VII. THE TREATMENT OF TRYPANOSOMIASIS

Experiments have been conducted under this head on all the various trypanosomic diseases described in the previous investigations, and include *T. brucei*, *T. evansi*, *T. equinum*, *T. equiperdum*, *T. dimorphon*, *T. gambiense* (various strains). In 1902-1903, while Governors Fellow of Pathology at McGill University, one of us (H. W. T.) made experiments with sodium arseniate, etc., on animals infected with *T. brucei* and *T. lewisi*. These tentative experiments have now been carried out on a large scale. As in the case of other workers we have tried a very large number of drugs to find them of no value in the treatment of infected animals. WENDELSTADT, LAVER and MESNIL, MUSGRAVE and CLEGG, all give long lists of drugs and chemicals used without avail. These we have retested, and have tried in addition sodium, potassium, and ammonium fluoride, fluorescene, chrysoidin, and various preparations of silver and mercury, especially the newer compounds. The results have coincided with the findings of other investigators. Up to March, 1904, the only drug of any value was arsenic; EHRLICH and SHIGA then published their results with a new dye named by them 'Trypanroth,' and these two preparations are the only ones found to be of value in the treatment of trypanosomiasis.

As animals have for a considerable period been known to be liable to infection with the various pathogenic trypanosomes, it was only natural to turn to the veterinary profession to see what drugs have in their hands proved of service in the treatment of these diseases. Of the countless drugs tried arsenic is the only one which has given encouraging results. Cases are on record where cures have been reported, but, on the other hand, many cases which have been treated by some form of arsenic and which have improved as long as the administration of the drug was kept up have shown parasites when it was discontinued and have succumbed to the infection. BRUCE records cases in which large doses of sodium arseniate were given, and so long as the treatment was continued parasites were hardly ever found in the blood, the animal retained its strength and weight and was able to work, but on the treatment being discontinued the parasites quickly reappeared and the animal began to fail. MOORE and CHICHESTER report the effect of the intravenous inoculation of cattle, after dosage the animals regained their weight, the yield of milk which had diminished became normal and the parasites disappeared, whilst animals which had not been treated quickly died. We have, therefore, evidence that arsenic is of use in prolonging the life of the animal. LAVERAN, in 1902, reported his results in treating rats infected with the Nagana parasites. The life of the animal was prolonged, the parasites disappeared, and the animal seemed to have recovered. When treatment was

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discontinued after a varying length of time the parasites reappeared, the disease pursued its normal course, and the majority of the animals died. Some few recovered, but the animals were not rendered immune, as LAVERAN proved by reinoculation.

He had, however, been able to keep infected rats alive for periods many times exceeding those of untreated animals. LAVERAN and MESNIL record very unfavourable toxic effects with the ordinary preparations of arsenic. At McGill and Liverpool, in our hands the experiments showed the same toxicity of the drug, the tendency to cause ulcers and extensive sloughing despite all asceptic precautions. The results obtained as to the prolongation of the animal's life was the same. On the arrival of natives suffering from Trypanosomiasis it was felt that some form of arsenic treatment was indicated. Subcutaneous inoculations of sodium arseniate caused so much pain that this form of medication was abandoned. BRODEN had reported the favourable results obtained by the administration of arsenic in the form of *Liquor Fowleri*. Resource was made to this form of the drug, but the surviving natives were sent back to the Congo before the effects of the different preparations could be determined.

Animals infected with the various pathogenic trypanosomes were subjected to treatment ; it was found that with sodium arseniate, the most useful form of arsenic, sloughing was prone to occur. In such a case the animal very often became so depleted by the ulceration that it succumbed to a secondary affection. Moreover, the use of arsenic in the form of sodium arseniate is all too apt to cause toxic effects. The administration of the preparation may be continued for some time, favourable results occur and then, unfortunately, toxic symptoms appear. If the drug be discontinued on account of the untoward symptoms the parasites very quickly make their reappearance and the animal dies. Moreover, despite medication being continued, the parasites which have disappeared will reappear and continue to be present and even augment. In such cases an increased amount of arsenic will not affect the termination of the disease. We have, therefore, to realise that the ordinary arsenic compounds when administered only produce a temporary, favourable effect, that, if long continued, the animals will die either from the parasite or from the arsenic or from both. Hence, some other compound is indicated. It is for this reason that the newer compounds of arsenic have been experimented with in order to find a preparation capable of being used over a long period and in high doses without producing toxic symptoms. Of the various preparations tried, a meta-arsenic anilin compound, atoxyl, has proved the most satisfactory, but it is not ideal. It is not non-toxic as dogs, kittens, guinea-pigs, and rabbits have shewn toxic symptoms and succumbed, but it is not so toxic as sodium arseniate. It does not produce the sloughing which so often follows the subcutaneous or intravenous inoculation of sodium arseniate, it causes no pain, and its administration can be continued over a period of many months even when used in extremely high doses.

It is the only remedy at present giving any prospects of a cure. In the treatment of cases a rational method of treatment must be adopted. It is useless, for instance, to only administer arsensic for a short period or until the parasites have apparently disappeared from the peripheral circulation. The drug must be administered in as high doses as possible, and it must be continued even after all the favourable signs are present, viz., disappearance of parasites from the blood ; increase of weight, improvement in the blood count and percentage of haemoglobin ; loss of the autoagglutination phenomenon of the blood corpuscles ; decrease in and more regular temperature. From time to time susceptible animals ought to be inoculated with large quantities of the patient's blood at least in amounts of 5·0 to 15·0 c.c. At the same time all aids in building up the physical condition of the individual should be used. If such a *régime* be carried out, and treatment be begun at an early period, the prognosis (based on the experience of treated animals) will be good.

With animals, on the other hand, the question whether treatment with arsenic is indicated calls for some consideration as LAVERAN and MESNIL, BRUCE, and others have pointed out that there is some danger of treated animals becoming a source of infection, by filling the position in these diseases occupied by the native in malaria. The arsenical treatment will cause a temporary disappearance of the parasites from the blood. Some animals may entirely recover, but the majority suffer a relapse, and these latter are then a source of infection for the surrounding country.

On the publication of EHRLICH and SHIGA's results in the treatment by trypanroth of rats infected with *T. equinum*, some of the dye was procured and a series of comparison experiments was made. The results coincide with the findings of the other workers. It is not a very safe drug. Ulceration is very apt to occur, the sloughing is often extensive and persistent. The tendency to cause nephritis is marked and hence its use, when continued, with arsenic is often apt to lead to fatal results, especially in guinea-pigs. High doses of the dye must be employed, and on account of its chemiotoxic properties intramuscular administration is the best method. Very often animals injected with large quantities of the dye appear to be in a stuporose condition which may wear off or persist up to death. Shortly after the injection of trypanroth the tissues commence to turn a bright red colour, and the secretions, urine, etc., are stained. The staining persists for a very long time ; the internal organs may show the staining of the dye for eight weeks or more after injection. We cannot claim to have cured any animal infected with the parasites of Surra, Nagana, Dourine ; the disease, especially in rats and mice, may be greatly prolonged, but the animals eventually die. Larger animals, especially dogs, cats, and monkeys, frequently show toxic symptoms with small doses. On the other hand, a bitch injected subcutaneously with fifteen to twenty c.c. of one per cent. trypanroth two and three times a week showed very little toxic symptoms besides a somnolent condition which lasted for about eight hours after each injection. The combination of trypanroth and

arsenic has given better results both in the hands of LAVERAN and ourselves.   The animals can live for a longer period ; the administration need not be so often repeated. The drawback is that the kidneys quickly become affected.   Animals infected with *T. dimorphon* do not seem to react well to arsenical treatment by itself ; trypanroth medication only causes the parasites to temporarily disappear ; the combination of arsenic and trypanroth causes the parasites to be absent for a longer period.   Various methods have been tried.   Administration of trypanroth and arsenic together, or the dye first, followed in twenty-four to thirty-six hours by arsenic or *vice versâ*.   It is the latter treatment which has given most satisfaction in our hands.   LAVERAN administers high doses of arsenic and follows twenty-four hours later with an intramuscular injection of trypanroth.

Arsenic has been tried in combination with the other dyes but without results.

Arsenic and human serum in the treatment of rats infected with Nagana and Surra have prolonged the life of the animals.   A guinea-pig inoculated with *T. Brucei* did not seem to improve, the serum was given in amounts of ten c.c. once a week, but no more serum being obtainable the treatment had to be stopped, and at the end of three weeks the parasites then reappeared.   Animals infected with *T. dimorphon* do not show as marked a reaction to serum treatment as do Nagana, Surra, etc.; the employment of arsenic and human serum does not appear to promise well.   Human serum and arsenic combined has been tried on animals infected with *T. gambiense* ; the control animals treated with arsenic alone showed as much, if not more, improvement as those with the combined treatment.

Arsenic and Baboon serum ; very little serum was obtainable, three mice infected with *T. gambiense*, *T. dimorphon*, and *T. Brucei*, respectively, were used.   The results were indefinite.

Other sera including horse, cow, sheep, goat, dog, cat, rabbit, and guinea-pig have been tried.   The sera were produced from both healthy as well as from infected animals which had the disease in a chronic form or were apparently cured. The sera were used alone or in combination with arsenic, with disappointing results, for in no case could a definite and permanent decrease be ascribed to the action of the sera.   Polyvirulent sera have also been tried without success.

ATOXYL EXPERIMENTS

The trypanosomes and animals used for carrying out the investigation were as follows :—

T. gambiense, in monkeys, dogs, puppies, kittens, rabbits, guinea-pigs, rats, and mice.

T. Evansi, in horse, dogs, rabbits, guinea-pigs, rats, and mice.

T. Brucei, in cow, rabbits, guinea-pigs, rats, and mice.

T. equinum, in dogs, rabbits, guinea-pigs, rats, and mice.

T. equiperdum, in pups.

T. dimorphon, in dogs, pups, rabbits, guinea-pigs, rats, and mice.

The drug was used only upon animals showing the effects of the parasites, such as loss of weight, anaemia, fever, and autoagglutination of the corpuscles, and no animal was used until its blood contained numerous parasites. The numbers of the parasites present differed according to the species of animal and the disease. In the majority of the experiments control animals which were not treated and had been inoculated at the same time as the treated animals were used. In all cases the control animals died.

Intravenous inoculation was used only on rabbits, all other animals were injected subcutaneously. Treatment was continued for one to three months or until increase of weight, diminution of the anaemia and entire absence of parasites from the blood, as far as microscopical examination could determine, was noted. At various periods susceptible animals were inoculated with the blood from a treated animal. When treatment had been discontinued for one to three months or longer, the animal was bled or killed and all the blood available was used to inject susceptible animals. Inoculated animals whose blood has given negative results after three to six months or after longer periods have been inoculated with virulent blood and have taken the disease, thereby showing that no immunity was conferred by the previous inoculation.

T. gambiense.—Rabbit, ♂, weight, 2,010 grammes. Parasites appeared on the twelfth day. On the forty-sixth day, numerous trypanosomes were present ; it had lost weight (1890 grammes). A blood count gave reds, 4,980,000 ; whites, 8,860 ; haemoglobin, sixty-seven per cent. For three-and-a-quarter months it received 1·0 c.c. of five per cent. solution atoxyl three times a week, gradually increasing the amount to 1·0 c.c. of ten per cent. solution. It then weighed 2,000 grammes. The blood count was : reds, 6,640,000 ; whites, 6,200 ; haemoglobin, eighty-eight per cent. The blood in quantities of ten c.c. was non-infective. The autoagglutination of the corpuscles was lost. Thirty-two days later it was very ill ; it was therefore bled to death, and the whole of its blood injected into a monkey. This monkey has never become infected. The *post-mortem* showed severe haemorrhagic cystitis, the bladder in parts being almost gangrenous and acute septic peritonitis, especially around the bladder. The spleen showed no congestion, but the connective tissue was slightly increased. The kidneys and liver were normal.

Rabbit, 889, inoculated October 26 ; weight, 1,760 grammes. Blood count : reds, 6,620,000 ; whites, 6,700 ; haemoglobin, eighty-nine per cent. Parasites were seen from November 8 up to January 10 ; the trypanosomes were always present, but in small numbers ; they then increased to eighteen to twenty to a field. The anaemia was pronounced, and loss of weight was noted. It then weighed 1,540 grammes. Blood count : reds, 3,880,000 ; whites, 11,800 ; haemoglobin, sixty-three per cent. It could hardly sit up, and remained most of the time lying down. This animal was given 0·8 c.c. of five per cent. solution atoxyl. At the end of eighteen hours the parasites were absent from the blood. Doses were given twice a

week, beginning with 0·5 c.c., and increasing to 2·0 c.c. of a five per cent. solution. The blood in large doses is non-infective. The animal is vivacious ; the coat is smooth and thick. Average weight, sixteen hundred grammes. Several guinea-pigs infected for about two months, and showing twenty to forty parasites to a field, have been treated. They were injected subcutaneously with 0·3 c.c. of ten per cent. solution. At the end of the fifteenth hour no parasites were seen. Treatment was 0·1 to 0·3 c.c. of five per cent. solution three times a week for two months. The animals all increased in weight. One died sixty-two days after treatment was discontinued. Three rats inoculated with its blood never became infected. The second and third pigs were killed at the end of eighty and one hundred days after stopping treatment, and the blood used to inoculate controls. No control has shown the parasites. Virulent strain. *Rhesus.*—Weight, 2,815 grammes. Inoculated November 16. Blood count : reds, 5,220,000 ; whites, 12,800 ; haemoglobin, seventy-eight per cent. On November 31 parasites were seen. Two days later, twelve to seventeen to a field were noted. The weight was 2,420 grammes. Blood count: reds, 4,600,000; whites, 7,200 ; haemoglobin, seventy-three per cent. The parasites counted per c.mm. gave 100,000. Oedema of the eyelids and bridge of nose was present. The animal was given 0·8 c.c. of ten per cent. solution atoxyl subcutaneously. Four hours later : reds, 4,450,000 ; whites, 24,800 ; parasites, 40,000 per c.mm. At the eighth hour : reds, 4,740,000 ; whites, 25,200 ; parasites, one to eighty-nine fields. Between the fourth and eighth hours the parasites were seen to become remarkably degenerated and deformed ; many phagocytes were present. At the twenty-fourth hour after injection a count gave reds, 5,050,000 ; whites, 46,000. The blood was negative. The leucocytes remained high for a couple of days, and then fell ; in none of the phagocytes could any remains of trypanosomes be found. Treatment, 1·0 c.c. of ten per cent. solution was given twice a week. The animal increased in weight. The autoagglutination of the corpuscles began to be less accentuated, and the number of erythrocytes and the haemoglobin rose. The local oedema disappeared. On the thirty-ninth day dysentery appeared, and the animal succumbed on the forty-eighth day after injection. The autopsy showed a very severe haemorrhagic and necrotic enteritis, with slightly enlarged spleen. Kidneys normal. Glands small ; inguinal group haemorrhagic. The blood was non-infective in amounts of 1·0 c.c., but infective if fifteen c.c. of pure blood was used. Unfortunately, the arsenic was discontinued on the appearance of dysentery. A second monkey, inoculated from the first *rhesus* just before treatment was begun, was treated with the same doses of arsenic ; the parasites disappeared in the same way, but the animal quickly succumbed to dysentery.

Many rabbits inoculated with this strain have been treated. It was found that unless treatment was started early that the majority of animals died as it was so exceedingly virulent. With these animals treatment was begun earlier and higher

doses given than with the standard 'Gunjur' strain. Despite treating the animals early some died. With this strain treatment had to be kept up longer. Some rabbits have survived eight months after injection, while all the controls have died in fourteen to thirty-six days. Guinea-pigs infected with this strain do not react so well to the treatment. Rats must be treated early and with high doses if treatment is to be successful. Mice infected with this strain react if treatment is commenced early enough.

T. brucei.—Guinea-pigs inoculated for two months. Average loss of weight one hundred and fifty to one hundred and ninety grammes. Parasites forty to sixty to a field. Initial dose, 0·4 c.c. five per cent. solution ; parasites absent in about eighteen hours. Treatment, two to three times a week, 0·1 of five per cent. solution. At end of two to three months treatment was discontinued ; the animals had increased in weight. At periods, one to two-and-a-half months after stoppage of arsenic, the animals were killed and their whole blood used to inoculate rats. None of the rats inoculated from these guinea-pigs have ever shown parasites. Some of the control rats inoculated during the first five weeks of the treatment have become infected after lengthy incubation periods. These treated guinea-pigs have shown increase of weight and lessening of the agglutination of the blood cells. Non-treated guinea-pigs have lived on an average forty to forty-five days. One treated pig had lived nine-and-three-quarter months since becoming infected, or five-and-a-quarter months since discontinuation of treatment.

Rats.—Treatment was begun two to three and four days after parasites appeared, when the animals were in the last stage of the disease and showed the semi-comatose condition which is so often met with. Rats of one hundred and twenty-five grammes in this last stage, and showing two hundred or more parasites to a field, have been given 0·5 c.c. of five per cent. solution. The parasites have been absent from the tail blood at the nineteenth hour. If treated twice a week with 0·3 of five per cent. solution, and the amount gradually increased, the parasites remain absent, the blood is non-infective and the animal puts on weight. Some have lived one hundred and twenty-six days from date of inoculation, and fifty-seven days after treatment was stopped ; they have succumbed to broncho-pneumonia or dysentery. The subinoculated animals have remained uninfected. Rats treated only once with 0·5 c.c. of five per cent. solution show parasites in their blood again in six to eleven days, and unless treatment be recommenced, the disease will pursue its natural course.

Cow.—This animal was inoculated subcutaneously from a guinea-pig. Parasites appeared on the sixth day but were very scanty. A severe enteritis occurred on the ninth day, but the animal survived the attack. The parasites slowly increased. Loss of weight and marked anaemia developed. Parasites continued to increase, the animal was then given 1·5 grammes of atoxyl in ten per cent. solution subcutaneously. The parasites disappeared slowly. Leucocyte count increased. Deformed degenerated

trypanosomes were seen at the end of a day. The parasites were absent from the blood ; 2·0 grammes of atoxyl was given twice a week. The animal was very anaemic, emaciated, and weak. It improved for a time. Unfortunately, our early departure prevented us making a study of this case, and, moreover, as there was no data as to the dose of the drug the administration had to be cautiously proceeded with.

T. evansi.—The results in the small laboratory animals are about the same as with Nagana. The animals have lived as long.

A horse was injected subcutaneously with blood from an infected rat ; many divisional forms seen. Incubation four days ; temperature rose on fifth day and parasites rapidly increased in numbers ; the animal eat little and stood with head hanging down. The numbers increased to forty thousand to the c.mm.; the haemoglobin fell from ninety-five to sixty-three per cent. ; no great change was noted in the red and white blood cell count. When definite loss of weight and anaemia had occurred and the parasites were about five hundred to the cover, 1·0 gramme of atoxyl in the form of a ten per cent. solution was given subcutaneously ; in thirty hours the parasites had disappeared. A slight leucocytosis was observed ; 2·0 grammes were then given and this was continued twice a week. The animal grew fatter and the blood count improved.

T. equinum.—Guinea-pigs react to treatment quickly and for a considerable time, but there is a tendency for some of them to show parasites in their blood one and two months after treatment has stopped. On the other hand, one treated for two-and-a-quarter months survived eight-and-a-half months after inoculation, treatment being stopped for two-and-three-quarter months. This animal was killed, and rats and guinea-pigs inoculated with its blood have never become infected. A rabbit which had been infected for twenty-eight days showed the loss of hair around the eyes and nose, and the purulent discharge from the eyes, nose, and urethra. The oedematous condition of the ears and genitals has by treatment with 1·0 c.c. of ten per cent. solution once every five days disappeared so far that all the external signs are wanting and no parasites are found ; it has, up to date, lived seventy-six days.

T. equiperdum.—The work has been entirely on pups ; these parasites have reacted in the ordinary manner, but the tendency of young puppies to manifest toxic symptoms has resulted in the experiments only prolonging the disease for one to two weeks longer than their controls. A bitch, inoculated January 14 ; positive January 20 ; large number first appeared on the 26th, temperature markedly irregular. Periodicity noticed ; usually parasites absent for one to three days, then a rapid increase. Animal became very anaemic and thin. The blood count before inoculation gave 5,860,000 reds ; 11,000 whites ; and ninety-seven per cent. haemoglobin. On February 25 the reds were 3,780,000 ; whites, 14,000 ; haemoglobin, sixty-five per cent. ; parasites were six to eight to a field. Trypanroth sixteen c.c. one per cent. solution administered. In thirty-six hours no parasites

were seen, but they reappeared six days later. Ten c.c. of one per cent. trypanroth was then given every eighth day ; the temperatute lowered. The parasites decreased but the anaemia persisted ; March 19, atoxyl 1·5 c.c. of ten per cent. solution subcutaneously given ; parasites disappeared to reappear eight days later. Despite large amounts of trypanroth the animal succumbed to the disease. A blood count, March 30, reds, 1,710,000 ; whites, 6,600 ; haemoglobin, forty per cent. ; trypanosomes, 24,000 per c.m.

T. dimorphon.—This parasite has proved harder to combat with atoxyl or any other form of arsenic than the preceding parasites. LAVERAN also notes this peculiarity. Guinea-pigs showing a severe infection react extremely badly, they appear to manifest a greater tendency to toxicity than do similar animals infected with Nagana or other trypanosomes. The disappearance of the parasites is often less rapid, sometimes they never absolutely disappear, an occasional one being seen in the preparation. In the endeavour to cause the typical reaction a fatal dose may easily be given. One guinea-pig out of twenty-two experimented with survived three months. This animal was only treated once a week with 0·3 c.c. of five per cent. solution. It improved considerably, but towards the last the parasites reappeared, and the animal succumbed. The autoagglutination of the blood-cells never completely disappeared ; the animal always exhibited some fever.

Rabbits.—React better to the drug, but the effect is more transient than with other trypanosomes. Higher doses have to be given. The life of the animal can be greatly prolonged, and for one or two months the general condition is improved. After that time parasites once more reappear ; by increased dosage they may again disappear. Towards the end larger doses have to be given than at first to produce the effect on the parasites. Here it may be noted as applying to all species of animals infected with *T. dimorphon* that the parasite in animals treated for some time will hardly be recognizable ; it presents a deformed appearance, its flagellated extremity is shortened so that scarcely any movement occurs. Blood from such an animal will be declared negative unless each field is gone over thoroughly. All peculiar bodies present must be examined, and over any such object minutes spent to determine if there is any movement. This point seems rather superfluous, but our experience is that the ordinary careful observer usually fails to find these degenerated, deformed parasites, and it is probably for this reason why so many negative diagnoses are made. Dogs and cats infected with this parasite show only a slight retardation of the disease ; the numbers are greatly reduced or absent, but tend quickly to reappear.

TRYPANRED

This dye has been mostly used in connection with animals infected with *T. equinum.* The results tally with those of EHRLICH and SHIGA, and the later report of LAVERAN and MESNIL. Some rats injected with this compound have lived as long as one

hundred and seven days; in one case sixty-three days without any treatment being given, this rat died from broncho-pneumonia, its blood was non-infective.   Mice have also shown the favourable results of this treatment, but they do not appear to be able to withstand large doses.[1]   Rabbits and guinea-pigs inoculated with this substance have had their lives prolonged for a considerable time, the parasites disappear completely, and if the administration of the dye be continued the trypanosomes may not be seen for thirty to sixty days ; but the animal does not show the favourable reaction which attends the administration of arsenic, the anaemia is very little lessened, very often it is increased, the weight is not recovered, the temperature remains irregular.   Very often if high doses are given toxic symptoms appear, these are characterized by more or less somnolence, the animals always appearing ' heavy,' there may be a slight discharge from the eyes.   The face is often puffy, the urine in such cases usually has albumen ; after a time these symptoms wear off.   They are especially marked in rabbits after intravenous injections.   Rabbits can withstand intravenous injections of 1·0 to 2·0 c.c. of one per cent. solution, and it appears as if the administration through a vein gives better temporary results, as the parasites, even *T. dimorphon*, disappear more quickly than by the subcutaneous method.   The disadvantage is that the anaemia appears more pronounced, and the toxic symptoms severer.   No rabbit or guinea-pig has been definitely cured.

Dogs and cats react fairly well to this dye, the life of the animal is prolonged, but no cure has been affected.   Towards the last, despite repeated injections of this dye, the parasites continue to increase, the animal becomes a living skeleton, and usually develops a purulent discharge from the eyes and urethra.

T. evansi, *T. brucei*.—Rats and mice inoculated with either of these parasites, and injected with this dye, live longer than infected controls.   The disappearance of the parasite is only temporary ; after four to six days the parasites reappear, and though they can be caused to disappear once or twice they sooner or later reappear, and the animal dies, sometimes at a time when only very few parasites are present in the blood.   With Nagana and Surra, inoculated animals which have been treated have not lived over thirty days, the majority dying in fifteen to nineteen days.

Rabbits and guinea-pigs react for a while, but the action on the parasites is not so marked as with Caderas infected animals.

Dogs and Cats.—The same results as with rabbits and guinea-pigs, only a very short prolongation of the disease is observable.

T. equiperdum.—The duration of the disease is only slightly affected by this dye.   If injected early in the disease when the pup shows hardly any parasites a considerable prolongation of the animal's life occurs, but once large numbers of

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1. Extreme care has to be exercised in administering th's solution. With rats and mice the preferable method seems to be to inject into the thick muscles of the hind legs. The hair is shaved off and the part disinfected before the injection. Guinea-pigs and rabbits ought to be treated in the same way. Dogs and cats can be injected in the deep muscles of both fore and hind limbs, in the back, or in the loose tissue of the neck. Monkeys are difficult as the skin is extremely sensitive, but the thighs offer the best site for inoculation.

parasites are present in the peripheral blood very little benefit occurs from a continuance of this treatment.

T. dimorphon.—The dye exerts a slight influence on this parasite, the numbers in the blood diminish, and many deformed ones are seen after a few days ; the organisms increase in numbers. In a few instances the parasites are absent from the peripheral circulation for a short time. Dogs show only transient improvement with a diminution of the parasites followed by a rapid increase in their numbers ; this phase lasts a few days only. Rats and mice show a temporary reaction, their lives may be prolonged for fourteen days ; at times the treated ones appear to succumb more quickly than the non-treated controls.

Rabbits and Guinea-Pigs.—The same temporary benefits are derived from injection of this dye.

Testing the action of the dye on the parasite by the addition of some of the solution to defibrinated blood containing trypanosomes, shows that with all of the different pathogenic trypanosomes a slight microbicidal action is manifested. The contact must be for some hours before the action will be noted ; it is, however, a definite action as control tubes show. EHRLICH and SHIGA record the preliminary injection of animals with trypanroth, and after a few days injecting the animal with some virulent blood. They believe that the dye forms anti-bodies. A goat was therefore regularly injected with the dye, and after some weeks some of the blood was drawn off; at first it was noted that the action of the serum did not differ from normal goat serum. As the animal got more under the influence of the dye it was noted that contact of living trypanosomes with this serum produced deleterious effects on the parasites which very often became deformed. If some of this serum was injected into mice with *T. equinum* a diminution in the number of the parasites was noted. Unfortunately, the injection of this dye for a long period caused the goat to become very run down, and the regular injection for a time had to be stopped. On the goat regaining its health it was once more given a course of injections with the dye. The serum had the same effects. In four mice the administration of this serum has prolonged the lives of the animals for thirty-one to forty-eight days. The serum is of a light, clear, fuchsin-red colour after five grammes have been given the goat. The amount of serum necessary is about 1·0 c.c. for a mouse of twenty-two grammes weight. As more of the dye is injected the amount of serum necessary is lessened. The experiments are too few in number to possess any practical worth. As the sodium arseniate and trypanroth had proved of use a combination of these two substances was tried for ten months.

Atoxyl-trypanroth combination has proved of benefit. With animals infected with *T. dimorphon* the results are more promising than in controls treated with either of these drugs separately. The parasites disappear rapidly, remain absent if the administration of the arsenic and the dye is pushed ; the animal increases in weight ;

the anaemia lessens, and the autoagglutination disappears. As mentioned before, trypanroth caused a nephritis, therefore, the combination of these two subjects is fraught with difficulties. In order to obtain results both drugs have to be pushed. It therefore often happens that the animal acquires a severe nephritis and succumbs. The effects of this combination have been noticed on other trypansomes.

The combined treatment offers a promising mode of medication, providing the untoward effect can be diminished or abolished. Very many of the smaller animals, especially guinea-pigs, have died from the effects produced by trypanroth.

ACTION OF ATOXYL AND TRYPANRED ON THE TRYPANOSOME

The action of atoxyl on the various trypanosomes has been studied, and after numerous observations, continued for the whole period during which the drug was administered, the effect appears to be as follows :—

On administration of arsenic compounds into an animal showing numerous parasites in the blood the following action on the trypansomes will be noticed. For the first three-and-a-half to four hours, depending on the dose used, very little change in the parasites can be noticed. Between the fourth and fifth hour the effect on the trypanosomes is evident. Some parasites appear to be swollen and their movement is less rapid. If now a series of blood specimens be examined at intervals of twenty to thirty minutes the following changes will be seen. The number of slowly moving trypanosomes increases, many parasites will be seen to be almost motionless. The protoplasm takes on a peculiar ground-glass appearance, and dark granules appear in the protoplasm ; very often a whole series of granules one behind the other, sometimes in pairs or all clumped together, are seen lying between the macronucleus and the anterior end or distributed through the whole body of the parasite. At the same time vacuoles are observed, oftentimes very large. The trypanosomes become deformed, assuming various shapes, the most common being a kite-shaped form with fairly long flagellum, and a tadpole-like one with hardly any free flagellum. These forms especially exhibit greatly impaired movements. At the same time a noticeable increase of the leucocytes is discernable ; phagocytes begin to appear, very often groups of five to seven will be seen. Up to this time (sixth to seventh hours) the trypanosomes, though decreased in numbers, are still present in considerable quantities. Suddenly in the course of an hour the numbers may drop from forty to two to three to a field or less ; coincident with this is a very marked increase in the number of leucocytes, especially phagocytes. From the ninth to the fourteenth and sixteenth hours the changes are less pronounced and rapid, the trypanosomes gradually disappear. At the eighteenth hour, provided the animal has been injected with the correct amount, the parasites are absent from the peripheral circulation and, even though the blood be centrifuged, none can be found.

My colleague, Dr. BREINL, and myself have observed a large number of experiments, examining the animals every one-and-a-half hours until the four-and-a-half hour, then every half hour until the parasites were almost absent, and continuing the observations hourly until the twenty-fourth to thirty-second hour after starting the experiment. From a series of these observations, we have determined that in hardly any of the forty-six continuously observed animals were parasites to be found after the eighteenth hour. Should, however, the drug be given in smaller amounts the process takes longer, lasting from thirty-six to forty-eight hours.[1]

Phagocytosis has been observed on three occasions. We have witnessed the engulfing of a trypanosome still alive though almost motionless, and on other occasions the ingesting of dead trypanosomes.

Observations on the effect of *Trypanred* on the trypanosomes show that almost the same changes take place, though the process is somewhat slower, usually requiring forty-eight hours or more ; leucocytosis also appears to be very marked in animals treated with this drug.

LEUCOCYTOSIS

In the observations on the action of arsenic and trypanroth on the trypanosomes, it was noticed that a marked increase of leucocytes occurred. Phagocytosis having been seen, it was determined to try the effect of hyperleucocytic agents on the parasites. In the work on the effect of diphtheria antitoxine on trypanosome-infected animals a certain amount of leucocytosis had been observed, but it was not marked. Advantage has, therefore, been taken of such agents as nuclein (this also affects the kidneys). Thanks to Professor SHERRINGTON, we were able to try the effect of colchicine on the infected animals. This substance causes a leucocytosis, but it only produces an increase of some six thousand in thirteen hours. Nucleinic acid acts in the same way. We have been induced to try the effect of administering the atoxyl or trypanred, or the combination atoxyl-trypanred, and following this treatment with the hyperleucocytic agent. Unfortunately, no better effects have been observed. This line of work ought not to be neglected, as it is quite evident that the leucocytes play a rôle in the decrease of the parasites. Animals which have been carefully observed every day show from time to time a decrease in the number of parasites in the peripheral blood. If the decrease be sudden and marked a vast increase in the number of leucocytes is determinable.

THE ACTION OF BACTERIA UPON TRYPANOSOMES

It has been noted in animals suffering from trypanosomiasis that should a localized abscess occur a certain decrease in the number of trypanosomes in that region can be made out. In rats the tail from snipping or pressure may become ulcerated or

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[1]. Blood films were made before beginning the experiment, and at every examination. The results of the examination of these stained films bear out the observations on the fresh blood films. The significance of the granules seen and the phagocytosis will be discussed in a fuller report.

gangrenous, in such a part very few parasites will be found although the blood above the area may be swarming. Moreover, the movements of the parasites are slow and show degeneration and deformity, whilst phagocytes will be found containing the remains of trypanosomes. Acting on these observations experiments have been made with various bacteria. Guinea-pigs suffering from tuberculosis seem to be somewhat more susceptible to trypanosome infection than healthy ones. Mice, already infected with trypanosomes, and then inoculated with *B. anthracis* and the *B. sui*, show at times a certain amount of retardation of the disease. The parasites do not appear to augment so rapidly. Ordinary cultures of pyogenic cocci are too virulent for the animal. If a very attenuated culture of *Staphylococcus pyogenes aureus* is inoculated with *T. dimorphon*, the animals show a marked diminution in the number of parasites, but they persist for some days. Unfortunately, the subcultures never act in the same way and show increase in the virulency. In one of some eight rats infected with *T. brucei* and then injected with *Leuconostoc mesenteroides* there was a lessening in the number of the parasites ; this animal was subsequently injected intraperitoneally with a culture of KLASSE's *Gonodiplococcus* of scarlet fever. The parasites disappeared, but the animal died one-and-a-half days later from a septic peritoneal infection due to the wounding of the gut. Cultures of *B. typhi*, *B. coli*, *B. diphtheriae*, so far as we have been able to observe, have proved of no avail.

In connection with these observations it is interesting to record the work of NISSLE ; unfortunately, only a review of his preliminary paper is available. He inoculated intraperitoneally a number of rats infected with the Nagana parasites with one-twentieth of a loopful of a *B. prodigiosus* culture grown on potato ; in twenty-four hours the trypanosomes disappeared, and some of the rats died from intoxication in half to three hours after injection. During the disappearance of the parasites he observed all kinds of endo-globular forms. It must be borne in mind that the injection of a culture causes a leucocytosis, and whether NISSLE's results depend only upon this or upon the toxic products of the bacteria remains to be seen. It is hoped that this question will not be neglected.

Conclusions

From the experimental work with various therapeutic agents the following conclusions can be made :—

1. That animals suffering from trypanosome infection react favourably to only a few agents, of which arsenic is the only drug which seems to exert a more than transient action.

2. That the greater the amount of arsenic introduced into the system of the animal the greater and more permanent the effect on the parasite.

3. That arsenic medication is indicated in the treatment of individuals suffering from Trypanosomiasis. That the treatment ought to be long continued and regularly

administered in as high doses as the case can stand.   That all aids to building up the system should be employed.

4.   That in trypanred we possess an agent of some use in the treatment of trypanosomiasis.   That certain trypanosomic diseases appear to be more amenable to its action than others.   That in the substance at present available there is need for improvement in order to abolish its toxic effects.

5.   That a combination of arsenic and of an improved form of trypanred would seem indicated in the further investigation of the cure of trypanosomiasis.

6.   That further efforts ought to be made to produce less toxic forms of arsenic suitable for injection.

7.   That the action of both arsenic and trypanred causes a hyperleucocytosis and that this condition has an effect upon the parasites.   In confirmation of this is to be recorded :—

 (*a*) The hyperleucocytosis coincides with the time when the parasites suddenly disappear from the peripheral circulation.

 (*b*) There is a smaller number of parasites present in necrotic, bacterially-infected areas than in the general circulation.

 (*c*) The effect of certain bacteria on animals infected with trypanosomes.

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SUPPLEMENTARY NOTES

On some Animals still under Treatment on Drs. Thomas's and Breinl's Departure

Nagana cow (page 55), died eleven days later. The haemoglobin was eighty per cent., and the agglutination of the red blood corpuscles remained absent. Death due to extensive hydatids and pulmonary tuberculosis.

Surra horse (page 56), died over three weeks later. The haemoglobin which had at first risen under the atoxyl to eighty per cent. had fallen to sixty per cent. just before death. The agglutination of the red blood cells remained constantly present. In the intervals between the administration of the atoxyl, the parasites would return, and were twenty-five per field on the fourth day after the dose in the last week but one. Two doses per week were therefore commenced. This kept the number of parasites down, so that one per five fields was the highest reached. The animal, however, had become too reduced, and died three days after receiving the third dose at the smaller intervals.

Autopsy.—There was anaemia of all the organs, otherwise (spleen not excepted) there were no striking naked eye changes. Patechial haemorrhages were found in the muscles of the chine. There was a retropharyngeal abscess the size of a child's head communicating with the nasal sinuses, but it appeared to have been of some standing and not to have caused death. The animal was able to swallow readily up to its death.

Rabbit 839 (page 53), kept in perfect health for another month, and as the animal went off its food for a day or two the last dose given was only half the usual one. Two days later, however, the animal died, parasites having been found in the blood the day preceding, twenty per cover, degenerated and almost motionless from the effect of the atoxyl.

Caderas rabbit (page 56), still alive, September 28, 1905. In perfect health. No discharges of any kind ; all hair regrown. No treatment since June 3. Blood negative since May 30 to microscopical examination. Its weight on April 25 was 1,770 grammes, and continued to fall till the end of May, when it was 1,190 grammes. Since then it has gradually increased to 1,795 grammes. It has now lived eight months. The head is usually kept over extended, which may be due to canker of the ears which was always present.

<div align="right">P. A. H. RADCLIFFE, M.B.</div>

I

PART II.   POST-MORTEM EXAMINATIONS WITH DESCRIPTION OF MACROSCOPIC AND MICROSCOPIC CHANGES

I.   EXAMINATION OF ORGANS IN MAN (four cases)

A.—Case of the Native, Kitambo

Death, June 12, 1904, eight p.m. ; *post-mortem* six hours later.

External appearances.—Body of middle height, very emaciated ; muscular development poor ; no exanthemata ; no oedema ; rigor mortis marked ; configuration of skull normal, markedly dolicho-cephalic. Conjunctivae and sclerae very pale and slightly jaundiced ; mucous membranes pale. The cervical and inguinal lymphatic glands were distinctly prominent. Thorax long ; intercostal spaces prominent ; abdomen retracted. External genitals normal. Superficial ulcers about three c.m. in diameter on the soles of the feet (probably produced by chiggers).

Brain and Spinal Cord.—The under surface of the scalp was very pale. Skull rather thin, the vessels on its inner surface occupied deep sulci. Circumference of skull fifty-two c.m. The dura mater adherent in places to the bone. The sinuses contained dark fluid blood and a few clots. The inner surface of the membrane was smooth and glossy. Cerebro-spinal fluid increased in quantity. The surface of the brain was of normal configuration ; the gyri appeared a little flattened. The superficial veins were very large, many were tortuous and they contained dark venous blood. The arteries were also engorged so that the whole surface of the brain was covered with a dark blue network, and greatly congested capillaries (Fig. 1). The leptomeninges were thickened, and in places whitish but not adherent to the brain. The pial vessels of the cerebellum were much congested and could be traced to the finest capillaries. The basal arteries showed no sclerosis. On section the brain substance showed irregularly distributed congested areas ; the basal ganglia showed puncta cruenta. The brain substance was doughy, soft, and very oedematous ; the ventricles were dilated ; the ependyma roughened ; the vessels very congested. The pons and medulla showed no macroscopic changes except congestion and some small haemorrhages about the size of pin heads. In the vertebral canal was a fair quantity of cerebro-spinal fluid. The inner surface of the spinal dura mater was smooth and glossy ; the pial vessels were enlarged and filled with dark blood ; the congestion was not equally distributed, being more pronounced in parts ; in others the vessels were only moderately filled. The cauda equina presented a striking appearance ; it was surrounded by a gelatinous tissue and its nerves were sheathed in networks of dilated vessels ; on section the congested vessels were seen as dark

red stripes entering the substance. In the region of the third dorsal segment, at the base of the right posterior horns, were four haemorrhages, the largest being about two mm. in diameter, and extending through two segments of the grey substance. The structure of the cord in the region of the haemorrhages appeared obliterated.

Thorax.—Diaphragm reached to the level of the fifth rib on each side. Both lungs were adherent to the diaphragm, but were free elsewhere ; parietal pleura normal.

Right Lung.—Pleura of the upper lobe normal ; that of the lower lobe showed dark blood coloured patches. The tissue of upper and middle lobes normal ; the lower lobe was congested and of a bright red colour.

Left Lung.—Under the pleura were numerous small haemorrhages. The lung tissue contained patches of congestion. The mucous membrane of the trachea was pale and covered with a small amount of clear viscid mucus. The thyroid gland normal. The nasal cavity and the sinuses in connection with it, as well as the tympanum, appeared normal. The pericardium contained a few c.c. of yellow tinged clear fluid.

Heart.—Size normal ; substance very pale and flabby. The chambers contained dark fluid blood and clots. The endocardium, the valves, and the intima of the aorta appeared normal.

Abdomen.—The cavity contained about two hundred and fifty c.c. of a light straw-coloured fluid.

Liver.—Of normal size ; its capsule here and there slightly thickened ; the parenchyma pale and friable.

The Gall Bladder.—Filled with thick, fluid, greenish bile.

The Spleen.—Enlarged (20 × 10 × 6 c.m.), the capsule very thick and partially adherent to the parietal peritoneum ; the pulp very dark purple-red and slightly diffluent ; on section, enlarged malpighian bodies could be seen in some parts, whilst in others no structure could be made out in the dark purplish mass.

The Kidneys.—Were of normal size, capsules stripped easily. Externally they retained the deep embryonic furrowings ; the cortex presented an appearance of fatty striation, otherwise they were normal.

The Bladder.—Contained nearly half a litre of cloudy urine. The mucous membrane was pale. The genital organs showed no changes. The suprarenals were greatly congested. Pancreas normal.

Stomach and Intestines.—The mucous membrane of the stomach was pale, that of the small intestine somewhat congested ; the large intestine being normal. The stomach contained a little mucus ; the small intestine was partially filled with yellowish-brown chyme, and here and there in the recesses of its folds a few anchylos-tomes were found.

Lymphatic Glands.—Mesenteric lymphatic glands were enlarged and showed haemorrhagic infiltration, others were normal. The inguinal glands were greatly

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enlarged, being six c.m. long and four c.m. broad. On cross section they appeared uniformly yellowish in colour.

Pieces were taken from all the organs and fixed in the usual fixing fluids :— Zenker's, Flemming's, hydrarg. perchlor., and alcohol. The brain and spinal cord were fixed in four per cent. formalin.

Microscopical Examination

The heart showed a fairly extensive small-celled infiltration in the endo-, epi-, and myocardium. The epicardial fat was well defined from the muscle, and in it was an accumulation of lymphocytes, large mononuclear and polymorphonuclear leucocytes. The infiltration extended along the connective tissue between the muscle fibres, and areas of infiltration were seen around some of the larger vessels in this situation, with a few mast cells and giant cells (these latter containing five to eight centrally-placed nuclei, or sometimes only fragments of nuclei). Here and there were large haemorrhages which extended from the perivascular connective tissue into the adjacent muscle, often destroying it. Frequently one could see, lying near a vessel wall, wedge-shaped patches of red blood corpuscles which were certainly the product of diapedesis, although no corpuscles were found in the wall itself. Many of the muscle fibres were smaller than normal, the striation was faint and around the nuclei were collections of reddish-brown pigment. These changes were for the most part equally distributed throughout the whole thickness of the myocardium, but in places were more marked in the left ventricle than in the right.

For the detection of bacteria, sections were stained by Löffler's methylene blue, carbol fuchsin (Pfeiffer), carbol thionin and by Gram's method. Only a very few bacilli (decolourized by Gram's fluid) were found. These were considered to represent *post-mortem* contamination.

Such parts of the lungs as appeared normal to the naked eye were found on microscopic examination to be hyperaemic. The congested vessels often contained a good number of white blood cells. The bronchi contained desquamated epithelium and exudation. The lymph tissue around the bronchi showed hyperplasia. The pleura showed various-sized extravasations of blood in its connective tissues, the blood vessels being very large. The pneumonic-looking parts showed typical catarrhal pneumonia, the alveoli were filled with red blood and exudation cells. The process had gone on further in some parts ; there the whole tissue formed a homogenous mass interspersed with red blood corpuscles and exudation cells. In the consolidated parts were a good number of cocci staining by Gram, and numerous Gram-negative bacilli and cocci.

The sections of the *liver* showed a thickening of the capsule. Between the chains of liver cells were many partly degenerated blood cells, decreasing in number as the centre of the liver was approached. The connective tissue was increased in amount

and contained a few lymphocytes. Many of the liver cells had no nucleus, the protoplasm was vacuoli., and in various stages of fatty degeneration. A few of the cells contained small clumps of yellowish pigment occupying more than half of the cell. The blood vessels which were very much congested, contained a limited number of white blood corpuscles.

The *kidneys* presented the picture of parenchymatous degeneration. The protoplasm of the epithelial cells showed granular degeneration. The blood vessels, especially in the cortical region, contained very many white blood cells, and here and there in close proximity to the vessel wall were numerous red blood corpuscles. In places only scanty remains of normal kidney tissue were visible, the field being occupied by well stained connective tissue, its meshes entirely filled with red blood corpuscles. The glomeruli showed the same congestion, distended capillaries sometimes forming more than two-thirds of the whole glomerulus.

The *spleen* in section showed a thickening of its capsule. The malpighian bodies were few in number and not prominent, and from them irregularly defined processes ran out into the surrounding tissue. There were two zones to be made out in a malpighan body, viz., an external zone consisting only of lymphocytes, and a central one consisting of large cells containing a vesicular nucleus. Amongst the latter were large non-nucleated cells (forty to fifty μ in diameter), the protoplasm of which stained a dull red with eosine, and in addition a few leucocytes with eosinophile granules. The trabeculae were hyperplastic, and the vessels showed endothelial proliferation. The congestion of the spleen was most striking, especially in the periphery. Interspersed in the tissue were red blood corpuscles, sometimes clumped together in dense irregular heaps, in which a few lymphocytes were also included. The red cells showed indistinct contours, and by their staining reaction were evidently degenerated. Here and there were small irregular necrotic areas, in which only a few connective tissue cells were present. The cellular elements of the organ included very numerous large hyaline cells, containing inclusions of red and white cells, a very few nucleated red cells in the larger vessels, and, in the tissue of the pulp, giant cells with numerous irregularly placed and irregularly shaped nuclei and a large protoplasmic body. Throughout the spleen are found mono- and polynuclear leucocytes with eosinophile granulation. Iron-containing pigment is occasionally seen, both intracellular and free. In the vessels and in the tissue itself occasional filaria embryos were met with.

Lymphatic Glands.—The most marked changes were found in the lymphatic glands. The vessels were found to be highly congested in accordance with the naked eye appearance. Side by side with normal glands, in nearly all groups, there were others which contained very numerous red corpuscles, lying free in sinus-like spaces partially filled with large phagocytic cells. In nearly all the lymphatic groups were also transitional forms between normal glands and those showing the above described changes. The changes bore no proportion to the size. In the same lymphatic gland

often one part would be quite normal, while another part showed typical sinus formation.

These enlarged glands in the first stage of their formation showed a thickened capsule, with bands of fairly thick connective tissue running in from it towards the centre and containing enlarged vessels showing distinct proliferation of their endothelium. In some places the lymphoid tissue does not reach right up to the capsule, but a lightly stained fine connective tissue meshwork intervenes, which contains, besides a few lymphocytes, a number of red corpuscles, large phagocytes, and iron-containing pigment granules. The nucleus of the phagocytes is peripherally placed, and their inclusions consist of the following :—numerous darkly-stained nuclei, the size of lymphocytes, and red blood cells which either stain yellow with VAN GIESON's method or appear merely as unstained vacuolar areas in varying number often filling the whole cell. The intervening spaces in the sinus are filled with coagulated fluid. Sinuses with the same contents as the peripheral one are found in very small numbers in the interior of the gland, recognizable at the side of the connective tissue strands by their light staining. The follicles are not so distinct as normally ; they are larger and not sharply defined from the surrounding lymphoid tissue.

The majority of the smaller glands presented a similar microscopic appearance ; frequently there were a good number of red corpuscles between the lymph cells, and the sinus system was more or less pronounced. Sometimes there occurred only a short peripheral sinus, with one or two central off-shoots ; in other places, the whole gland was interlaced by them. In some the follicles were normal, in others lightly stained, and contained, in addition to larger vacuolated cells and lymphocytes, a fair number of small cells two-thirds the diameter of a red blood corpuscle, which had an eccentric, deeply-stained, small nucleus and protoplasm staining faint pink with eosin. Besides, there were very many small nuclei both free and contained in other cells, staining darkly with haematoxylin. The blood vessels were much dilated, and contained, besides lymphocytes, a good number, as a rule, of large mononuclear and polymorphonuclear leucocytes and a few eosinophile cells.

Some of the larger lymph glands showed the change in a more advanced stage (see Fig. 6). In these, by a marked hyperplasia of the connective tissue, the lobules of lymphoid tissue became contained in a framework of connective tissue, producing thereby an alveolar structure. In the centre of the gland, an accumulation of connective tissue was extended by radiating septa to the capsule ; this was thin, and had large dilated vessels surrounded by numerous lymphocytes running in it. There were no fat cells in the connective tissue of the glands, showing that these were true lymphatic glands, and not new formations in the adipose tissue. The sinus formation was as described in the first stage. Around the congested vessels were nearly always deposits of red corpuscles with a little blood pigment. Follicles were not to be seen.

Fig. 7 shows this transformation in one of the mesenteric glands. A sinus is

seen not far from the capsule, filled with lymphocytes, and surrounded by red blood corpuscles, these latter being easily distinguishable from the blood vessels by the absence of endothelial cells.

The cellular elements included divisional forms of lymphocytes ; giant cells, resembling those of bone marrow ; large hyaline cells with included red corpuscles in all stages of degeneration, sometimes only appearing as vacuoles ; mono- and polymorphonuclear leucocytes with eosinophile granulation ; phagocytes ; and plasma cells.

In some of the glands were seen all stages between phagocytes containing red cells and eosinophile cells. In the first stage were phagocytes with their nucleus placed peripherally, and the protoplasm of the cell filled with red corpuscles, stained a peculiar colour with eosine. The eosine-stained red corpuscles became smaller and smaller in their including cells, whose protoplasm in a later stage seemed to be unevenly stained a brilliant red tinge. Later, the nucleus changes its form and becomes polymorphonuclear, and in other cells one can almost see the eosinophile granules crystalizing out from this red-stained protoplasm. Some of the eosinophiles are destroyed in the lymph glands by phagocytosis, whilst others appear to enter the circulation. New formation of lymphatic tissue was noted ; for example, in the adipose tissue around the axillary glands there was an accumulation of lymph cells between the fat cells, and in some places there was distinct follicle-like formation around the vessels.

The *Bone Marrow* of the femur in some places had normal appearance of fat marrow, but in others however the marrow was very cellular, the fat tissue being reduced to a few islets, and replaced by cellular tissue composed of normal marrow cells with very few nucleated red corpuscles, and a large number of eosinophile cells. The vessels were greatly congested and there were frequently haemorrhages around the vessel walls ; the blood corpuscles showing degeneration. In different parts there were varying numbers of phagocytes containing red blood cells. Blood pigment was only infrequently met with. Here and there the whole of the tissue was replaced by a homogenous substance containing very few cells, staining yellowish with VAN GIESON and red with eosine, and presenting the appearances of gelatinous degeneration.

Brain and spinal cord were fixed in four per cent. formalin and hardened in alcohol and sections were cut from the different regions. The sections were stained by haematoxylin, eosin and VAN GIESON, WEIGERT's method, WEIGERT-MARCHI, NISSL and UNNA, and for bacteria with LÖFFLER's methylene blue, carbolfuchsin, PFEIFFER, carbol thionin and GRAM.

The pia and arachnoid shewed a fair amount of pigmented cells, similar to those observable in skin, the pigment being dark-brown and granular. The large and small vessels were surrounded in a varying degree by small-celled infiltration ; sometimes the larger vessels had a quite normal wall. The small-celled infiltration consisted of

lymphocytes, phagocytes, with all kinds of inclusions, also a few larger mononuclear and polymorphonuclear leucocytes, and a large number of red blood corpuscles. The same infiltration was found about the cells of the pia and accompanying it into the cerebral sulci and to a certain extent into the brain cortex itself. In the brain the infiltration occurred chiefly around the vessels of the deeper parts of the brain, those of the cortex showing relatively slight changes.

The vessels in the deeper parts were very large and congested, the perivascular lymph space dilated, sometimes empty, at other times occupied by coagulated exudation or filled with lymphocytes. The proliferated endothelium projected into the lumen. The vessels in the more deeply situated parts of the brain were much more changed, especially in the region of the large grey basal ganglia. Here the vessels were surrounded by a thick layer of lymphocytes, filling up the perivascular space and extending into the brain substance. Amongst the exuded lymphocytes there were a good number of red cells. There were also cells resembling granulation-tissue cells, hyaline with a vesicular nucleus ; large cells (thirty to fifty μ in diameter) with protoplasm stained reddish with eosine and having one to three nuclei ; a few phagocytes and an occasional plasma cell.

The lumina of the vessels were often narrowed. Close to the vessels were various sized haemorrhages without any lesions of the vessel wall.

Very often, external to the layer of white cells which surrounded a vessel, was a second layer of red blood cells infiltrating the brain substance in the neighbourhood. In some places the brain was altogether destroyed through haemorrhage, but no blood pigment was seen, and red softening was marked. Around the infiltrated vessels was usually a proliferation of glia cells. The endothelium of the vessels was proliferated and the vessels contained many white blood corpuscles, sometimes in so large numbers that they seemed to fill the whole vessel like a thrombus. The infiltration was in general equally pronounced both around artery and vein, but sometimes the vein was more changed than the artery.

Here, and in other sections, where the vessel was seen in tangential section, red corpuscles were present in the media and adventitia. The ependyma of the lateral ventricle was proliferated, forming a dense fibrous layer.

The epithelial cells of the choroidal plexus approached the columnar in type and often had gaps between them.

Similar changes to those in the cerebrum were found in the pons, medulla, and spinal cord.

The pons on section appeared much congested, the vessels were irregularly sheathed with a layer of cells of the same nature as those above described ; the intervals between them being occupied by coagulated fluid. Throughout the section of the pons were seen various-sized haemorrhages ; the endothelium of the vessels was thickened, and there were a fair number of white blood cells in the lumen. Similar changes but

more pronounced were found in the medulla—small-celled infiltration around the vessels, with a dilated perivascular lymph space, filled with transudate sometimes containing lymphocytes. The vessels in the white matter were not so much altered as those in the grey. Haemorrhages were also present in far greater numbers in the grey matter though no pigment was seen.

The pia and arachnoid of the spinal cord was the seat of a well-marked small-celled infiltration, the meshes in the connective tissue of the arachnoid being often altogether obliterated by it. It is worthy of note that in this region the walls of the larger vessels were very often normal, whilst the small ones showed marked changes.

The spinal dura mater presented the same change as the cerebral. The congested vessels ran between the connective tissue, in places surrounded by a few lymphocytes, and here and there even small collections of lymphocytes.

The inflammation continued along the vessels into the fissures and white substance of the cord. Both fissures were often indicated by a dark blue band, this appearance being produced by the accumulation of exudation cells alongside the vessels running into the fissures. The changes in the grey matter of the cord were also much more marked than elsewhere. The periphery of the cord was very oedematous, and showed proliferation of the neuroglia. The grey matter was often intersected by a network of congested capillaries, which were surrounded by a thick sheath of cells (consisting of elements similar to those of the brain vessels). The congestion of the vessels was so great that the vascular supply of the cord was displayed in the sections almost like a diagram. The perivascular changes were irregularly distributed, now one, now another vessel showing them. Relatively, few white cells were found in their lumen, and but slight signs of endarteritis. The epithelium of the central canal was proliferated, as also the neuroglia adjacent to it.

The most marked changes were found in sections of the spinal cord taken from the level of the sixth cervical down to the third dorsal segment. In the seventh cervical region, the artery of the left posterior horn was surrounded by a layer of lymphocytes, four to six deep, with a layer of red blood corpuscles of the same width, external to it again. At the base of the horn a haemorrhage destroyed its substance in half its width. In the grey commissure also an abnormal patch was seen, consisting of two large arteries with veins and a number of capillaries, all sheathed in infiltrated connective tissue.

Fig. 8 represents a section of the cord taken two segments lower down. The artery of the posterior fissure is seen enclosed in small-celled infiltration in the inner third of its course. There is also a haemorrhage on both sides of it extending into the white substance of the cord, and destroying the adjacent medullated fibres, so that there are only a few bundles of fibres left, which appear in isolated groups in the haemorrhagic region. Other smaller haemorrhages are also seen in the posterior horns and in the grey commissure.

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All these haemorrhages were of quite recent origin, there being no trace of blood pigment. Below the third dorsal segment there were only some extravasated corpuscles around the infiltrated vessels. The other parts of the spinal cord all showed changes similar to those of the cervical part to a varying extent. Filaria embryos were present here and there in the vessels of brain and spinal cord.

The nerve fibres often showed alterations. The cerebral convolutions presented a marked degeneration of the supra- and intraradiar fibres. Usually there was slight degeneration of the fibres all over the brain, most marked around the larger foci of infiltrated vessels, the degeneration being both of older and more recent date. The fibres were often swollen and disintegrated. The pons and medulla showed degeneration of the medullated fibres irregularly distributed over the whole section, well marked around the infiltrated vessels, but most pronounced in the region of the haemorrhages, where Marchi's method showed an extensive recent degeneration. The spinal cord presented the same condition, but certainly more degeneration in the posterior than in the lateral columns, and less still in the anterior columns.

The large nerve cells of the brain and spinal cord which contained a fair amount of pigment, showed very marked changes, not confined to a certain group, but irregularly distributed in the whole central nervous system. The cells in the basal ganglia and of the anterior cornua of the cord were in an advanced stage of alteration. In the neighbourhood of normal nerve cells were others whose processes seemed to be broken away. They were spherical in shape with a dilated pericellular lymph space. They often showed chromatolysis, the Nissl bodies were nearly disintegrated, and a powdery mass occupied the whole body of the cell instead. Sometimes the chromatolysis was only peripheral, the Nissl bodies in the centre well preserved ; at other times, the change was only central, in the periphery the Nissl bodies being easily distinguishable, and the centre occupied by a homogenous mass. The nucleus was often normal, but at other times had an irregular contour, or was even not distinguishable at all. Here and there were nerve cells with vacuolated protoplasm. Some of the nerve cells were oedematous, others were shrunken, stained dark blue, and in a later stage represented by a number of disintegrated fragments ; they were pyknotic. At various levels in the cord, the number of the large anterior root cells seemed to be unequal on the two sides.

The nerve bundles coming off from the spinal cord also showed great dilatation of their vessels, sometimes forming more than half the bulk. Frequently there was slight degeneration of the fibres and the vessels were surrounded by a thin layer of lymphocytes. The spinal ganglia at the various levels showed changes analogous to those in the cord, viz., small haemorrhages between the ganglion cells, and slight round-celled infiltration of the vessels. Haemorrhages were also present in the surrounding fat.

In the peripheral nerves were sometimes signs of neuritis, small-celled infiltration in the endo- and perineurium, and slight degeneration of the fibres.

With the above-mentioned bacteria-staining methods, microbes were only found in a few sections of the central nervous system, although very many sections were examined. They consisted of a few long bacilli in short chains and groups, and a few large cocci. The specimen stained by GRAM's method showed no micro-organisms.

B.—THE SECOND CASE, TOMI ; DIED JUNE 17, 1904

Post-mortem three hours after death.

Length one hundred and fifty c.m. ; very emaciated ; slightly built ; skin dry ; desquamating ; no particular pathological changes ; Rigor mortis commencing in upper extremities. Skull dolicho-cephalic. Pupils unequal, the right one smaller. Left eye showed a corneal opacity one mm. in diameter. Right eye, corneo-scleral junction at its right lower quadrant, ill defined, with peri-corneal injection corresponding. Conjuctivae and sclera pale ; slightly jaundiced. Mucous membrane of lips and mouth very pale. Neck short, thin, its hollows well marked. Glands on both sides of the sterno-mastoid muscle visible as slight elevations. Thorax barrel-shaped ; deeply indrawn intercostal spaces. Inguinal glands much enlarged ; skin over them elevated. External genitals normal.

The under surface of scalp pale, here and there dark-blue, dilated veins visible. The circumference of skull fifty cm. ; skull cap thin with very numerous pacchionian depressions, especially along the coronal suture. Dura not tense, adherent to the bone ; inner surface smooth and glossy, no thickening nor haemorrhages ; all its sinuses filled with dark fluid blood. The amount of cerebro-spinal fluid was increased. The capillaries were injected ; the pia mater, especially along the edges and over the occipital lobe was opaque and thickened.

The base of the brain presented the same changes in the leptomeninges. Arteries not atheromatous. The gyri and sulci normal. The brain substance on cross section soft and oedematous. All over the cut surface numerous congested capillaries and a few small haemorrhages were visible.

The ventricles were somewhat dilated ; the ependyma smooth and firm. The spinal dura mater appeared normal. The spinal canal contained much yellow-coloured cerebro-spinal fluid in which were found numerous leucocytes and a few trypanosomes. The anterior and posterior spinal arteries with their corresponding veins could be followed as thick cords along the spinal cord and even traced to their last ramifications.

On the anterior surface of the cord, on the right side, was a spindle-shaped dark red-stained mass (seven mm. in breadth) extending from the sixth cervical to the third dorsal, sharply defined and resembling a blood clot (Fig. 4). The cauda equina was embedded in gelatinous tissue. On cross-section the central canal was dilated ; the structure of the cord was well preserved ; the vessels in the grey substance and

those entering the cord were much dilated. The congestion was less marked in the lumbar region. At places a greyish stripe of infiltration was visible at the sides of the vessels.

Tympanic and nasal cavities were normal.

Thyroid normal. Both tonsils were enlarged but not actively. The glands of the neck were enlarged (four cm. long, two cm. broad) ; on cross section they presented a partial haemorrhagic infiltration, being brownish-red in colour and speckled with grey points the size of a pin's head (Fig. 5). The connective tissue at the hilus was hyperplastic. The submaxillary lymphatic glands were also haemorrhagic. Lungs were free from adhesions and much congested. In the pleura of the right lung numerous haemorrhages were present. The peri-bronchial glands were enlarged, showing the same changes as the glands of the neck.

The pericardium contained six c.c. of yellowish, blood-stained serum. The heart was of normal size, pale, and flabby ; the epicardial fat well defined from myocardium. The cavities contained much dark fluid blood. The bicuspid valve was a little thickened, the other valves and aorta were normal. The mucous membrane of oesophagus and trachea was pale.

The abdomen contained a few c.c. of clear fluid. The liver was congested but otherwise appeared normal. The capsule was not thickened. The gall bladder contained much dark greenish bile. The spleen was increased in size (18 × 11 × 4 cm.), of a dark-reddish purple colour, hyperaemic, of solid consistence ; capsule thickened.

The malpighian bodies appeared hyperplastic on section, the medullary substance showed small white striae, but was otherwise normal. The bladder contained cloudy urine, its mucous membrane pale. External genitals normal. Stomach and intestine showed a little congestion. Slight enlargement of lymph follicles and PEYER's patches could be made out ; no cicatrices nor ulceration. The mesenteric glands were of a somewhat haemorrhagic appearance. The inguinal glands were the most enlarged (5 × 3 cm.), and showed the same changes as the cervical glands. Pancreas and supra-renals normal.

The bone marrow of the femur was reddish, with irregular greyish patches disseminated in it.

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On microscopical examination the organs showed lesions very similar to those of the previous case.

The *Epicardium* showed a varying amount of small-celled infiltration, most marked in the neighbourhood of the larger vessels and continuing along them into the myocardium. The infiltration consisted of leucocytes with a fair number of eosinophile cells and numerous red blood corpuscles. The endocardium also showed small-celled infiltration with similar constituents as in the epicardium. The striation of the muscle

fibres was frequently indistinct, and showed accumulations of brown pigment around their nuclei.

The *Lung* presented a remarkable congestion. The vessels of the alveolar septa were cork-screw shaped, projected into the alveoli and contained plenty of large mononuclear leucocytes. In some places were signs of commencing pneumonia. The alveoli were filled with a varying amount of exudation containing eosinophiles, and a large number of red blood corpuscles. There was striking hyperplasia of the lymphoid tissue around the bronchi. In the inflamed parts of the lung were a varying number of diplococci (stained by GRAM's method) and a few bacilli.

The *Liver* showed a little hyperplasia of the connective tissue. The cell rows were attenuated ; of the nuclei some were only faintly stained, others unstained ; the protoplasm being often merely an accumulation of various-sized fat droplets. No pigment was visible. Here and there were accumulations of red corpuscles between the liver cells.

The *Kidney* showed hyperaemia to such an extent that in some small places there was no epithelium to be seen, but only a delicate connective tissue with its meshes filled with red blood corpuscles. The glomeruli were often half-filled by dilated capillaries. Around the vessels were haemorrhages. Here and there parenchymatous degeneration was pronounced, the tissue being transformed into a homogeneous mass, with occasional tubules and a few epithelial cells left, and the whole traversed by numerous congested vessels containing many leucocytes.

The *Spleen* showed hypertrophy and hyperplasia of its follicles. The malpighian bodies were sharply defined and showed a light centre of large, irregularly-shaped cells with vesicular nuclei, other cells with much protoplasm and numerous (six to eight) irregularly situated, segmented nuclei, and a few mono- and polynuclear eosinophiles—all these cells being held in a fine meshwork of connective tissue. Around this centre is a corona of very densely-packed lymphocytes, while the external part is made up of the same interspersed with red blood corpuscles and polymorphonuclears. The congestion is very noteworthy, especially in the peripheral parts, where often nothing is visible beyond very thin sinus walls, surrounded by innumerable red cells. The sinuses contain many leucocytes, with a few phagocytes among them. The difference in staining reaction of the red cells is striking. In the VAN GIESON stained specimen, some of the corpuscles took the picric acid very intensely, others being only faintly or not at all stained. Sometimes they were clumped together and showed small iron-containing pigment granules ; these being found all over the spleen, and both free and intracellular, as for example, in the endothelial cells of the sinuses. The arteries show proliferation of their endothelium, and contain a fair number of leucocytes and a few megaloblasts. The spleen tissue contained phagocytes (with included red blood corpuscles and nuclei of lymphocytes) plasma cells, many eosinophiles, and giant cells of mononuclear type.

The *lymph glands* show generally the same changes as in the first case. The majority of the glands are affected, and all stages can be followed out from normal gland structure to that of haemo-lymph glands.

The following is a description of a typical haemo-lymph gland taken from one of the enlarged glands of the neck :—

There is a marked hyperplasia of the connective tissue, the capsule is thin and has enlarged vessels running in it. Underneath the capsule is the peripheral sinus, which does not extend round the whole gland, since at places the lymphoid tissue reaches the capsule. In the sinus are many red blood corpuscles, a few leucocytes and phagocytes and coagulated fluid ; from this sinus branches run towards the centre. The germ centres are in greater number than normally and are enlarged. They show nothing abnormal in their constituents except an infiltration of blood cells. The vessels often have an accumulation of red cells around them. The arteries inside, and to a less extent outside the glands show endarteritis. In some of the glands, chiefly in the periphery, are ill-defined strands of densely packed lymph cells. Similar patches, but smaller, are found in the central parts of the gland. A good number of phagocytes, a few giant cells, and a varying number of eosinophiles are found in the glands.

The vessels in the adipose tissue about the glands often have small new growths of lymphoid tissue around their walls.

Those lymph glands which appear slightly changed to the naked eye are found microscopically to be highly congested. Thick vessels enter at the hilus and divide into large branches running towards the periphery. Numerous free red blood cells are present among the lymphocytes throughout the glands and also there is a slight quantity of blood pigment giving an iron reaction.

The *long-bone marrow* in some places has the microscopical appearances of red marrow. It is very rich in cells, with but little fat. The vessels are very congested and contain a good number of leucocytes and a few nucleated red cells. The cellular elements present no abnormality, consisting of giant cells, eosinophiles, and a few phagocytes. Blood pigment is present in small quantity.

Eyes show microscopically nothing abnormal except a little infiltration at the corneo-scleral junction and in the cornea. The pial sheath of the optic nerve contains more cellular elements than normal, and the vessels of the nerve show well-marked small-celled infiltration.

Suprarenals are very congested. Enlarged vessels run in the capsule and between the cells separating them.

The *aorta* occasionally shows a little small-celled infiltration around its *vasa vasorum*.

The *central nervous system* presents much the same changes as in the case of ' Kitambo,' but as a rule in a less marked degree.

The leptomeninges are inflamed in varying degrees, the cellular infiltration being chiefly around the vessels, where it consists of a thick layer of lymphocytes ; some of the vessels however show no such infiltration around them. This infiltration is found along the vessels running in the sulci, and it is made up of lymphocytes, a few large mononuclears, phagocytes, with peripherally situated nuclei and all sorts of inclusions, a varying number of red blood corpuscles, and cells having the appearance of granulation tissue cells. The infiltration to a certain extent accompanies the vessels into the cortex itself, being usually more marked in its deeper portions, especially in the large grey ganglia. The perivascular space is in places dilated and contains transudate.

In this case also the changes were most marked in the basal ganglia, where a very extensive layer of infiltration was found around nearly all the vessels. The vessels contain a good number of white blood cells, and in the larger arteries sometimes the whole wall contains blood corpuscles interpolated among its own normal constituents.

The perivascular infiltration consists mainly of lymphocytes, a few large mono-nuclears, granulation tissue cells, and a few plasma cells. External to the layer of cells often occurs a large space filled with transuded fluid. Usually the endothelium of the larger vessels is normal, while that of the smaller ones is proliferated, the intima appearing as little projections into the lumen of the vessel.

The infiltration extends to the brain tissue in the neighbourhood of the vessels, destroying it. The neuroglia is markedly proliferated in places. There are only a very few capillary haemorrhages.

In the *choroid plexus* there are many gaps between the cells and a slight inflammation of the connective tissue. The cells themselves, however, show very little change. The *pons and medulla* show the same perivascular changes as in the cerebrum, with occasional capillary haemorrhages. The meningitis is most pronounced over the cerebellum.

The spinal cord and its membranes show the same changes as the brain. The pia and arachnoid are more or less infiltrated with round cells, the infiltration following the vessels into the substance of the cord. The pia shows a haemorrhage into its substance at the level of the first dorsal. The connective tissue in the pia is loose owing to extravasated red blood corpuscles.

The peripheral part of the cord is oedematous, and its neuroglia proliferated. Around the vessels of the cord the small-celled infiltration is much more marked in those found in the grey substance than in the white ; groups of six to ten vessels sheathed in a dense layer of lymphocytes are often observed. The neuroglia is a little proliferated. Various sized small haemorrhages are very often found in the grey matter, especially in the posterior cornua, often involving them to more than one-third their breadth ; no blood pigment present. The nerve roots are also the seat of small haemorrhages. The central canal is dilated, and its epithelial cells

proliferated. The vessels are generally very much congested, and show a thickening of the intima. The perivascular infiltration often seems to compress the vessels.

The *nerve fibres* show little degeneration, and that mostly around the infiltrated vessels.

The large *nerve cells* of the brain and cord are greatly altered. The changes occur in nearly all groups. Somewhat irregularly distributed side by side with normal cells are others which do not contain any Nissl bodies, and appear disintegrated into a dust-like mass. Sometimes only a peripheral zone of Nissl bodies remains, at other times they are only present in the centre so that all stages of chromatolysis are seen. Often the nucleus is well preserved, at other times it is not distinguishable at all. Irregular, fragmented cells are met with, their processes seeming to have been broken off. Their protoplasm, especially of the cells of the anterior cornua, is often quite filled with small vacuoles.

The *spinal ganglia* sometimes show the same small-celled infiltration around the vessels as in the nervous centre. Small haemorrhages are found here and there.

Many of the *peripheral nerves* were also examined. Some, such as the sciatic and median, appeared normal; others, for instance, some branches of the lumbar plexus, vagus, and others, showed a more or less pronounced small-celled infiltration of the peri- and endoneurium.

Very many sections of all the organs were examined for bacteria with the above-mentioned methods, and, although very carefully searched, yet only a small number of large bacilli and large cocci which did not stain by Gram's method were seen.

In the examination for trypanosomes in only a few sections of the brain, for example in the vessels of the choroid plexus, were a small number of trypanosomes found.

C.—The Third Case, Boyo

A black boy, fourteen years of age, died the 25th of May, at six p.m. The examination was not made until the 26th, at five p.m., twenty-three hours after death.

Body was very emaciated with sunken orbits and temples and deeply indrawn intercostal spaces. On the soles of both feet were numerous ulcers caused by chiggers. The limbs were much wasted. Rigor mortis was marked. Over the sacrum was a superficial bed-sore, ten c.m. in diameter. The skull was dolichocephalic, measuring fifty c.m. at circumference. The *dura mater* very adherent to the skull, which was of normal thickness, having deep sulci for the vessels and numerous small depressions for the pacchionian bodies. The dura appeared normal, and its sinuses contained a fair amount of clotted blood. The surface of the brain was of normal configuration, the veins of the pia very congested and filled with dark clotted blood, and showed opaque white bands along the vessels and thickenings in places.

The base of the brain was normal ; the brain substance firm, and the vessels of the grey matter slightly congested ; ventricular system dilated, its ependyma glossy, smooth and firm, showing congested vessels.   The cerebellum presented the same congestion of its leptomeninges (Fig. 2), but appeared otherwise normal ; pons and medulla macroscopically normal.   The cord showed very congested vessels, the anterior and posterior spinal arteries appearing as thick coiling threads (Fig. 3).   On cross section, hardly any change was seen except dilation of the central canal.   The tympanic cavity was normal.   Diaphragm on both sides reached the fourth rib.

Thyroid was a little enlarged, but appeared normal on section.   The mucous membrane of the tracheae was swollen, congested, and of a dark red colour.   Nearly the whole of the pharyngeal mucous membrane as far down as two cm. above the glottis was ulcerated, and covered with a stinking greenish-grey coating.   The mucous membrane of the nasal cavities showed the same condition, and were filled by a greenish cheesy mass.   The ulceration extended along the soft palate to the middle of the roof of the mouth.   The uvula was oedematous and swollen.   The mucous membranes of the frontal, ethmoidal sinuses and of the antrum were normal.

The *glands of the neck*, especially at the posterior border of sterno-mastoid, were enlarged (some three cm. in diameter), quite free, and on cross section presented a greyish meshwork filled with reddish-coloured tissue.   Some of the glands, however, appeared normal.

Both *lungs* were free and not adherent.   The visceral pleura was thickened and showed a few haemorrhages.   The right lung was generally congested, with here and there patches of consolidation the size of a hazelnut.   The mucous membrane of the main bronchus was livid, while the smaller bronchi contained pus.   The left lung showed less consolidation than the right, and its main bronchus was only slightly affected.

The *pericardium* contained a few c.c. of yellowish-coloured serum.   The heart muscle was pale and flabby.   Endocardium, valves, and intima of the aorta normal. The peribronchial glands were slightly enlarged and anthracosed.

The abdominal cavity contained about two hundred c.c. of cloudy fluid.   Liver was of normal size, pale and friable, the lobular arrangement indistinct.   The gall-bladder full of dark brownish gall.

The *spleen* was enlarged (16 × 11 × 4 cm.), its capsule thickened, its tissue dark red in colour, very soft and easily torn.   The malpighian bodies distinct and prominent and of a greyish colour.   The urogenital system appeared normal.   The mucous membrane of the stomach and intestines was pale ; the latter contained a few examples of *Trichocephalus dispar* of both sexes.

The *mesenteric glands* were slightly enlarged, some being soft and haemorrhagic and others quite normal.   Glands of the groin also enlarged (two-and-a-half cm.

L

long and one-and-a-half broad), reddish in colour on section.   Pancreas normal. Suprarenals congested.   The bone marrow of the femur was yellowish in colour, and showed several reddish islets.

<div align="center">Histology</div>

The microscopical examination of the *heart* showed an inflammation of the epicardium of some standing.   The myocardium showed patches of infiltration around its vessels, which were more numerous in the superficial parts, the muscle fibres being partially destroyed in their neighbourhood.   The infiltration consisted of lymphocytes and numerous cells with abundant protoplasm and small round nuclei. The muscle fibres in places were fragmented.   Haemorrhages occurred all through the muscle.

The *lungs* presented the appearance of catarrhal pneumonia ; the alveoli were filled with blood cells and exudation, the vessels in the consolidated regions congested, and contained many white cells.   The mucous membrane of the right bronchus was desquamated, and in the lumen was a purulent exudate.   The lung structure had disappeared in some places, being replaced by exudation containing necrotic particles. The left lung showed similar changes, but to a less extent.   Its bronchi contained a fair number of eosinophile cells.   The lung tissue and the bronchi were filled with numerous bacteria and cocci, of which many stained by Gram's method.

The sections of the *liver* showed congestion, which was more marked in the periphery than in the centre.   The connective tissue around the vessels was increased and infiltrated with lymphocytes.   The liver cells were atrophied and filled with fat droplets and their nuclei had disappeared.

The section of the *kidneys* showed cloudy swelling.   In its congested vessels are many white corpuscles.

The *spleen* showed considerable alterations of its structure.   There was a striking congestion of the whole organ.   In the section stained with polychrome methylene blue, stained areas of lighter appearance with a few nuclei in them and darker stained ones consisting of lymphocytes occurred.   The light stained areas were the congested parts.   The endothelium of the arteries was proliferated.   The follicles were hyperplastic, and from them processes extended into the pulp tissue around.   The cellular elements were as follows :—Normal spleen cells, numerous phagocytes, giant cells with a polymorphus nuclei and large protoplasmic bodies, and, in addition, a good number of large cells with peripherally placed nucleus and very vacuolic protoplasm. Iron containing pigment, both intracellular and free, was found all through the organ.

The *lymphatic glands* presented the same microscopical appearances as in the other cases, but the changes were not so extensive.   Besides congestion, some of the glands showed the same sinus formation as has been described above.   These sinuses were filled with red blood corpuscles and large phagocytic cells.   Glands with this sinus

formation were found in all the groups. Very often the lymphoid tissue was not confined by the capsule, but was also found external to it, and lymphocytes were also interspersed in the adipose tissue. The germ centres in some of the glands were normal, but in others they were broadened out with numerous gaps in their continuity, whilst in others still they were not recognizable at all. The lymphoid tissue was very often interspersed with a large number of red cells, so that in the specimen stained with methylene blue lighter areas of unstained red cells and darker ones of lymphoid tissue were met with. The vessels often had accumulations of red cells around them, and in their lumen many white corpuscles.

Among the various cells found inside the sinus and outside it were very numerous phagocytic cells with red blood corpuscles, which in the specimen stained with modified Laveran's method, were deeply stained orange ; other cells were mono- and poly-nuclear leucocytes with eosinophile granules ; cells of the size of large mononuclear leucocytes having small nuclei and basophile granules ; giant cells in limited numbers ; occasional cells such as are found in bone marrow, of an irregular oblong shape, staining a peculiar dark blue colour with haemotoxylin and containing one or more vacuoles, the protoplasm presenting a meshwork with one or two darker-stained spots in it ; and a few plasma cells. Blood pigment, which gives the Prussian blue reaction, was present in nearly all the glands.

The sections of the *bone marrow* of the femur showed the ordinary constituents of marrow ; nucleaed red cells were present in small number. The vessels were con-gested, and around them were very often accumulations of red blood corpuscles. Some parts presented typical gelatinous degeneration. In one place in the medullary substance of the left suprarenal was an infiltration of lymphocytes. The testes and epididymis were normal.

Brain and spinal cord showed the same changes as in the previous case, but to a far less extent. The pia and arachnoid over the cerebellum and over the convexity, more especially, of the cerebrum, presented a small-celled infiltration. The vessels were highly congested. The small-celled infiltration accompanied the vessels into the cerebral cortex. Many of the vessels of the cortex, however, were free from this perivascular infiltration, and only showed enlargement of the perivascular lymph spaces. The vessels of the grey basal ganglia were the most affected. The perivascular lymph spaces were occupied by a cellular accumulation consisting of lymphocytes, red blood cells, and a few plasma cells. The same changes were observed around the vessels of pons and medulla, while in the cord they were very little marked ; here the vessels have often a much-enlarged perivascular lymph space with a few lymphocytes in it. The cord showed a gliosis of its central canal. The nerve fibres presented very little degeneration, while the nerve cells, however, showed different stages of chromatolysis and pyknosis, more pronounced in the brain, however, than in the cord. The bacteriological staining methods revealed a varying number of large

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bacilli and large cocci, which did not stain by Gram's method, in all the organs. They are to be regarded as the result of *post-mortem* contamination.

Trypanosomes were not seen either in the organs or in the central nervous system.

D.—The Fourth Case, Disasi

Well-built, with a fair amount of adipose tissue. Externally, nothing of note. Brain and coverings normal. Right tympanic cavity filled with pus. The whole of right lung consolidated. The lower part of the left upper lobe with the upper part of the lower lobe were congested, and showed small haemorrhages in the pleura.

Liver somewhat enlarged and congested.

Kidneys normal.

Spleen enlarged. Capsule thickened. On cross section the pulp was found soft and showed no particular changes.

All the *lymphatic glands* were enlarged, some being dark-red with pin-head sized grey patches here and there.

The mucous membrane of stomach and intestines pale ; jejunum contained a few ankylostomes.

Microscopical Examinations.—Sections of the *lungs* showed typical fibrinous pneumonia and very numerous diplococci, staining by Gram.

The *spleen* was very congested.

The *lymph glands* presented the same conditions as in the sleeping sickness cases, only they contained far more free blood corpuscles among the lymphoid tissues. The examination of the *brain* and *cord* did not show any of those changes which were found in the sleeping sickness cases, although a large number of sections of different parts were examined. The perivascular lymph spaces were much dilated. There was neither haemorrhage nor small-celled infiltration in them, but they were often filled with transudation fluid containing one or two free lymphocytes. The large nerve cells only showed such changes as are caused by hyperthermia.

The cerebral vessels contained a large number of diplococci staining by Gram, and here, as well as in the tissues of the brain itself as in that of the cord, were a great number of large bacilli in small clumps and short chains, not staining by Gram.

II. EXAMINATION OF ORGANS OF ANIMALS INFECTED WITH *TRYPANOSOMA GAMBIENSE*

We examined the organs of a large number of animals such as monkeys, dogs (including puppies), rabbits, guinea-pigs, rats, and mice, who had succumbed to infection, with various strains of *T. gambiense*.

The brains of some of the monkeys in which the infection had run a longer course showed a marked congestion of the vessels, both in the meninges and in the brain itself.   Some brains, especially those in which the infection had run a rapid course, were very anaemic.   Those of the latter group showed little or no micro-scopical changes ; those of the former groups, however, presented marked changes affecting the vessels.

The section of one of the chimpanzees' brains showed extreme congestion, especially in the basal ganglia.   Fig. 9 is a section from the optic thalamus of this brain.   It shows a vessel with a large perivascular space distended with red blood corpuscles and among them a fair number of leucocytes.   The endothelium is a little proliferated.   The same changes were also observable in many of the neighbouring vessels.   The meninges did not show any changes.   The pons, medulla, and spinal cord were normal.   A *Rhesus* monkey presented, in the region of the left central gyrus, a haemorrhagic cicatrix.   Its surface was depressed below the level of the surrounding brain, and was of a yellowish colour.   Microscopically it showed destruction of the brain tissue, with much pigmentation at the bottom of the softening.

Two of the infected baboons microscopically examined showed congestion of the vessels of the brain and spinal cord.   Microscopically there were haemorrhages around the vessels of the grey matter of the brain, a good number of lymphocytes being present among the red cells.   The grey matter of the spinal cord showed many localized haemorrhages.   A few of the *Rhesus* monkeys, dying after infection of long duration, showed perivascular changes of varying extent in the brain and spinal cord.   Those cases which showed perivascular changes also showed changes in their nerve cells similar to those in the human cases.   The processes of the cells were often broken away.   Sometimes no nucleus was to be found, and the protoplasm appeared vacuolic.

In the more chronic cases of trypanosomiasis in dogs, rabbits, and guinea-pigs, haemorrhages were present in the grey matter of brain and cord in a limited number.   The vessels also often contained a large number of white blood corpuscles, and leucocytes were also found in the haemorrhages.   The meninges showed nothing abnormal except a few small haemorrhages.   Animals dying more rapidly from the infection did not show these changes in the central nervous system.   The other

organs showed lesions which varied with the intensity and duration of the infection. The heart often showed all the signs of a haematogenous myo- and epi-carditis. The lungs were often highly congested ; the parietal and visceral pleura had often small haemorrhages. In every case there was great congestion of the liver vessels. They contained a large number of leucocytes. There was proliferation of their endothelium, and they were often surrounded by small-celled infiltration. The liver cells showed fatty infiltration, and, at a later stage, fatty degeneration. Far advanced parenchymatous degeneration was frequently found. The kidneys did not show anything of note, except great congestion of the vessels and smaller and larger haemorrhages between the tubules.

The *spleen*, however, showed great changes. In almost all animals it was more or less enlarged, sometimes up to thrice its normal size. It was generally of a dark purple-red colour, and had a tense capsule. In cases dying after an acute course of infection its pulp was very soft, and the follicles large and very prominent. Micro-scopically, the hyperaemia was striking, being more pronounced at the periphery than towards the centre. In some cases small necrotic areas were present.

The spleen cells generally took the stain deeply. The follicles were hyper-plastic and irregularly defined, with processes running out from them. They may be described as follows :—a centre of lightly-stained cells having the appearance of granulation cells, around it a dense layer of lymphocytes with numerous red blood corpuscles interspersed. The vessels running in the trabeculae showed proliferation of their endothelium. There were a large number of phagocytes with included red cells and here and there were leucocytes with eosinophile granules. The red blood corpuscles accumulated in the spleen often showed all the signs of degeneration. Blood pigment, giving the iron reaction, was also present, both intracellular and free.

The spleen of those animals which had died after a more chronic infection was in the majority of cases much enlarged, dark, and of firm consistence. The malpighian bodies, however, were not so prominent, but rather seemed to be lessened in number and in size. On microscopic examination, the trabecular system showed much hyperplasia. The whole organ was very hyperaemic, many of the blood cells showing degeneration. The phagocytes and leucocytes with eosinophile granules were increased in number. Some cases had a large amount of blood pigment.

The *lymphatic glands* were usually enlarged, and their stroma hyperplastic. In nearly all the groups besides glands of normal aspect there were others reddish-brown or dark-brown in colour, which presented intersections of greyish bands corresponding to the hyperplastic connective tissue. On microscopical examination they showed sinus formation throughout the gland. The sinus contained many red and white blood corpuscles, phagocytes, which had included pigment, and the granular remains of red blood corpuscles. The lymphoid tissue often had many free blood corpuscles inter-spersed through it, along with a varying number of eosinophile cells, pigment, and

TRYPANOSOMES, TRYPANOSOMIASIS, AND SLEEPING SICKNESS

giant cells. In some glands the lymphoid tissue was reduced to smaller or broader bands, between which were lightly stained strips of finely reticulated connective tissue having in its meshes a varying number of blood cells and large hyaline cells packed with pigment and very numerous free pigment granules.

The long-bone marrow sometimes presented the appearance of red marrow. Microscopically it occasionally showed slight gelatinous degeneration.

In animals having large numbers of trypanosomes present in their peripheral blood at the time of death trypanosomes were easily detected in the sections of the organs (Fig. 10).

They were found both singly and in groups in the lumen of the vessels along with numerous leucocytes. Only in the spleen, however, were they found outside the vessels among the tissue cells. For staining the trypanosomes we used a modification of LAVERAN's method, which we found to give the best results for tissue imbedded either in paraffin or celloidin.

The section is stained in a mixture of

> 1 c.c. Borrel blue
> 4 c.c. Eosin (1 in 1,000 solution)
> 6 c.c. Distilled water.

Stain for half-an-hour or a little longer. The section will now be of a dark-blue colour. Wash for a short time in distilled water, and differentiate with orange tannin (Unna), wash again well in water till it appears purple-red in colour, then pass rapidly through absolute alcohol into xylol and mount in *neutral* Canada balsam. A very easy method of dehydrating is to use aniline oil. The sections after differentiation with orange tannin and washing with water are fixed upon the slide. The excess of water is removed by blotting paper, and pure clear aniline oil is added two or three times for a very short time. After removing the oil, the sections are passed through xylol and mounted in the balsam. Both methods by careful management give the same good results. The protoplasm of the trypanosome is stained orange, and the chromatin of a red-violet colour.

The flagellum is unstained. Thin sections are needed to get good results. Thick sections stain blue throughout.

GAMBIAN HORSE TRYPANOSOMIASIS

For studying the lesions caused by *T. dimorphon* in the organs of experimental animals, microscopical examinations were made of monkeys, dogs, rabbits, guinea-pigs, and rats.

Macroscopically, the leptomeninges of brain and spinal cord were very highly congested in all the more chronic cases, showing dilated capillaries in a meshwork of large dark veins. Cross sections showed the same congestion, especially in the grey matter, in which were also a varying number of haemorrhages. The cerebro-spinal

fluid was generally increased in amount. The lungs were normal, the heart flabby. There was generally effusion in the pericardium and pleural cavities. The liver was usually much congested, its vessels enlarged and filled with both dark fluid and clotted blood. The capsule was very often thickened.

The *spleen* in all the animals was very much enlarged, being sometimes four or five times its normal size. On section it often showed haemorrhages, the pulp being dark-red in colour. The follicles were usually hyperplastic and prominent.

The *kidneys* appeared normal.

The *lymph* glands were enlarged as a rule, and whilst few appeared normal, most of them were light or dark-brown in colour with greyish intersecting bands of hyperplastic connective tissue.

The *bone marrow* in most cases was dark-red in colour with a few grey islets in it.

Microscopical Examination

In some of the vessels of the grey matter of the brain and spinal cord, the perivascular spaces were filled with red blood corpuscles, pigment granules, and a few leucocytes. The tissue around the vessels was often destroyed.

Spleen. The most striking feature was the extreme congestion which, in the majority of the cases, was most marked in the periphery, where sometimes nothing could be seen but red blood corpuscles with a few spleen cells here and there amongst them. The follicles were enlarged, and consisted mostly of leucocytes. In sections from the periphery, the cells often seemed to be arranged in cords along fine bands of connective tissue. The space between the cords was packed with red blood cells and phagocytes. Nearly all sections showed large numbers of hyaline cells containing red corpuscles and pigment. The more chronic the case the more blood pigment was found in the spleen, so that in all old cases the whole of the organ was filled with large granules, of which a fair number were intracellular. In some sections eosinophile cells were present in large numbers. There were also more or less extensive haemorrhages, the large ones destroying the tissue right up to the capsule, thus accounting for the ruptured spleen sometimes found. The vessels showed great proliferation of their endothelium, so that in places they seemed to be obstructed.

The *lymphatic glands* presented marked changes. Very often the lymphoid tissue was reduced to small cords traversing the gland. Beneath the capsule was a small layer of lymphocytes. The spaces between the bands of lymphoid tissue network contained a few lymphocytes, hyaline cells with two or more nuclei, cells with a large quantity of included pigment, giving the iron reaction, and very many large free pigment granules (see Fig. 11).

It is remarkable that in some cases in which the spleen contained only traces of pigment the lymph glands were practically filled with it. The amount of pigmentation in the glands and spleen increases with the duration of infection.

The *kidneys* were normal in most cases, sometimes they showed parenchymatous degeneration.

The *liver* cells were often wasted, sometimes showing fatty degeneration, and had no nucleus or only a faintly stained one. Blood pigment was often to be found in the liver.

The other organs showed nothing abnormal, except congestion of vessels.

Surra, Nagana, Etc.

We examined microscopically the organs of rabbits, guinea-pigs, and rats infected with *T. brucei* and *T. evansi*, and the organs of young dogs infected with *T. equiperdum*.

In the organs of animals infected with *T. brucei* we found in the brain and spinal cord congestion and a few haemorrhages around the vessels, as for the other organs, we can only confirm Baldwin's findings. We could not find a periodical variation of the pigment of the spleen, but the amount of pigment increased with the duration of the infection. In acute cases there were very often haemorrhages in the spleen destroying its tissue, and often extending up to the capsule. The liver showed small necrotic areas in places.[1] The lesions in Surra and Mal de Caderas were of the same nature, but showed slight variations. The amount of pigment in the spleen in these two latter diseases was always very small. Trypanosomes in groups were found in nearly all vessels.

The organs of one pup infected with Dourine were normal, as were the brain and spinal cord. The spleen was not much enlarged, and was of normal microscopical appearance. The lymph glands were greatly enlarged, however, the enlargement being due entirely to the new formation of lymphoid tissue.

Summary

The pathological histological changes of the brain and spinal cord in cases of sleeping sickness were first described by Mott, Warrington, and the Portuguese Commission. Low and Mott describe the case of a European. Mott has recently, in a lecture on the cerebro-spinal fluid, recorded the changes found in some more recent cases.

The changes described by all the authors are the small-celled infiltrations around the vessels of brain and spinal cord, especially remarkable through the presence of plasma cells. Some of the authors, especially Mott, have described diplococci as being present in the vessels of the central nervous system. In our three cases of sleeping sickness a few cocci and bacilli, negative to Gram, were seen in the brain, cord, and organs, but they were so few in number that they could be considered as due

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1. This observation is confirmed by Neporogny and Yakimoff. Modifications du foie dans les Trypanosomiases experimentales (Recueil des Travaux dédiés a M. Lukianoff a l'occasion de son jubilé scientifique. St. Petersbourg, 1904).

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to *post-mortem* contamination.   The cases died during the summer months ;   the earliest autopsies were performed four hours after death.

We may draw attention to the fact that large quantities of pure blood or cerebro-spinal fluid taken during life from these cases were inoculated intraperitoneally into monkeys, guinea-pigs, rats, and mice, and intravenously into rabbits without causing any septic infection.   As much as twenty c.c. of pure blood has been inoculated, and cerebro-spinal fluid in the amount of ten c.c.   Cultures were also made from the blood and cerebro-spinal fluid of two of the cases of sleeping sickness, and were negative, with the exception of one or two tube and flask cultures, which showed a growth of *Staphylococcus albus*.

Undoubtedly a large number of sleeping sickness cases do not die from the original infection, but rather from some secondary infection, such as pneumonia, septic meningitis, etc., caused either through septic processes starting in the oral or nasal cavities or due to the generally debilitated condition, a result of the trypanosome infection.   Among the experimental animals a heavy mortality occurs when any epidemic occurs among them.   Haemorrhagic lymph glands have been described by DUTTON, TODD, and CHRISTY in their series of sleeping sickness cases.   The two first observers have also described such glands in their experimental animals infected with *T. gambiense* and *T. dimorphon* (Senegambia report).

In all the three cases of sleeping sickness, and in the case of trypanosomiasis, and in many of the experimental animals infected with *T. gambiense* and *T. dimorphon*, haemorrhagic lymph glands have been found.   These glands have all the characters of the haemo-lymph glands, as described by ROBERTSON, CLARKSON, VINCENT and HARRISON, WARTHIN, DALTON, KELLY, and other observers.   These authors describe them as normally present, but in small numbers, in human beings and animals, and always in the neighbourhood of large blood vessels.   We find that these glands are present in large numbers, and all stages between normal and haemo-lymph glands are to be seen, especially in the human cases.   Necrotic areas were present in the spleens of our human cases and some of the animals.   The spleens of all were intensely congested.   Blood pigment giving the iron reaction has been found in the spleen and glands of these cases.   It would seem from all these facts that an extensive destruction of the blood corpuscles occur and, as the bone-marrow is always degenerated, that there is not a sufficient formation of new cells.

Animals infected with various strains of trypanosomes derived from sleeping sickness and trypanosome fever cases show similar changes in their nervous system and organs as those described in sleeping sickness cases ; and these changes depend largely on the duration of the disease ; and, moreover, similar changes are described above in animals infected with *T. dimorphon* (but only after the disease has continued for a long time). Further, SIVORI and LECLER describe in their paper, *La Surra Americane* (Mal de Caderas), haemorrhagic areas in the grey substance of

the posterior cornua and small-celled infiltration around a blood vessel in the lumbar region of a horse naturally infected with the malady (it had shown paraplegic symptoms).   Neuritis has been described in animals infected with *T. equiperdum*.

From these facts one must conclude that the lesions in the brain, cord, and organs can be produced by the trypanosomes.   We have only found these changes occurring in animals infected with the parasite for some time, and in whom there were evidences of the duration of the disease, such as anaemia, loss of weight, etc. It would, therefore, appear that only in the chronic infected animals do the lesions occur.

In the ' trypanosome fever ' case we have not found the same appearances in the brain and spinal cord as in our sleeping sickness cases, probably because the disease was not long enough established to produce the changes around the vessels of the nervous centres.

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ADDENDUM (see p. 13)

Note on the effect produced on rats by the trypanosomata of Gambia fever and sleeping sickness, by H. G. Plimmer, F.L.S., communicated by Dr. C. J. Martin, F.R.S. Received December 1, 1904. Read January 19, 1905.—*Proceedings of the Royal Society*, T. LXXIV, No. 504, February 24, 1905, pp. 388-390.

Synopsis of the Paper :—

Gambian Fever. Fourteen rats

Parasites appeared about four weeks after inoculation. Duration of disease, two months twelve days. Parasites present in large numbers towards end. Paralysis. Nervous symptoms, none ; before death, animal heavy and apathetic.

Sleeping Sickness. Three rats

Parasites never found in the blood. Duration, six to nine months. Paralysis—

Both hind legs May 12, death May 23
,,   ,,   Aug. 2, ,, Aug. 30
,,   ,,   Aug. 28, ,, Sept. 8.

Post-Mortem

Spleens not enlarged. No microscopic lesions. The blood was citrated and centrifugalized, and the organs were mashed and washed with normal salt solution and centrifugalized, but in no case were any trypanosomata found. Portions of the extracts of liver and spleen and spinal cord were injected into other rats, but up to the present these show no sign of illness. *In an addendum, December* 14, 1904. Two of the three rats inoculated developed paraplegia. The blood examination of these animals fails to discover any trypanosomata. Apart from the paralysis the animals show no sign of ill-health. In the mashed spinal cord of each of the rats the characteristic trypanosomata were found in small numbers, but none were found in the brains which were examined in the same way.

Mr. Plimmer concludes

1. ' These experiments go to show that the two diseases—Gambia Fever and Sleeping Sickness—from which the organisms were obtained, are distinct ; the duration of the disease, the symptoms and the *post-mortem* appearances being quite different. It is evident that these two organisms are quite separate and distinct, as their different effects on similar animals indicate. The fact of the clinical observation that Gambia fever not infrequently appears to terminate with all the symptoms of sleeping sickness may quite possibly be explained by a double infection. For, in both rats and monkeys, the one trypanosoma does not interfere with the other, but the more active organisms—that of Gambia fever in the case of rats and monkeys—kills in about the same time, whether inoculated before, with or after that of sleeping sickness.'

2. ' There can be no question, from the above experiments, of the susceptibility of the rat to the trypanosoma of sleeping sickness.'

3. ' These experiments show that the inoculation of the trypanosoma of sleeping sickness into rats gives rise to no obvious symptoms for many months, nor are trypanosomata discoverable in the blood by microscopic examination. But after a period of from six to nine months paraplegia occurs, leading to the death of the animal ; and *post-mortem* the organisms are found only in the spinal cord. The organisms are thus in rats, as sometimes in man, entirely confined to the nervous system ; where, as in monkeys, they are, in my experience, always generalized at some period of the disease.'

In this laboratory more than seventeen rats have been used in the research. The following tables show the behaviour of the parasite in a *few* of the rats infected with the various strains. The third column gives the record of the parasite as proved by microscopical examination.

Explanation of Signs

+ + + Very numerous, fifty or more to a field.

+ + One or more to a field.

+ Fairly numerous.

− Scanty.

o Absent.

p. Periodicity, the parasites disappearing or diminishing to reappear or increase.

Example — +, p, −, o, read :—

' +,' the parasites were fairly numerous at first.

' p,' periodicity then occurred, the numbers diminishing or disappearing to reappear.

' −' the parasites became less numerous, and

' o' at death the blood was negative.

Unless ' o' is recorded, understand parasites seen at death.

From these figures it is evident that the results of the inoculations of our many different strains are absolutely opposed to those of PLIMMER. As noted before, the strains used by PLIMMER are two of those used by us and Professor LAVERAN. The record of the direct inoculations and sub-inoculations of blood from ' Kitambo,' and of blood and cerebro-spinal fluid from ' Tomi,' *show that no such a feature* as no trypanosomes appearing in the blood but in the spinal cord, and later on, paralysis occurring was observed.

Mr. PLIMMER claims that in man the organisms are sometimes entirely confined to the nervous system. Careful examination of the blood in such cases would reveal the parasites. As pointed out under Periodicity of the Parasite in the Natives (see p. 10), the daily examination of the blood for many days is necessary before such a decision can be arrived at.

Until Mr. PLIMMER can show that he has been able to obtain his original results on a large series of rats inoculated with the Uganda and other strains of Sleeping Sickness, the question may well be raised : ' Must such a dogmatic conclusion, as this result of only seventeen experiments, be accepted as to the differentiation of the parasite of " Uganda " Sleeping Sickness from that of " Gambian Fever "? ' The figures of this laboratory are entirely opposed to such a conclusion.

Uganda Sleeping Sickness

Incubation—days	Duration—days	Record of parasites in the blood
20	210	−, o
14	140	−, p
14	127	−, p
9	129	−, p
5	11	+
13	61	+, −, p
5	29	+
4	33	+ +, −, p, +
5	76	+ +, −, p
4	42	+, p

Congo Sleeping Sickness—Direct Inoculations

Incubation—days	Duration—days	Record of parasites in the blood		
18	17	+, −, p	Kitambo	
18	86	+ +, p, +		
13	35	−, p		Blood
13	92	−, p, +	Tomi	
11	54	−, p, o		
19	63	−, p, o		
10	51	−, p		Cerebro-spinal fluid
9	47	+, p		
12	68	+, p, −, o	Tomi	
12	42	−, p, +		

Congo Sleeping Sickness
Subinoculation of Strains from Congo Free State Expedition

Incubation—days	Duration—days	Record of parasites in the blood
10	72	−, p
21	44	−, p
5	70	+, p, o
19	84	−, p
4	187	+, −, p, o

'Congo Fever.'—Direct Inoculations

Incubation—days	Duration—days	Record of parasites in the blood	
9	112	−, p, o	
9	191	−, p, +, o	
11	24	+, o	Mpangila
8	58	−, p, o	
10	168	−, p, o	
7	35	+	
5	18	−, +	
11	36	−, p, o	Disasi
4	19	−	
14	51	−, p, +	
9	57	−, p	
9	39	+, p	
12	50	+ +, p, o	Banja
14	90	+, p, −, o	
14	101	+, p, −, o	

'Congo Fever.'—Subinoculations

Incubation—days	Duration—days	Record of parasites in the blood
14	26	−, p
7	24	−, p, o
10	38	−, p, o
8	102	−, +, p, −
$6\frac{1}{2}$	82	+, p, o
2	76	+ + +, p, +
2	53	+ +, p, −, o
9	49	−, p
9	33	−, +
12	69	−, p, +
15	94	−, p, + +

'Gambia Fever.'—Subinoculations

Incubation—days	Duration—days	Record of parasites in the blood
Gunjur strain		
7	106	−, p, +
3	22	+, p, + +
7	134	+, p, −, o
6	70	+, p, + + +
1	72	+ +, p, −, +
1	35	+, p, −
10	33	−, +
6	72	+, p, + + +
'Q' strain		
8	365	−, p, o
'Lammin' strain		
18	366	−, p, o, +

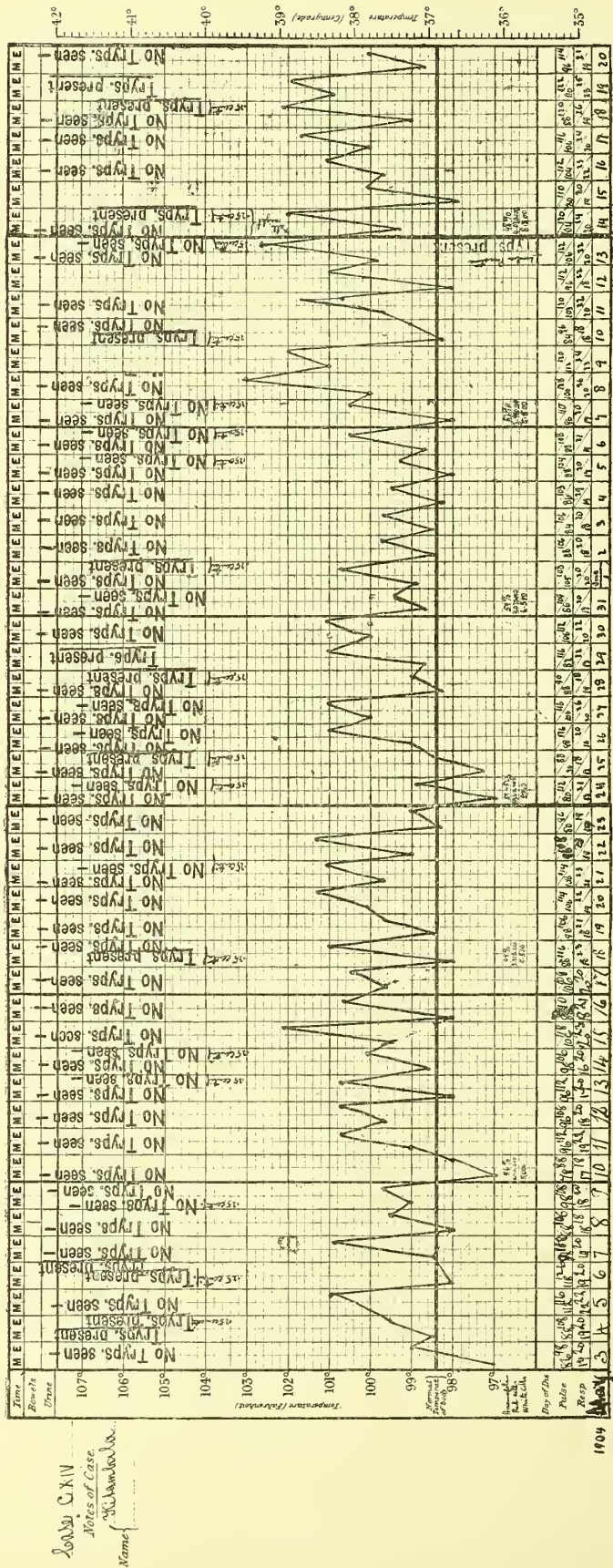# GLAND PUNCTURE IN TRYPANOSOMIASIS

Fig. 1.   Commencing at 9 a.m. on June 14 the blood was centrifuged at three hour intervals for a space of twenty-four hours.   Parasites were found only at 9 and 12 a.m.   Unless it is otherwise indicated the examinations noted were always made with fresh coverslip preparations of finger blood

Fig. 2.   Commencing at 9 a.m. on June 14 the blood was centrifuged at three hour intervals for a space of twenty-four hours.   Parasites were found only at 9, 12, and 6 a.m.   Unless it is otherwise indicated the examinations noted were always made with fresh coverslip preparations of finger blood

GLAND PUNCTURE IN TRYPANOSOMIASIS[*]

COMPARED WITH OTHER METHODS OF DEMONSTRATING THE PRESENCE OF THE PARASITE

FOURTH INTERIM REPORT

FROM THE EXPEDITION OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE TO THE CONGO, 1903

BY THE LATE

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THE great need of a good routine method of searching for the parasite in persons supposed to be infected with trypanosomes has long been apparent to us.

When the investigation of human trypanosomiasis was commenced it was thought, from the analogy of similar diseases in animals, that the microscopical examination of body fluids, particularly blood, and their experimental inoculation into laboratory animals would be the two methods which might be employed in the examination of suspected individuals.[1] Animal inoculations were soon found to be impracticable because of the uncertain and variable pathogenicity of *T. gambiense* to all ordinary laboratory animals,[1,2] and the examination of the blood alone was relied upon for diagnosis. Unfortunately, the parasites appeared only periodically in the peripheral circulation of even known cases, and often a prolonged search of many preparations was without result or only revealed a single parasite. Therefore repeated and tedious examinations of many preparations were necessary before a suspected case could, even tentatively, be said to be uninfected.

It was found that if infected blood were mixed with some diluent which prevented coagulation and centrifugalized, the parasites were deposited, along with the white corpuscles, between the red cells and serum.[3] Various methods of centrifugalizing large (5 c.cm.) or small (·5 c.cm.) quantities of blood were devised. During the past two years we have employed a simple method of centrifugalizing small quantities of blood which has given very satisfactory results. An inspection of the accompanying charts (Figs. 1 and 2) will suggest how frequently blood negative to coverslip examination has been positive when examined by this method.

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The apparatus necessary are an ordinary alcohol lamp, a high speed centrifuge, a few capillary pipettes, a diluting fluid—we used a 1·5 per cent. solution of sodium citrate in normal saline—and a special bulb-shaped tube capable of containing ·25 to ·5 c.cm. (Fig. 3). These tubes are easily drawn out in the flame of a bunsen from easily-fusible glass tubing having an internal diameter of a little more than five milli-metres. They are afterwards cut with a file to a length which fits the haematocrit arm of the centrifuge.

It is very necessary for the successful use of these tubes that each step should be done quickly. The patient's finger must be well pricked so that the blood flows freely and in fair quantity. The longer arm of a perfectly clean tube—if it is greasy blood will not enter—is gently touched to the drop of blood and the tube allowed to half fill by capillarity—breathing through the tube just before using it will cause the blood to enter more easily. The tube is then quickly transferred to the diluting fluid and allowed to almost, never totally, fill itself. The filled tube is gently rotated so as to thoroughly mix its contents, and then, held so that there is an empty space at either end, the extreme tip of the longer arm is placed in the flame of an alcohol lamp and allowed to seal. If the mixture of blood and diluent becomes coagulated by heat, or if an air bubble forms in the tube before it is sealed, it will be found better to commence again than to attempt to remedy either defect. The best results are

Fill to here —
serum + diluting fluid
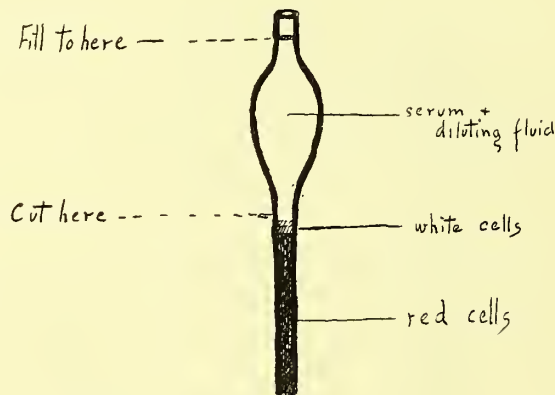Cut here --
white cells
red cells

Fig. 3

obtained from tubes so prepared if they are first centrifugalized rather slowly and later at a much higher speed, for instance, for five minutes at eight to nine revolutions per minute of the handle of DELAND's centrifuge, and then for two minutes at sixty to seventy (about eight thousand revolutions per minute). At the end of this time a well-marked white ring will have formed above the red cells. The undesired, over-lying, clear fluid can be quickly removed by gently scratching the tube at about three millimetres above the white ring with a file and then breaking off the bulb by a smart tap from the same tool. Any superfluous diluting fluid is first removed, and the

white cells carefully drawn up together with a small quantity of serum by means of a capillary pipette. If the operation is well done only sufficient material for one coverslip preparation is obtained.

Although such methods are exceedingly efficient, periods do occur during which the most careful examination fails to find trypanosomes in the blood of even advanced cases of sleeping sickness in whom parasites have previously been very numerous. (Fig. 1). Much less, then, can once or twice repeated negative examinations of blood from a merely-suspected person be accepted as proving non-infection.

The introduction of lumbar puncture in cases of sleeping sickness by CASTEL-LANI supplied a new and valuable diagnostic method, and not infrequently the deposit derived by centrifugalizing from fifteen to twenty c.cm. of spinal fluid showed parasites in individuals from whose blood they had been persistently absent.[4] In early cases of sleeping sickness, however, the conditions were frequently reversed, and trypanosomes were found in the blood, while examinations of the cerebro-spinal fluid remained negative.[5] Neither method, then, can be entirely relied upon. In addition, both spinal puncture and the centrifugalizing of blood require too much time and technique for either to ever become a commonly employed diagnostic method.

The examination of spleen pulp and serous fluids (hydrocele) gave, sometimes startling,[4] but no constantly positive, results.

Although it is scarcely worthy of being considered a diagnostic method, we mention a phenomenon[3] frequently observed in ordinary vaseline-sealed coverslip preparations of fresh blood from cases of trypanosomiasis. The red cells, instead of forming rouleaux as normally, run together into formless clumps and huge agglomerate masses. The condition is easily recognized macroscopically, and the sandy, granular appearance of the preparation is very characteristic. Although this condition is not always seen in infected bloods, and is sometimes observed in preparations from cases who have never been infected, still it has so constantly been associated with the presence of trypanosomes that bloods which 'agglutinate' in this manner are looked upon with the greatest suspicion. More than once has the 'agglutination' encouraged the continuance of an ultimately successful search for parasites. Only once[4] have we had the opportunity of observing a patient (European) from whose blood trypanosomes, once present, have finally disappeared. In this instance autoagglutination of the red cells disappeared with the parasites.

One of the great interests of the note[5] from Capt. GREIG and Lieut. GRAY, recently communicated by BRUCE, was that it suggested a new* and possibly more perfect method of demonstrating the parasite in early cases of trypanosomiasis.

By a comparison of the results obtained from the simultaneous examination of blood, gland juice, and cerebro-spinal fluid from a series of fourteen suspected, though very early, cases of 'sleeping sickness,' we satisfied ourselves that, as a rule, trypanosomes

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* Kanthack, Durham, and Blandford[3] had already noted that parasites might occasionally be present in the glands, though absent from the blood, of animals infected with *T. brucei*.

were present in the few drops of fluid drawn by a hypodermic syringe from the enlarged glands of infected persons ; that parasites could be frequently demonstrated by this method where careful centrifugalizing of peripheral blood had failed ; and that the parasites were often seen in as great numbers in the preparations of gland fluid as in the preparations of the sediment obtained by centrifugalizing many (twenty) cubic centimetres of cerebro-spinal fluid.

These results were most encouraging, and it was resolved to test the method further by using it in the examination of apparently healthy persons. Twenty-two natives, coming from districts where the disease occurred, but all absolutely unsuspected of being cases of 'sleeping sickness,' were chosen because of their more or less enlarged glands. These were punctured and trypanosomes were found in the fluid aspirated in eight instances. A simultaneous examination of fresh coverslip preparations only detected two of these cases, and by centrifugalizing the blood only one additional case was revealed. Lumbar puncture was done on two of the cases found to be infected by gland puncture and the spinal fluid shown to be in every way normal. Although the largest cervical glands have usually, for convenience sake, been those punctured, parasites have been found, with equal facility, in glands no larger than peas and in glands from other groups.

Only twice during the examination of a series of thirty cases of trypano-somiasis did a single examination of gland fluid fail to demonstrate the parasite which other methods of examination showed to be present.

On seven occasions (in twenty-six cases) it was successful when centrifugalizing the blood had failed, and it was thrice positive (sixteen cases) where examinations of the cerbro-spinal fluid had given negative results.

Although a general glandular enlargement is very common among African negroes, it seems possible that, at all events in infective areas, persons with much enlarged lymphatic glands must, other causes being absent, be regarded as possible cases of trypanosomiasis. For example, fresh coverslip preparations of blood from a squad of twenty-six healthy soldiers were examined. Two men were infected. Both had been previously chosen, with three others, for gland puncture because of their enlarged cervical glands. Unfortunately, further examination of these cases was not permitted.

A small herd of cattle was established near Coquilhatville in 1902 by the Congo Government. The cattle suffered a good deal from an unrecognized chronic wasting disease, ending in death. The veterinary surgeon (M. Bertolotti) in charge had made an excellent report to the Governor upon the clinical aspects of the disease, and there seemed to be some analogy between the symptoms which he described and those observed in Gambian horses and cattle infected naturally, or by inoculation, with *T. dimorphon*.[1]

When we arrived in Coquilhatville only one cow out of a herd of forty odd

head showed obvious signs of disease. The first examination of its blood was negative. Ten days later the blood was again centrifugalized, and trypanosomes were found. The fluid from a superficial neck gland was examined at the same time, and parasites, in fair numbers, were seen to be present. Three days later the animal was killed and an autopsy performed. Parasites, though once more absent to ordinary coverslip preparations of blood, were again found by centrifugalizing. They were also seen in the juice of a hyperaemic mesenteric gland, which was examined almost immediately after the animals death, in about the same numbers as at the previous examination of cervical gland fluid. After this positive result it was extraordinary that many preparations, made one or two hours after death from glands taken from various parts of the body, should have all been negative, while preparations from blood, pleural, pericardial, and peritoneal fluids all showed active parasites.

This observation has a most interesting parallel in the results of our autopsies on cases of sleeping sickness.[4] Only once,[4] and that in an autopsy done within an hour after death, have trypanosomes been found in gland fluid taken *post-mortem* from cases of human trypanosomiasis, but living parasites have very frequently been seen in the various serous fluids.

The bloods and gland fluids of several other animals from this herd were examined with negative result.[*]

We conclude that :—

(1) The examination of glandular fluid, though not infallible, is a very efficient means of detecting the presence of trypanosomes,

(2) Because of its simplicity gland puncture will be found a very useful routine diagnostic method.

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[*] The neck glands of two cows, infected with trypanosomiasis, since examined at Nouvelle Anvers, were found to contain parasites.

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4.   DUTTON, TODD, AND CHRISTY.   Memoir XIII, 1904, Liverpool School of Tropical Medicine.

5   GREIG AND GRAY.   *British Medical Journal*, 1904, May 18, p. 1252.

F$_{IG.}$ I

Brain of a case of sleeping sickness (Kitambo).   (See p. 66)

Fig. 2

Fig. 3

Cerebellum and cord of a case of sleeping sickness (Boyo).   (See p. 81).

Fig. 4

Cord of a case of sleeping sickness (Tomi).   (See p. 75).
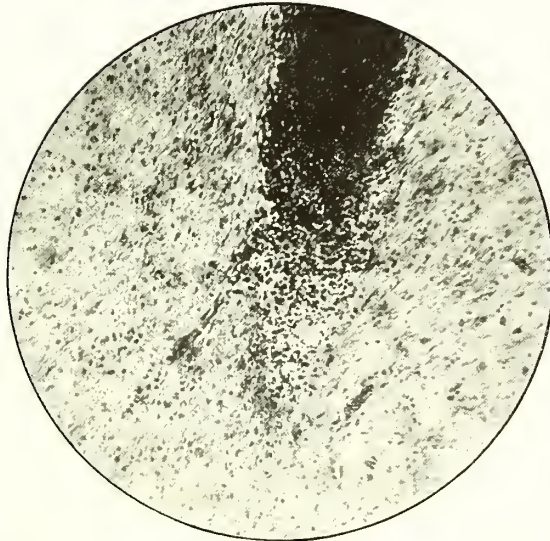
Fig. 5

B

A

C

Lymphatic glands from a case of sleeping sickness (Tomi).   (See p. 76).
To show hyperplasia of the connective tissue.
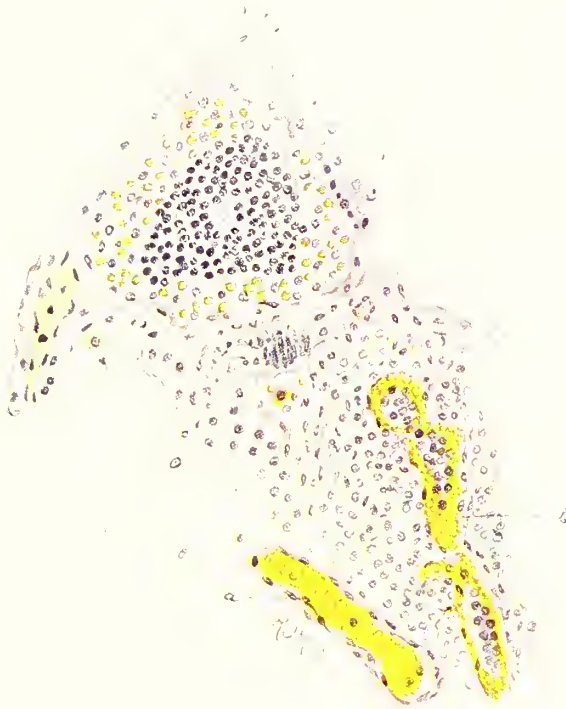(A) and (B) Cervical ; (C) Retroperitoneal

FIG. 6
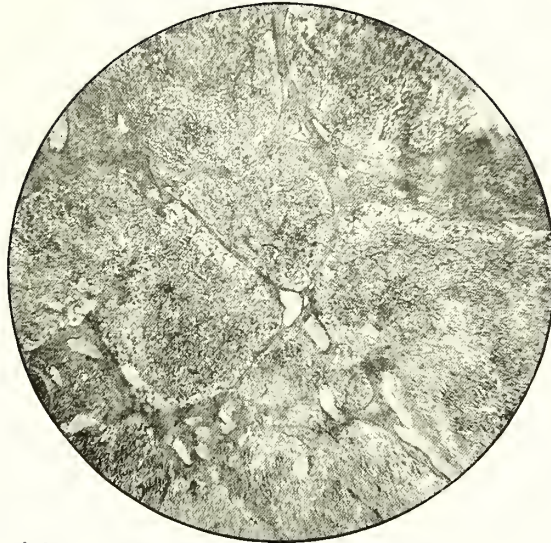
Section of cervical gland from a case of sleeping sickness (Kitambo), x 60.　(See p. 70)
To show hyperplasia of connective tissue

FIG. 9

Section of brain (*Thalmus opticus*) of chimpanzee infected with *T. gambiense*, x 300.　(See p. 85)
To show small-celled infiltration and haemorrhage around a vessel

FIG. 7

Section of mesenteric gland from a case of sleeping sickness (Kitambo), x 90.   (See p. 70).
To show sinus formation.   The sinus contains phagocytic cells and red blood corpuscles
(a) blood vessel ; (b) sinus

THE NATURE OF HUMAN TICK-FEVER IN THE EASTERN PART OF THE CONGO FREE STATE

THE NATURE OF HUMAN TICK-FEVER IN THE EASTERN PART OF THE CONGO FREE STATE

WITH

NOTES ON THE DISTRIBUTION AND BIONOMICS OF THE TICK

BY THE LATE

J. EVERETT DUTTON, M.B.

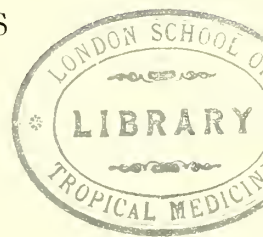(WALTER MYERS FELLOW OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE)

AND

JOHN L. TODD, B.A., M.D. McGILL

AND AN APPENDIX ON THE EXTERNAL ANATOMY OF ORNITHODOROS MOUBATA

BY

ROBERT NEWSTEAD, A.L.S., F.E.S.

PRICE **7 6** Nett

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PREFACE

Four months ago we were in possession of the main facts stated in this paper. Illness and death retarded the gathering of information and the recording of our observations. It is only now possible to publish a part of our work. At the end of November we both fell ill with recurrent fever. Dr. Dutton's illness was severe, and it was not until the middle of January that his convalescence obviously began. Unfortunately, he over-rated his strength, and even before his fever subsided commenced once more to work, harder and for longer hours than ever. On the twenty-first of February his fatal illness commenced, the twenty-seventh of February he died, after four days' unconsciousness.

For almost a year and a half Dr. Dutton and I have worked together in the Congo. This communication represents only a small part of the results of our observations. Other reports, based on our common work, must soon be written by me alone.

May any weakness in all be recognised as mine, and in nowise due to my absent comrade.

J. L. T.

KASONGO, March 28, 1905.*

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* On March 24, 1905, we received the "British Medical Journal" for November 26, 1904, containing the note of Ross and Milne on tick-fever. They are to be congratulated on their discovery. The present paper will help to complete their observations.

THE NATURE OF HUMAN TICK-FEVER IN THE EASTERN PART OF THE CONGO FREE STATE

BY THE LATE

J. EVERETT DUTTON, M.B., Vict.

(WALTER MYERS FELLOW OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE)

AND

JOHN L. TODD, B.A., M.D McGill.

On the twenty-sixth of November, 1904, we sent a message to the Committee of our School saying that a spirochaete was the pathogenic agent of the human tick-fever which occurs in the Oriental province of the Congo Free State, and that we had been able to infect monkeys with spirochaetes through the bites of ticks.

In the countries of Africa where it occurs the human tick is generally found to have an evil reputation among the natives. To its bite is attributed a sickness of longer or shorter duration, which may end in death. Accounts of the sickness are mainly derived from the writings of travellers.

Livingstone was the first (4, 5) to describe a " human tick disease " in Portuguese South Africa. In the beginning of 1903 Manson, in his work on Tropical Diseases, (1) was able to sum up in two short pages the known facts of the African disease.

Since our arrival in the Congo our attention has often been called to the human tick and to its evil properties. We found that natives who knew the arachnid had, as usual, a decided dread of it ; but it was not until we had left Stanleyville, on our way up the Congo to Kasongo, that we constantly encountered the tick, and saw, for the first time, cases of the disease. We were at Nyangwé, an infective centre, from November 13 to 22, 1904. Here we collected a large number of ticks for experimental purposes, and were fortunate enough to obtain an autopsy on the only fatal case of " tick-fever " that we have seen. We reached Kasongo on November 23, and have here seen further cases, and have been ourselves attacked by the disease.

From this clinical material, from transmission experiments with ticks, and from information and reports received from residents in the Congo, we have been able to show that " tick-fever " in the Oriental province is a relapsing fever, produced by a spirochaete, probably identical with *Spirochaete Obermeieri*, and that this organism can be transmitted by the bite of the tick.*

In addition, we have in one experiment been successful in transmitting the spirillum by the bites of young ticks newly hatched in the laboratory from eggs laid by infected parents.

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* We are not in a position to identify the species of ticks which we found in the Congo. All that we have seem to be the same as those we found in the Lower Congo, and judged to be *Ornithodoros moubata*. Some of our specimens have been taken home by Dr. Christy, and have been identified by Mr. R. I. Pocock, of the British Museum as *Ornithodoros moubata*.

The History of Human Tick-fever in the Oriental Province of the Congo Free State.

Livingstone mentions being annoyed by human ticks while at Nyangwé, in 1871 ; but he does not speak of them as producing disease (2, 3), although he was well acquainted with their pathogenic properties (4, 5). Dr. Hinde, who accompanied the expedition which drove the Arabs from this part of the Free State in 1892-94, saw, at Kasongo, ticks and sick persons who attributed their illness to the bites of these acarids (6). Although he lost some of his own men from the same cause, he believed that the tick was harmless, and the natives had died through the force of an ignorant superstition. His opinion has been shared by practically all the Europeans knowing this district whom we have met. Each told us of the " *Kimputu* "— the local name for the tick—and of the imagined deadliness of its bite. Almost no one believed that there might be some truth in the natives' belief. As a result, all who reported ill through " *Kimputu* " bites were at once suspected of malingering.

It is not altogether difficult to see how such a mistake could perpetuate itself. To a casual observer the human tick resembles the ordinary " harmless " cattle tick.

As Dr. Hinde justly observed (6), ills which have no connection with the tick are attributed by the natives to its bites. The real nature of tick-fever has probably escaped recognition by physicians—microscopes have not been used—through this fact, and because, as seems probable, atypical cases— such as those described below, " No. 7 " and J. L. T."—may not infrequently occur. Among all the natives whom we have questioned, only one has certainly recognised the recurrent nature of tick-fever.

In the more typical cases the symptoms of a disease which prostrates the patient on one day and leaves him free from fever on the next must have puzzled many, who could not suspect their specific nature, were it not that to most persons in this part of Africa every fever is malarial, and quinine is at once given. The naturally falling temperature of relapsing fever might thus be easily made to furnish the " therapeutic proof " of its malarial nature. Our own experience illustrates how easily mistakes might occur in diagnoses uncontrolled by the microscope, and based on only a single observation made, perhaps, between the attacks. Since we have been in the Oriental province twenty-four reputed cases of tick-fever have come, or have been sent, to us. The symptoms of seven of these patients were explainable on other grounds. Six were infected with trypanosomata, one with malarial parasites. Seven of the remaining cases gave histories similar to that recorded below in Case 3, and no cause for their illness was discoverable during the one or two days that they were under our notice. In only ten did we find spirochaetes. We have in addition seen spirochaetes in two soldiers, new-comers, who had never heard of the " Kimputu." In all we have had under observation twelve natives infected with spirochaetes.