodium salt of acetyl-p-amino-o-oxyphenylarsenic acid and is readily soluble in water. Fourneau 270 was given subcutaneously by Ledentu and Daude (1926) in doses of 1.5 Cg. to 5.0 Cg. per kilo of body weight at weekly intervals. It was found to be a powerful trypanocide, its action in the second stage being superior to that of atoxyl and comparable to that of tryparsamide. Vaucel (1929) reached similar conclusions.

VI. THERAPEUTIC STUDIES AT YEJI

In the treatment of human trypanosomiasis at the Medical Research Institute, Yeji, the routine was as far as possible similar to that used by Maclean in Rhodesian sleeping sickness: viz., five injections each consisting of 1.0 G. of Bayer 205, in three or four weeks, followed with or without a month's interval, by a course of tryparsamide. Unfortunately it was often difficult to get patients to continue with the course after a few injections, on account of the great improvement which they experienced in their physical condition. When mental symptoms were pronounced, tryparsamide was given first, as the general consensus of opinion appears to be that this drug is more efficacious in such cases than is Bayer 205. In one such case the improvement noticed after two weekly injections was wonderful - from being a raving lunatic the patient became a normal, intelligent girl.

Treatment with Atoxyl.

Following the method used in the French Congo, it was also decided to test the use of atoxyl in early cases without nervous symptoms. The treatment consisted of six full subcutaneous doses of atoxyl with 10 days' interval between each injection, 2.0 Cg. per kilo. of body weight, with a maximum of 1.0 G., being given per dose.

The results are summarised in the following table:-

TREATMENT BY ATOXYL

Case No.	Method of Diagnosis	Results of Treatment
31	Animal Inoculation	Trypanosomes found by animal inoculation. Died of sleeping sickness.
36	Gland Puncture	Negative to all tests.
37	Animal Inoculation	Negative to all tests
38	Animal Inoculation	Trypanosomes in triple centrifuged blood.
39	Triple centrifuged Blood	Negative to all tests.
40	Animal Inoculation	Trypanosomes in triple centrifuged blood.
41	Animal Inoculation	Negative to all tests.
45	Gland Puncture	Trypanosomes in triple centrifuged blood.
46	Triple centrifuged Blood.	Negative to all tests.
47	Triple centrifuged Blood.	Negative to all tests.
50	Animal Inoculation	Trypanosomes in triple centrifuged blood.
57	Triple centrifuged Blood	Negative to all tests.
58	Triple centrifuged Blood.	Clinical relapse, but negative to all tests.

One of the cases treated by atoxyl developed toxic jaundice during the course of treatment. This quickly cleared up, however, and treatment was resumed with good results.

7/6/30. Case No. XLVI.

Lamatu, female 18 years of age, married. No nervous symptoms. Complains of severe headaches and occasional fever. Small cervical glands: gland puncture negative. Finger blood negative. Fairly numerous trypanosomes in second sediment of triple-centrifuged blood. Confirmed by animal inoculation 7/7/30. Adhesion test + + - - - .

7/6/30 atoxyl gr. 15 subcutaneously.

17/6/30 do. do. do.

27/6/30 do. do. do.

7/7/30 do. do. do.

17/7/30 Toxic jaundice present. Sclerotics tinged: bile in urine: faeces clay coloured. Atoxyl withheld.

28/7/30 atoxyl gr. 15. Jaundice much improved.

4/8/30 do. do.

20/8/30 Test for cure. Triple-centrifuged blood negative, gland puncture negative, animal inoculation negative, finger blood negative. Adhesion test + - - - - .

Of 13 cases all of which completed the course of treatment with atoxyl, 7 appear to be cured. All 13 cases became

negative to methods which would be used in an ordinary survey, namely gland puncture and fresh finger blood. No case of eye trouble occurred. The tests for cure were usually made within a month of completion of the course of treatment. The results indicate that a considerable diminution of infectivity takes place after atoxyl, but the method cannot be relied upon to cure. The advantages in favour of treatment by atoxyl are (1) the drug is administered subcutaneously and can be given much more quickly than either tryparsamide or Bayer 205, and (2) the drug is much cheaper than either of the other two. These advantages are, however, of little weight in view of the relatively small percentage of cures obtained (53%), and the ever-present danger of toxic jaundice.

Treatment with "4002".

Four rather advanced cases were treated with a new preparation, "Höchst 4002" received from the firm of I. G. Farbenindustrie, Höchst-am-Main, Germany. "4002" is a white powder, easily soluble in water. It is an arsenical preparation, the formula of which is not disclosed.

The following are clinical histories of four illustrative cases:-

Case No. LXV.

Mahama of Prang. Male, aet. 35 years.

Has lived at Attababu and Prang: a lorry driver's

collector. Suffers from impetigo. Sleeps whenever he sits down, and is said to be "crazy". Illness of several month's duration. Only one small unpuncturable gland in the neck.

27/8/30 Finger blood negative. Triple centrifuged blood, final sediment, trypanosomes present. Adhesion test + + + - - .

27.8.30	"4002"	0.5 G. intravenously
5.9.30	"4002"	0.5 G. intravenously
15.9.30	"4002"	0.5 G. intravenously.

Seen six months later he was in good health, and negative to all tests.

Case No. LXVII.

Adama (female) of Prang. Aet 17 years.

Complains of weakness and headaches. For past four months has exhibited symptoms of somnolence. One small gland in neck. Trypanosomes found in triple-centrifuged blood, third sediment. Treatment consisted of two injections of "4002" 0.5 gramme and one of 1.0 gramme. She relapsed later, and was given an injection of tryparsamide.

Case No. LIII.

Braima (male) of Prang. 25 years of age: farmer.

For the past four months has shown a tendency to lapse into sleep whenever he sits down. Complains of headaches and

fever. Takes food well, and is well-nourished. One small gland on right side of neck, not puncturable. Many trypanosomes in final sediment of triple-centrifuged blood.

Adhesion test + + + + + (23.6.30).

23.6.30 "4002", 0.5 G. intravenously.

22.7.30 "4002", 1.0 G. intravenously. Improvement in clinical condition. Facies still typical of sleeping sickness.

29.7.30 "4002", 1.0 G.

5.8.30 "4002", 1.0 G. Adhesion Test + + + + - . Triple-centrifuged blood negative, fresh blood negative.

15.8.30 "Sobita" gr. IV intramuscularly.

22.8.30 Feels very well, and looks well. Refuses injection.

Two months later he was brought back paralysed. After two further injections of 1.0 G. each of "4002" he was able to walk quite well, but he became insane.

Case LXXIV:

Alassan of Ejura - aged 11 years. Symptoms of sleeping sickness of two years' duration. Cervical glands not greatly enlarged, but trypanosomes found by gland puncture. Adhesion test + + - - - .

He was given seven injections of 0.5 G. "4002" at weekly intervals, and his symptoms disappeared after the sixth injection.

Ten days after the seventh injection trypanosomes were found in centrifugalised blood. He then received three injections of "4002" 0.5 G. and one of 2 G. in two weeks. Trypanosomes were again found in centrifugalised blood, and so he was put on Bayer 205.

Conclusions: The immediate symptomatic results of intravenous treatment with "4002" are dramatic, but the drug cannot be relied upon to sterilise the blood, and relapse may occur after a short time.

Treatment with Tryparsamide.

The following protocol illustrates the action of tryparsamide on a case showing maniacal symptoms.

30.6.30 - Case No. LVI.

Mata Mallam, female, 16 years of age, unmarried.

Symptoms of acute mania of 4 months' duration. She had tried to commit suicide by throwing herself down a well. Slept whilst waiting at the laboratory. Difficult to inject on account of her violent struggling. Numerous trypanosomes in first sediment of centrifugalised blood. Adhesion test + + - - - . No enlarged glands. Pupillary reflexes normal, knee jerks active, plantar reflex flexor in type.

Diagnosis confirmed by animal inoculation 23.7.30.

30.6.30	Tryparsamide 2 G.	intravenously.	
7.7.30	"	"	slight improvement mentally.
14.7.30	"	"	dramatic improvement. Quite normal mentally.
21.7.30	"	"	improvement maintained.
28.7.30	"	"	" "
4.8.30	"	"	" "
25.8.30	"	"	" "
1.9.30	"	"	" "
2.9.30	Triple centrifuged blood negative, fresh blood negative. Adhesion test + + - - - .		

This patient appeared to be completely cured. In March 1931 she was still free from symptoms.

24.6.30 - Case No. LV.

Bavia, male, 48 years of age. Farmer. For the past four months he falls asleep whenever he sits down. Unable to work at his farm. Finger blood negative. Gland puncture negative. Triple-centrifuged blood negative on 21.4.30, but trypanosomes found in third sediment on 24.6.30. Animal inoculation positive. Adhesion test + + - - - .

24.6.30	Tryparsamide 2.0 G.	intravenously	
1.7.30	do.	do.	do.
8.7.30	do.	do.	do.
15.7.30	do.	do.	do.
22.7.30	do.	do.	do.
29.7.30	do.	do.	do.
29.8.30	do.	do.	do.

As a result of treatment this patient was able to resume his occupation of farmer, and became negative to all tests.

Six cases completed a course of tryparsamide alone, receiving from 12.0 G. to 16.0 G. of the drug in weekly doses of 2.0 G. intravenously. Four appeared to be completely cured: the other two, who were rather advanced cases when treatment commenced, were much improved. All were well at the end of one year.

One case received 45 grains of atoxyl followed by 8.0 G. of tryparsamide with apparent cure.

Treatment by Bayer 205 followed by tryparsamide.

Three cases completed a full course of Bayer 205 followed by tryparsamide. These appeared to be completely cured and were negative to all tests after the completion of their course of treatment. The following is an illustrative case:-

Case No. XLIII.

Kojo Battaw. Male, 22 years of age, fisherman. Cervical glands enlarged, gland puncture negative. Finger blood negative. Third sediment of triple-centrifuged blood positive, confirmed by animal inoculation. Patient complains only of fever and headaches. Adhesion test + + - - - .

6.6.30 Bayer 205 1.0 G. intravenously.

13.6.30 do. do. do.

20.6.30 do. do. do.

15.7.30 Tryparsamide 2.0 G. intravenously.

22.7.30 do. do. do.

29.7.30 do. do. do.

8.8.30 do. do. do.

15.8.30 do. do. do.

22.8.30 do. do. do.

29.8.30 do. do. do.

12.9.30 Negative to all tests. Adhesion test + + - - - .

Thirteen cases received incomplete courses of Bayer 205 followed by tryparsamide, or of tryparsamide alone. The improvement noticed by the patients after two or three injections was so remarkable that unfortunately they regarded themselves as being cured. Their homes were so widely scattered, and so inaccessible to mechanical transport, that I could not possibly visit them to carry out the treatment.

Death after, or during treatment.

Case No. XXXI, (diagnosed microscopically), died of sleeping sickness after a full course of atoxyl. Several other cases died during treatment with tryparsamide. These were advanced, somnolent cases, in which the presence of T. gambiense had not been demonstrated microscopically.

VII. THE CEREBRO-SPINAL FLUID IN HUMAN TRYPANOSOMIASIS

Historical.

For long the French Colonial physicians had divided sleeping sickness into two stages, (a) the first stage, when the parasites are confined to the blood stream and to the lymphatic glands, and (b) the second stage, when the cerebro-spinal fluid is invaded by the trypanosomes. Lefrou and Ouzilleau (1922) drew attention to the alterations found in the cerebro-spinal fluid in cases of hyman trypanosomiasis. In the opinion of these two observers, the only alterations of significance in the composition of the cerebro-spinal fluid during the second stage of trypanosomiasis are (1) the pleocytosis and (2) the increase in proteins.

The pleocytosis at first is ushered in by an increase in lymphocytes, followed by the appearance of small and large mononuclear cells, the small variety constituting from ten to twenty per cent. of the cells present, and the large type never exceeding five per cent. The actual number of cells present increases as the disease advances, and a stage may be reached when the number of cells per cubic millimetre may be from one to two thousand.

The total protein content also increases as the disease progresses, but in general the quantity of protein is less variable and less subject to fluctuation than the number of

cells present. The total protein content is rarely more than 0.1 per cent. Along with this increase in total protein, there is also an increase in the proportion of globulin present. Lefrou and Ouzilleau define a pathological meningeal reaction as existing when there are found upwards of twenty cells present per cubic millimetre and more than 0.015 per cent. of total protein. They further state that in those cases in which the cells number less than fifty per cubic millimetre and in which the protein is less than 0.02 per cent., trypanosomes are not found in the cerebro-spinal fluid by centrifugalisation, and there are no clinical manifestations denoting involvement of the central nervous system.

Sicé (1930) urged that the treatment of all cases of human trypanosomiasis should be controlled by repeated lumbar puncture and examination of the cerebro-spinal fluid. In reply to those who urged the danger of such examination he showed that in only one case out of 1,350 lumbar punctures could death be attributed to the results of this procedure, and in a further series of papers, he attempted to assess the value of this procedure in the treatment of human trypanosomiasis. The points of importance in the analysis of the cerebro-spinal fluid are (1) the cellular reaction, (2) the increased total protein content, (3) the presence of trypanosomes, and (4) the colloidal benzoin reaction. The cerebro-

spinal fluid, according to Sicé (1930), "may be regarded as a mirror, in which are faithfully reflected all the successive attacks carried out by the flagellate against the central nervous system." His conclusions are (1) human trypanosomiasis may be diagnosed by lumbar puncture, (2) in every case this method enables the physician to arrive at an exact prognosis, (3) it enables the physician to institute a methodical and efficacious treatment, and (4) it is absolutely essential in the treatment of human trypanosomiasis.

Laboratory Investigations at Accra.

During the first quarter of 1930, and again during the period October 1930 to July 1931, while Clinical Pathologist attached to the Gold Coast Hospital, Accra, I was fortunate in having wards for the treatment of medical cases admitted to the hospital. As a matter of routine I adopted the principle that the blood of every patient admitted to the hospital be examined for blood parasites, those observed being malarial parasites, the spirochaetes of relapsing fever, trypanosomes and microfilariae. In the first period, January to March 1930, 347 blood films were examined for parasites, and in no cases were trypanosomes found. From October 1930 to July 1931, 2,673 blood films were examined, and trypanosomes found on three occasions.

The cerebro-spinal fluid of every patient who presented nervous symptoms came under review, a close co-operation

being maintained between the medical staff of the hospital and the pathologist (myself). One hundred specimens of cerebro-spinal fluid were examined, ninety-nine being obtained by lumbar puncture and one by cisternal puncture. Six cases of trypanosomiasis were found, including three who also showed parasites in the blood. All six had enlarged cervical glands, and trypanosomes were found in each case by gland puncture.

The routine followed in the examination of the cerebro-spinal fluid included the observation of the physical character of the fluid, the cell count, the Nonne-Apelt test for globulin, the estimation of the total protein, the reaction with Fehling's solution, the estimation of the chlorides, the Wassermann reaction, and the examination of the centrifugalised deposit for trypanosomes. The cell count was performed using the technique and chamber of Fuchs and Rosenthal.

Analysis of 100 Cerebro-Spinal Fluids.

Group I. Eight frankly purulent fluids, seven being cases of pneumococcal meningitis and one meningococcal.

Group II. Eleven syphilitic cases with negative cerebro-spinal fluid Wassermann reactions and positive blood Wassermann reactions.

Group III. Nineteen cases of cerebro-spinal syphilis, with positive cerebro-spinal fluid and blood Wassermann reactions.

Group IV. One cerebro-spinal fluid obtained by lumbar puncture and one by cisternal puncture from a case of spinal cord tumour, in the upper thoracic region.

Group V. Thirty-nine normal cerebro-spinal fluids, i.e., with five or less than five cells per cubic millimetre, and negative blood and cerebro-spinal fluid Wassermann reactions.

Group VI. Twelve cases in which the cerebro-spinal fluid shewed an increased cell count. Of these, two cases, with 1500 and 34 cells per cubic millimetre, respectively, were found to have brain abscess. In the remaining ten cases both blood and cerebro-spinal fluid Wassermann reactions were negative and the chlorides were within normal limits. Nothing further of diagnostic import could be obtained from the cerebro-spinal fluid.

Group VII. Nine cases of human trypanosomiasis. The following tables give a comparative synopsis of results obtained.

Illustrative Examples of Cerebro-spinal Fluid Examination in Cases of Human Trypanosomiasis.

	Case A	Case B	Case C
Colour	clear	opalescent	opalescent
Pressure	slight increase	normal	normal
Cells per cubic millimetre	150 lymphocytes	260 lymphocytes	135 lymphocytes
Total Protein	0.08%	0.05%	0.04%
Nonne-Apelt	+ +	+ +	+ +
Chlorides mgm. per 100 cc.	730	740	730
Fehling's Solution.	reduced	reduced	reduced

	Case A	Case B	Case C
Cerebro-spinal Fluid Wassermann Reaction	-ve	-ve	-ve
Blood Wassermann Reaction	-ve	-ve	-ve
Trypanosomes Present	Few	numerous	few

It became early apparent that the cerebro-spinal fluid findings were remarkably similar in character, although not perhaps in degree, contrasting cases of cerebro-spinal syphilis and those of human trypanosomiasis. All the cases of trypanosomiasis, before treatment, showed a greatly increased protein percentage, the actual figures ranging from 0.04 per cent. to 0.085 per cent. Both in cases of cerebro-spinal syphilis, and in syphilitic conditions in which the cerebro-spinal fluid Wassermann reaction was negative, there was also an increased protein percentage, the figures ranging from the normal 0.015 per cent. up to 0.12 per cent. The highest protein percentage obtained in my series was actually 0.62 per cent., from a case of spinal cord tumour (Froin's syndrome).

With regard to increased cell content, there is again a resemblance between cerebro-spinal fluids from cases of syphilis and of human trypanosomiasis. Fluids with positive

Wassermann reactions varied as to cell content from 7 to 1120 cells per cubic millimetre, and fluids from cases of trypanosomiasis varied from 135 to 610 cells per cubic millimetre.

The Nonne-Apelt Globulin test was positive in both conditions, and the chlorides in all cases were within normal limits.

Analysis of the laboratory findings convinced me that even with greatly altered cerebro-spinal fluid, with pleocytosis and increased total protein, the differential diagnosis between syphilis and trypanosomiasis, a difficult matter on the Gold Coast under ordinary circumstances, is impossible apart from (a) demonstration of the presence or absence of trypanosomes in the blood, gland juice or cerebro-spinal fluid, and (b) the presence or absence of a positive Adhesion Test.

Possibly an exception might be made to this rule in cases showing advanced symptoms of sleeping sickness, which then are unique both in character and degree, and in addition, as Castellani and later, Bruce and Nabarro showed, trypanosomes are found in the cerebro-spinal fluid in a large percentage of cases (70%).

The effects of treatment on the cerebro-spinal fluid of six advanced cases of trypanosomiasis at the Gold Coast

Hospital was investigated. On account of the reputed influence exercised by this drug upon the meningeal reaction, all were treated with tryparsamide, receiving weekly injections of 2 G. intravenously.

Table Showing Comparison of Cerebro-spinal Fluid in Cases of Human Trypanosomiasis before and after Treatment with Tryparsamide.

Case D.	Before Tryparsamide	After Tryparsamide (14.0 G.)
Date	2/3/31	13/4/31
Colour	opalescent	water-clear
Pressure	slight increase	normal
Cells per cubic millimetre	610 lymphocytes	20 lymphocytes
Total Protein	0.085%	0.025%
Nonne-Apelt	+ +	faintly positive
Chlorides mgm. per 100 cc.	720	720
Fehling's Solution	reduced	reduced
Cerebro-spinal Fluid Wassermann Reaction.	negative	negative
Blood Wassermann Reaction	negative	negative
Trypanosomes Present.	few	nil

Case E	Before Tryparsamide	After Tryparsamide (16.0 G.)
Date	13/3/31	4/5/31
Colour	opalescent	water-clear
Pressure	normal	normal
Cells per cubic millimetre	212 lymphocytes	8 lymphocytes
Total Protein	0.07%	0.02%
Nonne-Apelt	+ +	negative
Chlorides, mgm. per 100 cc.	710	720
Fehling's Solution	reduced	reduced
Cerebro-spinal Fluid Wassermann Reaction	negative	negative
Blood Wassermann Reaction	negative	negative
Trypanosomes Present	few	nil

Case F	Before Tryparsamide	After Tryparsamide (14.0 G.)
Date	5/2/31	23/3/31
Colour	slightly turbid	water-clear
Pressure	slight increase	normal
Cells per cubic millimetre	382 lymphocytes	91 lymphocytes
Total Protein	0.06%	0.035%
Nonne-Apelt	+ +	faintly positive
Chlorides, mgm. per 100 cc.	720	720
Fehling's Solution	reduced	reduced
Cerebro-spinal Fluid Wassermann Reaction	negative	negative
Blood Wassermann Reaction	negative	negative
Trypanosomes present	few	nil

In one case the cell count dropped from 382 to 91 per cubic millimetre in six weeks (14 G. of tryparsamide having been given) and the total protein from 0.06% to 0.035%. In a second case the cell count dropped from 610 to 20 cells per cubic millimetre and the total protein from 0.085% to 0.025% in a similar period, and in a third case the cell count per cubic millimetre fell from 212 to 8, and the total protein

from 0.07% to 0.02% in a period of slightly over two months.

These three patients were ultimately discharged from hospital well, and were able to resume their normal occupations, as was also a further case. Two other cases died during treatment. Treatment by tryparsamide produced great improvement in four cases out of six, whilst it failed completely in the remaining two cases.

My observations have led me to the conclusion that examination of the cerebro-spinal fluid is not only a valuable guide as to the progress under treatment of cases showing cerebro-spinal symptoms, but that this procedure is also of considerable value as an aid to diagnosis, although not so important in this respect as gland puncture or the examination of triple centrifugalised blood.

VIII. THE ADHESION TEST

In March 1930 I took over the duties of Pathologist at the field laboratory, Yeji, with the object of continuing the research which was then being carried out on the subject of trypanosomiasis, both human and animal. The possibilities which lay in the use of the adhesion phenomenon as a method of diagnosis in human trypanosomiasis had aroused my interest, and as my predecessor had carried out only a few adhesion tests, I resolved from the outset to make this one of the chief subjects of investigation at Yeji in an endeavour to estimate the value of this reaction as applied to the diagnosis of human trypanosomiasis in the Gold Coast.

Historical.

The earliest work on this subject was carried out by Russian workers, who termed it variously the "Rieckenburg", "Thrombocytobarin" or "Beladung" reaction. The phenomenon had been observed first of all by Rieckenburg (1917). While investigating the trypanocidal effects of certain drugs, e.g. trixidin (30% emulsion of antimony trioxide in oil), in the course of his experiments Rieckenburg found that by mixing the fresh blood of a rat, which had been cured of a trypanosome infection (nagana), with citrated broth and adding to the mixture a suspension of live trypanosomes belonging to the same

strain, an interesting phenomenon resulted. Within a few minutes the trypanosomes became thickly coated over with blood platelets, so that their movements became slow and laborious.

In Rieckenburg's technique a rat is inoculated intraperitoneally with a suspension of T. brucei. When in the course of a few days the blood is swarming with trypanosomes, the animal is treated with a sterilising dose of some trypanocidal drug e.g. trixidin. Three or four days later a drop of blood is mixed with a drop of citrate solution to prevent coagulation, and to the mixture trypanosomes of a homologous strain are added, the whole being well mixed together on a slide and covered with a cover glass. The reaction is observed under a microscope with the highest power dry system.

First of all the phenomenon of agglomeration takes place. Two trypanosomes join themselves together by the posterior (non-flagellated) extremities. Other trypanosomes continue to attach themselves to the first pair in such a way that eventually a rosette is formed consisting of a variable number or organisms, all of which are firmly attached by their posterior ends, the anterior ends remaining free and motile. Several hundreds of individuals may go to make up such a rosette. Secondary rosettes may be formed by the grouping of primary clusters. Not all the organisms in the preparation are united in such agglomerations, some remaining free throughout

the period of observation. The rosettes finally break up into their individual components, which gradually detach themselves and move away, or, on the other hand, the trypanosomes may die and disintegrate whilst still in the state of agglomeration.

This phenomenon was described as early as 1900 by Laveran and Mesnil, and was called by them agglomeration or agglutination, the terms being interchangeable. Agglomeration may be brought about in either of two ways. (1) It occurs spontaneously in infected defibrinated blood preserved on ice after a varying interval of time, but not before the end of two or three days at the earliest. (2) It may be caused in a few minutes by the addition to the defibrinated blood or serum containing the trypanosomes, of an equal volume of the normal serum of certain animals (dogs, rabbits, sheep, horse or fowl), but more especially by the addition of the serum of rats which have been previously immunised by the injection of homologous trypanosomes.

In addition to the phenomenon just described, Rieckenburg observed that the individual trypanosomes become gradually covered over with, and are eventually almost hidden underneath a coating of adherent blood platelets. Occasionally a leucocyte similarly adheres to a trypanosome but the red blood corpuscles do not disturb the picture because they usually

sink to a lower level than the flagellates and so lie in a different plane. Rieckenburg found it possible to obtain the reaction only when using citrated plasma. The use of serum from immune blood which had been allowed to clot, or the addition of blood platelets from normal or from immune animals could not elicit the reaction, although agglutination of the trypanosomes took place as before. He concluded that this newly observed reaction was different from, and not merely a variation of, agglomeration, and that it depended upon some specific qualities resident in the blood platelets of the immune animal. The reaction could also be obtained by using the blood of animals during a chronic infection in the course of which the trypanosomes disappeared from the bloodstream without any therapeutic measures having been taken.

By using various strains of trypanosomes, Rieckenburg found that the reaction was strongly specific, and that by means of it he could distinguish serologically between those strains of trypanosomes present in the blood of an experimentally infected animal at the commencement of infection, and those which brought about the relapses: i.e., he could differentiate between starting and relapse strains of trypanosomes.

No further work appears to have been done on the subject until Kritschewsky and Tscherikower (1925) of Moscow turned

their attention to the phenomenon in order to investigate its mechanism and nature. Intent upon discovering the exact role played by the blood platelets of the immune animal, they were quite sceptical of Rieckenburg's statement that the platelets possessed peculiar properties, which, in conjunction with the "agglutinin" present in normal blood, brought about the coating of the trypanosomes with blood platelets. Kritschewsky and Tscherikower experimented with three factors, (1) the blood plasma, freed from all formed elements, of an immune animal (i.e. one that had been cured of a trypanosome infection), (2) the homologous trypanosomes free from blood platelets and (3) the blood platelets of a normal animal. They proved that (a) immune plasma is essential to the reaction and cannot be replaced by normal plasma, (b) blood platelets from an immune animal are not essential, but can be replaced by platelets from a normal animal. In their opinion the reaction depends upon the presence in the blood plasma of immunised animals of anti-bodies to which they gave the name "Thrombocytobarins" on the analogy of haemolysins and bacteriolysins. These thrombocytobarins differ from most other anti-bodies in that they are not present in normal blood, requiring previous infection and immunisation for their elaboration.

Kritschewsky and Tscherikower could not obtain the

reaction with immune serum which had been obtained after clotting of the blood. They concluded as a result of further experiments that the "thrombocytobarins" are thermostable and depend upon the presence of normal serum for their activation.

Brussin and Beletsky (1924), working at the same laboratory as Kritschewsky and Tscherikower, applied the Rieckenburg reaction to the differentiation of relapse strains in trypanosome infections. Brussin (1925), inspired by Kritschewsky, demonstrated that the "thrombocytobarins" are also present in the blood of animals during the course of spirochaetal infections, and that therefore the reaction has a much wider field of application than had previously been realised. Working with Spironema duttoni in experimentally infected mice, Brussin obtained a positive reaction by taking the blood from an animal after the crisis, (whether spontaneously or therapeutically induced), and adding to it a suspension of homologous spirochaetes. Here also the specificity of the reaction was proved. Mice infected with Russian relapsing fever gave a positive Rieckenburg reaction only with Spironema obermeieri, and conversely, mice infected with African relapsing fever gave a positive reaction only with Spironema duttoni. Starting and relapse strains of spirochaetes could be differentiated by means of suitable experiments.

Krantz (1926), using mice experimentally infected with Sp. duttoni, confirmed the work of Brussin and in addition, added the following facts to our knowledge of the reaction:- (1) An injection of dead spirochaetes fails to produce in an experimental animal the antibodies necessary for the causation of the Rieckenburg reaction. (2) Immune plasma retains its power of producing the reaction for at least two months if kept in an ice chest. (3) Immune serum obtained after clotting of the blood gives a positive reaction, - a result which first Rieckenburg and subsequently Kritschewsky and Tscherikower had failed to obtain. (4) Substitution of bacteria for blood platelets gives a positive reaction, the spirochaetes being covered with masses of adherent bacteria.

Davis and Brown (1927), working with T. equiperdum appear to be the first British workers to have studied the Rieckenburg reaction. Whereas the earlier Russian workers laid stress upon their inability to produce the reaction when working with serum obtained from the coagulated blood of immune animals, Davis and Brown obtained positive results using such serum in the case of trypanosomes infections as that with which Krantz had obtained a positive reaction in the case of spirochaetes. Their experiments prove that, as regards experimental trypanosomiasis, a certain period after the sterilization of the animal's blood must elapse before

the appearance of the antibodies necessary for the production of the reaction, five days being the minimum period. Earlier than this a positive result cannot be obtained.

From the studies of Davis and Brown three facts are proved. (1) The antibody concerned exhibits a high degree of thermo-stability. The sera of immune mice, rabbits and guinea-pigs withstand a temperature of 65° C. for two hours without loss of power to produce the Rieckenburg reaction. (2) Blood platelets play only a mechanical role in the production of the reaction, being replaceable by a colloidal inorganic suspension such as gamboge. (3) Complement is not necessary for the production of the reaction. Davis and Brown suggested the use of the term "Adhesion Phenomenon".

Miss Leupold (1928), working at Frankfort, made use of the Rieckenburg reaction in the investigation of relapse strains in trypanosome infections. Accepting the previous work of Kritschewsky that the property of producing the phenomenon lay not in the blood platelets of the immune animal, but in the serum, she was able, like Davis and Brown, to produce a positive reaction in every case when using serum obtained after coagulation of the immune animal's blood. She further experimented to discover if it were possible to produce the elaboration of the reaction-producing antibodies in a normal animal by passive immunisation. After infecting mice with T. brucei, and at the height of the infection

sterilising the peripheral circulation with Neosalvarsan, she then killed and bled the animal and obtained the serum by centrifuging the blood after the clot had formed. When this serum was mixed with the homologous trypanosome-containing blood, in the presence of citrated saline, the Rieckenburg reaction was obscured by the other phenomenon of agglomeration. She therefore modified her technique by inoculating 0.25 cc. to 0.5 cc. of the immune serum into normal mice. Twenty-four hours later these mice contained sufficient antibodies in their serum to give the Rieckenburg reaction without producing any agglomeration of the trypanosomes. This property was retained for a varying period of weeks according to the dose of serum injected. Similarly immune serum obtained from rats and young dogs could be used in the passive immunisation of mice for the production of the adhesion phenomenon.

In her subsequent experiments Leupold made use of the specificity of this reaction in proving that the trypanosomes responsible for the first relapse in different experimental mice were not serologically identical. She conducted a parallel series of experiments making use on the one hand of the adhesion phenomenon and on the other hand of the "reinfection" method, i.e. the method by which certain animals are infected with trypanosomes, and then cured,

sufficient time being allowed to elapse for the formation of antibodies in their serum, after which the animal is re-infected with trypanosomes. If the re-infecting parasites now multiply in the blood, it is inferred that no antibodies corresponding to that strain of trypanosomes are present in the serum, and therefore that the new strain of trypanosomes is serologically different from the original strain. Trypanosomes do not develop when the two strains used have been homologous. Using the Rieckenburg phenomenon on the one hand and the reinfection method on the other, Leupold completely proved the specificity of the adhesion phenomenon, and further, obtained evidence of its extreme delicacy.

The antibody content of the immune serum can be estimated by means of the adhesion phenomenon. In every adhesion test one does not see all of the trypanosomes laden with blood platelets, for where sera of weak antibody content, or sera from mixed infections are employed, there are found trypanosomes without one single adherent blood platelet in close proximity to others completely covered with platelets. Comparing the results of reinfection experiments with adhesion tests, Leupold found that sera, in which the antibody content is not sufficient to protect the animal from reinfection can nevertheless give weakly positive adhesion reactions, but that the adhesion reaction is never negative when the antibodies are sufficient to prevent infection taking place.

Johnson and Lester (1929) in Nigeria for the first time made use of the test in conditions apart from animal experiment, by applying the adhesion test to the blood of wild game suspected of harbouring T. brucei, and also to the blood in cases of human trypanosomiasis. Two hundred cases of sleeping sickness (not all microscopically diagnosed, however), were tested against T. gambiense by the adhesion test, using as controls ten individuals definitely known not to be suffering from trypanosomiasis. The cases were divided into five groups. Group (1) before treatment, group (2) during treatment, group (3) within one year after treatment, group (4) from one to three years after treatment, and group (5) from three to five years after treatment. Their results may be tabulated as follows:-

Group	Adhesion Test		
	Positive	Doubtful	Negative
1	31	3	6
2	39	2	5
3	51	2	8
4	27	8	13
5	0	1	4

Of the cases proved microscopically to be harbouring trypanosomes in the first three groups, the positive results were 90 per cent., 86.5 per cent., and 84.6 per cent. re-

spectively. In similar cases in group (4) the number of positive results fell to 57.8 per cent.

Johnson and Lester conclude that (1) the adhesion test is of value as an aid to diagnosis in human trypanosomiasis and (2) the adhesion reaction gradually disappears after a varying period following infection and treatment. It is suggested that the reaction may be an indication of acquired immunity, and that as this is lost, re-infection may occur.

EXPERIMENTAL STUDIES

The Technique of the Adhesion Test:

The reagents required for the performance of the adhesion test are (1) a suspension of trypanosomes and (2) the blood to be tested.

The suspension of trypanosomes is usually obtained from a rat. A drop of blood obtained from the tail of an experimentally infected animal is examined wet under a coverslip by direct illumination with a x6 eyepiece and a one-sixth objective, a suitable blood being one which contains twenty to thirty trypanosomes per field. The animal is then chloroformed, the heart blood drawn off and diluted with an equal quantity of citrated saline (1.0% sodium citrate, and 0.85% sodium chloride). This suspension is then centrifuged slowly

for five minutes to throw down the red blood corpuscles, a hand-turned centrifuge being employed. The upper clear layer contains the trypanosomes.

The Blood to be Tested.

Davis and Brown (1927) found that the reaction took place if serum were used instead of plasma. Johnson and Lester (1929) in Nigeria used citrated plasma, the cells being thrown down by rapid centrifuging. In my work (vide p. 119), after certain preliminary experiments, I adopted the use of serum.

Setting up the Test.

Following the technique of Johnson and Lester, equal quantities of the trypanosome suspension and of the plasma or serum to be tested, were mixed in an agglutinating tube and incubated for thirty minutes at 37° C. Thereafter a drop of the mixture was pipetted on to a slide, covered with a coverslip and examined for the presence or absence of adhesion, with a x8 eyepiece and a one-sixth inch objective by direct illumination. As a control, a tube of trypanosome suspension alone, without immune fluid was incubated at 37° C. for thirty minutes, and a drop of this examined.

Readings.

In interpreting the results of the test, I adopted a

modification of Brussin's method of five plus or minus signs, viz:-

+ + + + + = all trypanosomes have adherent platelets.

+ + + + - = only single trypanosomes are free from platelets.

+ + + - - = half, or practically half of the trypanosomes have adherent platelets.

+ + - - - = less than half of the trypanosomes have adherent platelets.

+ - - - - = only single trypanosomes have adherent platelets.

- - - - - = no trypanosomes have adherent platelets.

In order to obtain a working knowledge of the adhesion phenomenon, the tests were first of all carried out with the plasma of laboratory animals which had been infected in the usual way with T. gambiense, and whose blood had been sterilized at the height of the infection with Bayer 205, with atoxyl or with tryparsamide, the optimum dose being found by experiment.

On examining microscopically an adhesion test set up in accordance with this technique, one sees in the field a variable number of trypanosomes, blood platelets, red blood corpuscles and an occasional leucocyte. If the test be positive, a varying number of the trypanosomes present in each field are seen to be covered with numerous blood platelets. Occasionally a leucocyte might be seen adhering to the

trypanosome, but never a red blood corpuscle. Not all the trypanosomes are covered with adhering blood platelets, a varying number being quite free. The parasite still maintains its activity despite the adhesion of the platelets, and wriggles about as before, although it does not move so freely across the field.

No notice is taken here of the phenomenon, commonly met with, of agglomeration, referred to by Laveran and others. When it is present, large clusters of actively wriggling trypanosomes are seen to be attached to each other by the posterior (non-flagellated) ends, forming rosette-like masses. This phenomenon may be encountered, irrespective of whether the adhesion test is negative or positive, and has no bearing on the result of the test.

In carrying out the adhesion tests, several strains of T. gambiense were used, all of which had been isolated from patients seen at Yeji. None of them had been passaged through laboratory animals for more than three months before the series of tests commenced. During the course of my experiments some of the original strains developed an extreme virulence towards the laboratory animals, killing the latter in three days, so that some of the original strains were lost in which event I carried on with new strains of T. gambiense isolated from freshly diagnosed cases of sleeping sickness.

TABLE NO. I

Examples of Adhesion Tests carried out with the plasma of immunised laboratory animals.

Test No.	TRYPANOSOMES			IMMUNE FLUID			READING
	Source	Strain	Passage	Source	Treatment	No. of days after treatment.	
1	Rat 265	XV	6th	Rat 118	Control (never infected)		-----
2	Rat 208	XVI	8th	Rat 169	Bayer 0.0005G.	25	+++--
3	Rat 265	XV	6th	Rat 242	Trypars. 0.1 G.	5	++---
4	Rat 265	XV	6th	Rat 246	Trypars. 0.1 G.	5	++---
5	Rat 284	XVI	10th	Rat 272	Atoxyl 0.2 grain	5	++---
6	Dog J3	XVI	11th	Rat 282	Trypars. 0.1 G.	3	-----
7	Dog J3	XVI	11th	Rat 276	Do.	3	-----
8	Rat 204	XIII	6th	Rat 232	Do.	8	++++-
9	Rat 294	XIII	6th	Rat 266	Atoxyl 0.25 grain	13	++++-
10	Rat 294	XIII	6th	Dog J2	Bayer 205 0.1 G.	6	+++++
11	Rat 294	XIII	6th	Dog J3	Bayer 205 0.1 G.	6	+++++

The results of eleven adhesion tests on laboratory animals are tabulated. Experiment No. 1, a control, was carried out on a rat which had been inoculated with infected blood, but in which trypanosomes had never appeared. This test was negative.

Tests No. 6 and 7 were also negative, having been performed only three days after the sterilization of the animals' blood. David and Brown had already stated that at least five days must elapse between the sterilization of the blood, and the elaboration in the serum of the antibodies necessary for the production of the adhesion phenomenon. My experiments bear out their results.

The other eight tests were positive, Nos. 10 and 11 being very strongly so.

My next step was to carry out a series of adhesion tests upon cases of human trypanosomiasis which had been proved by microscopic diagnosis. Before finally deciding upon the adoption of a definite technique, I proceeded to obtain an answer to certain questions. (1) Is serum as reliable as plasma for the carrying out of the adhesion test? (2) will successful results be obtained if a drop of serum be mixed with a drop of trypanosome suspension, upon a slide, the drop mixture being incubated at 37° Centigrade for thirty minutes before being examined for adhesion? (3) will successful results be obtained if a similar procedure be adopted,

substituting plasma for serum?

TABLE NO. II

A series of tests to compare results when using immune serum from cases of human trypanosomiasis on the one hand, and immune plasma on the other, incubating both serum and plasma in agglutinating tube and on glass slide respectively at 37° C. for 30 minutes.

Test No.	Trypanosomes		Immune Fluid		How Incubated	Result of Test
	Rat No.	Strain No.	Source	Nature		
1	218	XVI	Case No. 3	Serum	In tube	++---
					On slide	++---
				Plasma	In tube	++---
					On slide	++---
2	218	XVI	Case No.34	Serum	In tube	+++--
					On slide	+++--
				Plasma	In tube	+++--
					On slide	+++--
3	307	XVII	Case No.39	Serum	In tube	++++-
					On slide	++++-
				Plasma	In tube	+++--
					On slide	+++--
4	307	XVII	Case No.35	Serum	In tube	++++-
					On slide	++++-
				Plasma	In tube	+++--
					On slide	+++--
5	253	XIV	Case No.36	Serum	In tube	+++--
					On slide	+++--
				Plasma	In tube	+++--
					On slide	+++--
6	265	XV	Case No.32	Serum	In tube	++---
					On slide	++---
				Plasma	In tube	++---
					On slide	++---

The experiments prove that (1) results obtained by performing the test by means of a large drop on a slide, correspond with the results obtained when incubating the mixture of serum or plasma and trypanosome suspension in an agglutinating tube and (2) the test may be performed with serum as well as with plasma. In two instances the adhesion test was more strongly positive when using serum than when plasma was used. This may be explained by the fact that the serum is undiluted before its admixture with the trypanosome suspension, whereas the plasma is diluted with an equal volume of citrated saline before being added to the trypanosome suspension. In the remaining tests the results were identical. The glass slide technique using serum was therefore adopted in my subsequent experiments.

Using serum from cases of human trypanosomiasis in the adhesion test a new feature was observed, in that not only blood platelets, but actually red blood corpuscles, were adhering to the trypanosomes. This did not occur in the controls, i.e. preparations containing no immune fluid, but merely a drop of trypanosome suspension, which were always put up when adhesion tests were being performed. Therefore, in putting up the test I always withdrew from the clotted blood a quantity of red blood corpuscles along with the drop of serum to be tested, and came to regard the test thus modified as a red-cell adhesion test, although none of the

previous writers had observed this phenomenon. Apparently it only occurred in human cases of trypanosomiasis, as it did not appear in the series of tests conducted with the plasma of dogs and rats.

The adhesion test was performed (1) on the blood of all new cases in whom the presence of trypanosomes had been proved microscopically before commencing treatment. (2) On a certain number of cases of human trypanosomiasis during treatment, (3) on a necessarily limited number of cases from one to three months after the conclusion of a course of treatment, (4) on a large number of Africans who attended for treatment of yaws, leprosy or other tropical conditions, and in whom by the most exhaustive investigation, the presence of trypanosomes was as far as possible excluded and (5) on two healthy Europeans (the assistant Entomologist and myself).

TABLE NO. III

Adhesion Tests performed on microscopically proved cases of human trypanosomiasis before treatment:

N.B. T.C.B. = Triple centrifuged blood.

Case No.	Date	Method of Diagnosis	Result
36	29/3/30	Gland Puncture	Plasma + + - - -
39	28/4/30	T.C.B.	Plasma + + + - -
37	12/4/30	Animal Inoculation	Serum + - - - -

TABLE NO. III (Contd.)

Case No.	Date	Method of Diagnosis	Result
38	20/4/30	Animal Inoculation	Serum + + - - -
39	28/4/30	T.C.B.	do. + + + + -
40	17/5/30	Animal Inoculation	do. + - - - -
41	17/4/30	do. do.	do. + - - - -
42	21/5/30	do. do.	do. + + - - -
42	15/5/30	do. do.	do. + + - - -
43	29/5/30	T.C.B. + Animal Inoc.	do. + + - - -
44	5/6/30	T.C.B.	do. + + - - -
45	7/6/30	Gland Puncture	do. + + + - -
46	7/6/30	T.C.B. + Animal Inoc.	do. + + - - -
47	27/5/30	T.C.B.	do. + + + - -
47	28/5/30	do.	do. + + + - -
48	10/6/30	do.	do. + + - - -
49	12/6/30	do.	do. + + + - -
50	23/4/30	T.C.B. + Animal Inoc.	do. + + - - -
50	22/6/30	do. do.	do. + - - - -
51	16/6/30	T.C.B.	do. + + - - -
52	27/5/30	Animal Inoculation	do. + + + - -
52	28/5/30	do. do.	do. + + + - -
53	15/5/30	T.C.B.	do. + + + + -
53	23/6/30	do.	do. + + + + +
54	23/6/30	do.	do. + - - - -
55	24/6/30	do.	do. + + - - -

TABLE NO. III (Contd.)

Case No.	Date	Method of Diagnosis	Result
56	30/6/30	T.C.B.	Serum + + - - -
57	3/7/30	do.	do. + + - - -
58	14/7/30	do.	do. + + - - -
59	30/7/30	do.	do. + + - - -
60	31/7/30	do.	do. + + - - -
61	31/7/30	do.	do. + - - - -
62	11/8/30	do.	do. + + - - -
63	18/8/30	do.	do. + + - - -
64	21/8/30	do.	do. + + - - -
65	27/8/30	do.	do. + + + - -
66	27/8/30	do.	do. + + - - -
67	28/8/30	do.	do. + - - - -
68	25/8/30	do.	do. + - - - -
69	2/9/30	do.	do. + - - - -
70	2/9/30	Fresh Blood	do. + + + - -
71	4/9/30	Gland Puncture	do. + + + + -
72	5/9/30	do. do.	do. + + - - -
73	10/9/30	do. do.	do. + + - - -
74	12/9/30	do. do.	do. + + - - -

Adhesion Tests were performed on thirty-nine new cases of human trypanosomiasis, diagnosed by myself at the field

laboratory at Yeji. There were no negative results. If the reactions of "one plus", i.e. + - - - - , be classified as doubtful, there are nine such results.

With regard to these doubtful cases, there may be three possibilities. (1) The infection may be minimal and the tissues of the host may not be reacting strongly to the invading trypanosomes. (2) The individual may be recovering from the infection, either naturally, as it must have been in the above cases, since treatment had not yet been instituted, or, as will arise during the consideration of two subsequent tables, as a result of treatment by trypanocidal drugs. (3) The trypanosomes against which the reacting serum is being tested, may be serologically different from the strain which is at the moment circulating in the patient's bloodstream.

In my opinion a + - - - - reaction must be taken as indicating that the individual is either suffering from a trypanosome infection, or that he has so suffered in the recent past. All the authorities studied, emphasise the specificity of the reaction. The question of a non-specific reaction does not arise.

As the strength of the adhesion reaction diminishes and approaches the stage at which only single trypanosomes show adhesion, then doubt must arise as to whether we are dealing

with an active infection, and we are faced with the question as to whether we should institute treatment or not. The question of the relationship between the strain of trypanosomes used in the test, and the strains responsible for the antibodies circulating in the immune serum, is also difficult. One can only state that in the Gold Coast we are dealing with strains of T. gambiense, so that possibly this difficulty could be overcome by testing the doubtful serum against several different strains.

TABLE NO. IV.

Results of Adhesion Tests in Cases of Trypanosomiasis during Treatment.

Date	Case	Diagnosis	Result	Treatment Received Previous to Test. Drug	Amount
28/3/30	XXVII	Clinical	- - - - -	Tryparsamide	6.0 G.
28/3/30	V	Triple Centrifuged Blood.	- - - - -	Bayer 205 Tryparsamide	10.0 G. 10.0 G.
29/3/30	III	Animal Inoculation	+ + - - -	Bayer 205 Tryparsamide	9.0 G. 12.0 G.
6/5/30	XXXIII	Clinical	+ - - - -	Bayer 205 Tryparsamide	10.0 G. 10.0 G.
29/3/30	XXXIV	Triple Centrifuged Blood	+ + + - -	Bayer 205	12.0 G.

TABLE NO. IV. (Contd.)

Date	Case	Diagnosis	Result	Treatment Received Previous to Test. Drug	Amount
29/3/30	XXXV	Animal Inoculation	+ + - - -	Bayer 205 Tryparsamide	10.0 G. 4.0 G.
8/5/30	XXXVII	Animal Inoculation	+ - - - -	Atoxyl	30.0 grains
28/3/30	XXVIII	Fresh Blood	+ - - - -	Bayer 205 Tryparsamide	10.0 G. 8.0 G.
28/4/30	XXXV	Animal Inoculation	+ + + + -	Bayer 205 Tryparsamide	10.0 G. 8.0 G.
7/4/30	XXXII	Triple Centrifuged Blood	+ + - - -	Bayer 205 Tryparsamide	10.0 G. 10.0 G.

In this series of tests we have patients at various stages of treatment. There are two negative results, one in a case diagnosed on clinical grounds, and the other in an old-standing chronic case, a long time after treatment had commenced, and whose treatment had been irregular.

There are three "one plus" or doubtful results, one diagnosed on clinical grounds, one by finding trypanosomes in the finger blood, and one by animal inoculation.

TABLE NO. V

Results of Adhesion Tests performed on a series of cases immediately after conclusion of treatment.

Case	Method of Diagnosis	Treatment	Result	State at end of Course of Treatment.
III	Animal Inoculation	Bayer 9 grammes Tryparsamide 14 Grammes.	+----	Negative to all tests.
XXXVI	Gland Puncture	Atoxyl 81 grains	+++--	Negative to all tests.
XXXVII	Animal Inoculation	Atoxyl 60 grains	+----	Negative to all tests.
XXXIX	Triple Centrifuged Blood	Atoxyl 84 grains	+----	Negative to all tests.
XLVI	Triple Centrifuged Blood.	Atoxyl 90 grains	+----	Negative to all tests.
XLI	Animal Inoculation	Atoxyl 90 grains	++---	Negative to all tests
XLII	Animal Inoculation	Bayer 3 grammes. Tryparsamide 16 grammes.	++---	Negative to all tests.
LIII	Triple Centrifuged Blood.	"4002" 3.5 grammes.	++++-	Negative to all tests.
LVI	Triple Centrifuged Blood.	Tryparsamide 16 grammes.	++---	Negative to all tests.
LVII	Triple Centrifuged Blood.	Atoxyl 90 grains	++---	Negative to all tests.
LVIII	Triple Centrifuged Blood.	Atoxyl 90 grains	++---	Negative to all tests.

TABLE NO. V. (Contd.)

Case	Method of Diagnosis	Treatment	Result	State at end of Course of Treatment.
XLIII	Triple Centrifuged Blood.	Bayer 3 grammes. Tryparsamide 14	++----	Negative to all tests.
XLVII	Triple Centrifuged Blood.	Atoxyl 90 grains	++----	Negative to all tests.
XLVIII	Triple Centrifuged Blood.	Bayer 3 grammes. Tryparsamide 12 grammes.	+----	Negative to all tests.
LI	Triple Centrifuged Blood.	Atoxyl 45 grains. Tryparsamide 8 grammes.	++----	Negative to all tests.
LX	Triple Centrifuged Blood.	Tryparsamide 12 grammes.	++----	Negative to all tests.
LXI	Triple Centrifuged Blood.	Tryparsamide 14 grammes.	++----	Negative to all tests.
XXXVIII	Animal Inoculation	Atoxyl 72 grains	+----	Trypanosomes in Triple Centrifuged Blood.
XL	Animal Inoculation	Atoxyl 84 grains	+----	Trypanosomes in Triple Centrifuged Blood.
XLV	Gland Puncture	Atoxyl 90 grains	++----	Trypanosomes in Triple Centrifuged Blood.
L	Animal Inoculation	Atoxyl 72 grains	+----	Trypanosomes in Triple Centrifuged Blood.

Here we have a series of 21 cases after the conclusion of treatment. 10 of the cases had been given a full course

of treatment by atoxyl with, on the whole, disappointing results in that trypanosomes were still found in their blood.

The foregoing table suggests that (1) there is no correlation between the intensity of the adhesion test and the nature or quantity of the drugs employed in treatment, and (2) the intensity of the adhesion test seems to be diminished after, and as a result of, treatment.

The adhesion test was performed using the blood of a series of individuals in whom trypanosomes could not be demonstrated, and who clinically did not exhibit any symptoms of trypanosomiasis.

No. of tests performed		66
No. of tests negative	(- - - - -)	56
No. of tests doubtful	(+ - - - -)	6
No. of tests positive	(+ + - - -)	3
	(+ + + - -)	1

Of a total of 66 tests performed, 85% were negative, 9% were doubtful, and 6% were positive. Possibly, under repeated examination, the four positive cases might have been proved to harbour trypanosomes. As it was, however, careful examination of these and of the six doubtful cases failed to reveal any trypanosomes. It must be remembered that all these individuals came from areas where they were continually

open to infection from the bites of infected Tsetse flies. Two of the positives were fishermen on the Volta, a third was an Agricultural Department official whose duty it was to examine kola at Yeji ferry, the very heart of an endemic centre of trypanosomiasis, and the fourth was one of the attendants who looked after my experimental animals. All of them had been repeatedly bitten by Glossina Palpalis, the probability being that they had received sub-infective doses of trypanosomes, but that in each case the invading organism had been successfully overwhelmed by the antibodies elaborated by the tissues of the host.

A further series of experiments was made in order to find out whether the serum would retain its property of producing a positive adhesion reaction, if some time were allowed to elapse between the collection of blood from the patient and the carrying out of the test. My object was to determine if it were feasible to perform the test upon sera collected by medical officers at various stations and forwarded by them to a central laboratory.

The method employed was to carry out the adhesion test upon sera and to repeat the test on the following day. No ice was available.

TABLE NO. VI.

Adhesion Test performed on fresh blood and repeated on same specimen 24 hours later.

Test No.	Immune Fluid	Date	Result	Remarks
1	Case 39	28/4/30	+ + + + -	
		29/4/30	+ + + + -	
2	Case 35	28/4/30	+ + + + -	
		29/4/30	+ + + + -	
3	Case 34	27/5/30	+ + + - -	
		28/5/30	+ + + - -	
4	Case 44	5/6/30	+ + - - -	
		6/6/30	+ + - - -	
5	Case 45	7/6/30	+ + + - -	
		8/6/30	+ + + - -	
6	Case 46	7/6/30	+ + - - -	
		8/6/30	+ + - - -	
7	Ateni	1/5/30	+ - - - -	Clinical Case of Sleeping Sickness.
		2/5/30	+ - - - -	
8	Kodjoe	27/5/30	+ + - - -	Clinical case of Sleeping Sickness.
			+ + - - -	
9	Bale	1/5/30	- - - - -	Control.
		2/5/30	- - - - -	

These results show that keeping the serum for twenty-four hours did not invalidate the results. On the other hand whilst I was at Yeji, several sera of suspected cases of trypanosomiasis were sent to me both from the Medical Research Institute at Accra, and from Medical Officers at other stations for testing. Some of these specimens proved to be badly contaminated, and on being set up with a suspension of trypanosomes, lysis of the trypanosomes resulted, and no reading was possible. This was also the experience of Lester and Johnson.

Red Cell Adhesion Test.

The reaction as now carried out was really a red cell adhesion test, although in all the literature to which I had hitherto referred there was no mention made of red cell adhesion.

Several degrees of adhesion were observed, both as regards the proportion of trypanosomes which had red cells adherent to them, and as regards the number of red cells adherent to the individual trypanosome. With regard to the latter variation great differences were noticed. In some cases only one red cell would be seen adherent to the trypanosome, either to the body or, sometimes, to the flagellum, being lashed about with the movements of the latter. On the other hand in the majority of cases, the trypanosome

might be observed to be completely covered with adhering red blood corpuscles. So dense would be the wriggling mass of red cells that only by careful focussing could any trace of the trypanosome be seen in the midst of the adhering corpuscles. The motion imparted to the mass of red cells is, however, sufficiently characteristic to prevent the phenomenon being mistaken for a simple case of agglutination of the red cells.

Between these two extremes, varying intermediate degrees of adhesion were observed. In reporting on the tests no notice was taken of trypanosomes with a single red cell adherent to them. Such an adhesion might conceivably be purely mechanical.

In performing the adhesion tests upon the series of human cases, this red cell adhesion phenomenon was of so constant a character that I came to regard it as a typical reaction. It did not occur in my first few cases where the immune fluid tested was obtained from laboratory animals.

Johnson and Lester (vide supra p. 111) state quite clearly that the adhesion reaction consists of adhesion of blood platelets or foreign particles to the trypanosomes in saline suspension when incubated with the plasma of the immune animal. It is strange that they missed the red cell adhesion phenomenon.

When I handed over to my successor at the end of September

1930, I informed him of the red cell adhesion, but it was not until later that I found and studied the article by Duke and Wallace on red cell adhesion. Working at Entebbe in Uganda, these investigators evolved a new technique as follows. They mixed equal parts of (1) the immune blood (or blood to be tested), and (2) of the blood which contained the trypanosomes and (3) of 2% citrated saline, in a small tube. From the resulting large drop, two coverslip preparations were made on the one slide. There it was allowed to stand for ten minutes before being examined. They discovered that for red cell adhesion the red cells of a Primate must be present.

They explain the occurrence of the partial reactions, i.e., + + - - - to + + + + - as being due to the presence of a mixed population of trypanosomes, only some of which are serologically homologous with the reacting blood. If for instance, by means of a trypanocidal drug, all the trypanosomes circulating in the blood stream of an animal are destroyed within a very short space of time, there is a correspondingly large elaboration of antibodies corresponding to the particular trypanosomes present at that time. If the blood now be tested against that particular strain of trypanosomes, one might expect a very strong adhesion reaction. On the other hand, Duke and Wallace point out with regard to their human cases, that as the African population

is continually being subjected to infection with a large number of different strains of trypanosomes, only a partial reaction is obtained, an observation which would apply equally to the Gold Coast.

A still later paper appeared by Wallace and Wormall (1931), also working at Entebbe, in which the following very instructive conclusions are reached.

(1) The red cell adhesion phenomenon is due to the presence in the blood of the infected animal of "adhesin", a substance which appears during the course of an infection.

(2) Red cell adhesion has been obtained with the blood of Primates only.

(3) Centrifuging the trypanosome suspension does not destroy the power of trypanosomes to adhere to red cells.

(4) Trypanosomes which have been freed from plasma by centrifuging and washing give good adhesion when fresh serum or plasma is used. There is no reaction when the adhesion-containing serum is very old, or if it has previously been filtered through a Berkefeld filter candle, or if reacting serum has been heated at 56° Centigrade for 30 minutes.

(5) In addition to red cells, trypanosomes and adhesin, some other factor is necessary which is present in the plasma

and serum of most normal animals.

(6) For Red-cell adhesion there are required, (a) red cells of a primate, (b) adhesin, (c) trypanosomes of a strain related to that which gave rise to the formation of the adhesin and (d) a complement-like component.

SUMMARY:

142 Adhesion Tests were performed on human blood, 68 on cases proved microscopically to be trypanosomiasis, and 66 on cases not proved, which acted as controls. The following table shows the results of the tests.

Reactions	Proved Cases	Cases not Proved
+ + + + +	1	0
+ + + + -	5	0
+ + + - -	11	1
+ + - - -	37	3
+ - - - -	20	6
- - - - -	2	56
Total	76	66

The proved cases include a very few early cases: the negative cases include a few very suspicious clinical cases in whom trypanosomes could not be demonstrated.

There remains a residue of persons who do not seem to be suffering from trypanosomiasis, but who give positive adhesion tests. As few people in this area escape exposure to infected tsetse at some time or other, I suggest that

sub-infective doses of trypanosomes might be able to produce a positive adhesion test. Alternatively, the residue of symptomless positive reactors represent transient infections quickly dying out, or they have only mild relapsing infections which give a positive adhesion test.

In the course of my investigations no relationship was observed clinically between the intensity of the adhesion test and either the duration or the strength of the infection. The impression gathered from the material at my disposal was that after a complete course of treatment the intensity of the reaction tended to diminish.

There is a possible fallacy in the test, as suggested by Davis and Brown, that the reaction may be vitiated either by the animal used as the source of the trypanosome strains having in its own blood the substance causing the reaction, or owing to it having had previous trypanosomiasis, its blood may contain residual trypanosomes which are resistant to the phenomenon. The first possibility has been guarded against by setting up on each occasion on which the test was performed a control with the suspension of trypanosomes alone. The second contingency could not occur in my series of tests, because the trypanosomes which were used were obtained from rapidly developing infections in rats, which infections very rapidly proved fatal.

Our final conclusion, in the light of present evidence, must be confined to the assertion that clinically, a positive Adhesion Test is nothing more than proof, but nevertheless a substantial proof, that the patient has had a previous trypanosomal infection or, at the moment of testing, has actually trypanosomes in his blood.

In any assessment of the value of the Adhesion Test as an aid to the diagnosis of trypanosomiasis it must be borne in mind that whilst trypanosomiasis is fairly common in the Gold Coast in certain endemic areas of the Northern Territories and Northern Ashanti, it is a condition which presents considerable difficulties in diagnosis. Cases therefore which are clinically the least degree suspicious and which give a positive Adhesion Test, should be regarded in the same light as proved cases of trypanosomiasis and treated as such. Where time is a factor of importance, as it would be in the case of a travelling Medical Officer, the Adhesion Test can be carried out in a much greater number of cases than can triple-centrifugalisation of the suspected blood, this latter procedure being in my experience the method by which more than half of the positive cases of trypanosomiasis were diagnosed in the past. The maintenance of several strains of trypanosomes in a travelling laboratory should not present insuperable difficulties.

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