case was diagnosed 0.5 g. of "antrypol" was given intravenously and the patient told to report for treatment at Kibigori on the Tuesday following. After the initial dose, weekly injections of 1 g. were given up to a total of 9.5 g. for the adult male, and proportionate doses for women and children. It was hoped to follow this course of antrypol with a course of tryparsamide, but the adverse propaganda effect of telling people they were required to attend for a further six weeks was so great that it was decided to rely on the antrypol alone. Little trouble was experienced in getting the Africans to attend for their first three or four injections, but after that, as their symptoms cleared up, it became increasingly difficult to persuade them to complete the course of treatment. This problem was not made any easier by the attitude of certain employers who withheld rations and pay on the days the employees attended for treatment. All cases living far from Kibigori were persuaded to come as in-patients at the Native Hospital, Kisumu.

Results

The investigation was completed just after the rains started in the middle of April, by which time all the farms had been visited as well as the native reserve areas. We examined 362 Europeans and Asians, and 11,533 Africans; 549 of the latter were suspect clinically and were further tested, with the results shown in the table.

	No. Found Positive			Total
	By Gland Only	By Gland and Blood	By Blood Only	
African males ..	73	6	4	83
" females ..	46	2	—	48
" children ..	27	—	—	27
Total ..	146	8	4	158

After the end of the rains a brief re-survey was carried out at Kibigori and at Songhor, the European farming district adjacent to Kibigori. Bush clearing had already begun at Kibigori, but reports from the Tsetse Control personnel seemed to show that the rains had tended to increase and spread the fly in certain areas. This re-survey showed that the disease was still active in the Kibigori area, and indicated the possible existence of a new small focus of infection. The figures for the re-survey were:

No. of Africans examined	1,893
No. of gland punctures done	81
No. of cases of trypanosomiasis found	43

The distribution of all the cases found was as follows:

Kibigori	134
Central Nyanza Reserve	24
Nandi Reserve	6
Farm areas	37

At the end of their course of treatment all cases were re-examined. Patients were regarded as cured if their glands had disappeared, or were fibrosed and negative on puncture, and if they also had a negative blood slide. Of the 201 cases of trypanosomiasis, 105 were treated and cured at Kibigori, and 18 at the Native Hospital, Kisumu; 6 patients died during their course of treatment, probably owing to intercurrent infection. Of the remaining 72 cases, 51 were defaulters after receiving anything from one to six injections, whilst the remainder were still under treatment when I handed over the control of treatment and investigations, which had by then become standardized.

Comment

During the course of the survey, through conversation with some of the very old Africans, the fact came to light that Kibigori had been the centre of a serious epidemic of human trypanosomiasis before the 1914-18 war. Owing to the suppressive measures taken before 1914 or to unknown natural factors, the disease had completely died out between that epidemic and the present outbreak, although the tsetse fly had apparently remained throughout the entire district.

The fly had apparently not been reinfected in spite of much movement of people through the area from regions where sleeping sickness was, and still is, endemic.

Although there was continual movement of the local population within the area itself, especially to and from Kibigori, which is the hub of the district, the fly in the Nyando basin seemed to be infected in only two places, Kibigori itself and at a small site some 15 miles away.

Nearly all cases seemed to be at an early stage. In no instance were glands found which exceeded the size of a hazel-nut. In only two cases was there any suggestion of involvement of the nervous system, and this consisted merely in some marked lethargy and some debility without any definite neurological signs. Both these cases were treated at the Native Hospital, Kisumu; they showed much improvement with antrypol; and after tryparsamide a complete cure resulted, both clinically and from examination of the C.S.F.

Trypanosomes were seldom found in the blood when gland puncture was positive, and even when found were few in number. This, combined with the result of the tests on rats, seemed to show that the infection was caused by *Trypanosoma gambiense*.

No cases were found in Europeans or Asians. The youngest African patient was about 6 years old. Compared with the numbers examined there was a higher proportion of cases among women than among men, perhaps because women went more often to the river to draw water.

With one exception there was no severe reaction to antrypol. The first case of all was over-treated, receiving 10 g. in less than five weeks. A severe exfoliative dermatitis and an acute nephritis developed, but subsequently a complete recovery was made. One case also developed an antecubital cellulitis following an injection of antrypol; recovery followed the application of moist heat.

I am indebted to the Honorary Director of Medical Services, Kenya, for his help and encouragement, and to Dr. W. W. Wilkinson, lately S.M.O., Kenya, for his most valuable advice during the survey.

DIAGNOSIS OF HUMAN TRYPANOSOMIASIS

BY

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While Cates and McIlroy (1951) showed clinical alertness in suspecting trypanosomiasis in a rather atypical case seen in England, their article indicates that routine methods of laboratory diagnosis are not well known there. Details of the methods developed over a number of years by the Gold Coast medical field units might therefore be of use to home practitioners. These units include mobile teams who examine entire populations and fixed centres at which cases attend. Cases found by the mobile teams are usually less advanced than those who come to the centres.

Blood Films

Trypanosomes may or may not be found at any stage of the disease. About 33% of the cases diagnosed by the mobile teams are found positive in one thick drop, but only 16% of those who attend at the fixed centres. Trypanosomes are most easily found in fresh blood examined with a 1/6-in. (4-mm.) objective; but if this method is inconvenient a thick film stained with 1:10 Giemsa for twenty minutes and examined with the oil-immersion objective will show them. It is best to begin at one corner and work through the entire film line by line on the mechanical stage.

Gland Puncture

Gland puncture was first described by Greig and Gray (1904). Our technique is based on instruction given us by Dr. A. MacPherson, a pupil of one of the original authors, and by the late Colonel E. Jamot, of the French Service. The clearest published account is that by McGowan (1951).

Needles should be of fairly wide bore or they will be blocked up, and should be short to facilitate manipulation; a convenient size is a bore of 0.8 mm. and length of 2.5 cm. Platino-iridium needles can be sterilized in the flame, but are so apt to break off at the joint that we find it more convenient to use steel ones. If the needles are boiled they must be dried before use, as water kills trypanosomes. To dry the needle, put it on a dry syringe with the piston withdrawn, blow out, take off the needle, draw piston back, blow out, and repeat until dry. Blow the stream of air against the face to ensure that it feels dry.

Do not sterilize the hands, but avoid touching the shank of the needle. Select a suitable gland. It is usually easier to find trypanosomes in the lower cervical glands than the higher, probably because the latter are more liable to sepsis from the head, and sepsis appears to be antagonistic to trypanosomes (MacPherson). Paint the area with iodine. Hold the needle by the butt. With the needle at about 45 degrees to the skin, aim at the thickest part of the gland, and try to get the point of the needle in the middle of it. Move the needle a little to see that the gland moves with it, and move the gland a little to see that the needle moves with it. Rotate the needle on its axis, push it a little further, then withdraw it a little (suction is not needed), and pull it out quickly. Apply iodine to the puncture. Fix the needle on the syringe with the piston withdrawn, and squirt on to a clean dry slide. With the needle move to one side the fragment of skin which is usually in the fluid. Apply cover-glass. Press the cover-glass gently to spread fluid.

Examine under the 1/6-in. (4-mm.) objective with the light partially cut down. The fluid consists of lymphocytes suspended in a clear medium. Sometimes red cells are present. If fat is present it may mean that the gland has been missed, but it is worth while examining the specimen, as there may be gland juice containing trypanosomes as well. Work through the entire specimen line by line on the mechanical stage. The appearance of the trypanosome "is very dramatic, and never fails to produce a thrill of discovery. It behaves like a tiny hooked trout kicking and thrashing among the lymph cells" (McGowan).

Blood Centrifugation

Our method is as near as we could get to that of Broden (1920), the modification being due to our having the simple two-speed hand centrifuge, whereas Broden's was of a different type. We proceed thus:

Take into a 10-ml. syringe 1 ml. of 6% sodium citrate in 0.9% saline. Fill the syringe with blood from a vein. Centrifuge for ten minutes on the low gear of the two-speed centrifuge. This brings the red cells down as a sediment, on the surface of which the leucocytes form a grey layer. Pipette off supernatant fluid into another tube. Examine the leucocyte layer and the first sediment for trypanosomes; occasionally trypanosomes come down in these (about 4% in leucocyte layer and 11% in first sediment). Re-spin supernatant on the high-speed gear for ten minutes, and pipette it off into a third tube. Examine the second sediment, which occasionally reveals trypanosomes (in 16% of cases). Spin the supernatant on high gear for twenty minutes. The third sediment often has trypanosomes (39% of cases). By his original method Broden found 80% of cases positive; we have found about 70% of all cases positive, but only about 3% of those negative by blood film and by gland puncture.

We have not had access to an electric centrifuge. Sicé (1937) states that centrifugation at high speed on the electric

centrifuge reduces the motility of trypanosomes. He advises that the speed be gradually increased, and should not exceed 3,000 revolutions a minute; after four to five minutes at that speed the supernatant should be centrifuged at 3,000 revolutions for 10 minutes and the sediment examined.

Animal Inoculation

This procedure sometimes reveals trypanosomes when other methods fail. In the Gold Coast we have found that the best animals for the purpose are the green *Cercopithecus* monkey and the pouched rat, *Cricetomys gambianus*—common here, but possibly hard to obtain in Europe. The cat and the dog are next; direct passage from man to a small rat or a guinea-pig succeeds only occasionally.

The blood of the animal should be examined for trypanosomes before the inoculation if there is any possibility of its having a natural infection; *Trypanosoma lewisi* occurs in rats everywhere. When the animal becomes positive a thin film should be examined to see that the trypanosome is of the *gambiense* type, and not *T. lewisi*.

When inoculating blood into an animal it is well to have an anticoagulant in the syringe, in case there should be any delay. We use about 10 ml. of the citrated mixture for a monkey, cat, or dog; 3–6 ml. for cricetomys; and 1 ml. for a small rat. The animal is held obliquely with head downwards and belly upwards, the skin picked up, and injection made into the peritoneal cavity at the base of the raised fold. The interval between inoculation and the appearance of trypanosomes in the peripheral blood of the animal varies from 6 to 49 days.

Kligler and Weitzman (1924) found that in experimental animals inoculated with trypanosomes a relapse could be produced by the intraperitoneal injection of olive oil. Comparative observations, too few in number to be conclusive, suggested that this method helped to establish the parasites inoculated from human cases (Saunders, 1930). The oil can be injected either before or after the blood; two days before is probably the best time.

Cerebrospinal Fluid

Trypanosomes.—Castellani and Chalmers (1919) state that centrifugation of 10 ml. of cerebrospinal fluid for 15 minutes nearly always shows trypanosomes in the sleeping stage; but Broden (1920) found them in only 4.5% of cases. We have found them in 0.15% of cases, but we centrifuge only about 4 ml. of the fluid. They are occasionally found in the fluid without centrifugation.

Cells.—A diagnosis of trypanosomiasis on a cell count alone, without finding the parasite, must be regarded as somewhat questionable. But, taking into account the difficulty of demonstrating trypanosomes in some proved cases, we and the French and Nigerian workers with whom we have discussed this consider that one is justified in treating as a case of trypanosomiasis a patient from an endemic area with suggestive symptoms who has a high cell count not otherwise explained; I would, very tentatively, put the limit here at about 30 cells. I do not think a diagnosis of trypanosomiasis is justified on a cell count of 15 or 20; I have kept under observation for some years a case with such a slightly abnormal count, and have found that the condition subsided without treatment. The chief value of the cell count is for the diagnosis, not of trypanosomiasis, but of the state of the patient's nervous system, which is from the point of view of treatment of equal importance. A definite diagnosis cannot be made on the type of cell present. The morula cell of Mott is suggestive; but, as Stefanopoulou *et al.* (1944) point out, it may occur in other conditions. In the discussion on their paper Lavier pointed out that other cells may occur in the fluid which can be distinguished from Mott's cells only by special staining.

Protein.—The use of protein estimation to diagnose trypanosomiasis is open to the same objections as the cell count; but it is of great importance in the diagnosis of the

condition of the nervous system. It is important to specify the method used in estimating protein, as the normal varies considerably with the different methods. After a series of parallel tests carried out with the help of pathologists of the R.A.M.C. (Hill, 1948), we decided that the Sicard-Canteloube method is the best for the purpose, as it gives a more accurate figure in the range of greatest importance—that just above the normal.

Adhesion Test

The test is based on the power possessed by the blood of infected animals to make trypanosomes of the same type sticky, so that platelets or blood cells adhere to them. In an endemic area we found that 91% of proved cases gave some degree of adhesion in this test, while 75% of negative controls gave none. Taking the strength of the reaction into consideration, we found that only 3% of the controls gave +++— or stronger, and only 11% ++— or stronger. The positive cases included some very early ones, and the negative controls included a few suspicious cases in which trypanosomes were not demonstrated. We found some evidence which suggested that the false positives might be due to tsetse injecting trypanosomes which were not numerous or vigorous enough to maintain themselves (Saunders, 1931).

Our technique was slightly modified from that of Johnson and Lester (1929). Blood from an infected animal, containing preferably 20–30 trypanosomes per field under 1/6-in. (4-mm.) objective, is taken into an equal volume of citrated saline and centrifuged slowly for five minutes. The supernatant suspension of trypanosomes is pipetted off. The plasma to be tested is prepared in the same way, or serum can be used. At first we set up the test in tubes, using equal volumes of the suspension and of the fluid being tested; later we found that two drops mixed on a slide gave the same results, and that incubation was not needed. A positive result is shown by platelets or blood cells sticking to the trypanosomes. In recording results we used the method of writing 1 to 5 crosses, as Johnson and Lester did, according to the number of trypanosomes seen with platelets or cells stuck to them.

Weak strains which do not produce numerous trypanosomes in infected animals were found to be unreliable for the test. In Nigeria starting or relapsing strains gave much the same result; in the Gold Coast our results were entirely erratic with relapsing strains.

Other Methods

Nelis (1944) and Fain (1944) found trypanosomes by sternal puncture when blood and glands were negative; but we have not found this method of value in the Gold Coast. Auto-agglutination of red cells certainly occurs in trypanosomiasis—but also without trypanosomes. We have not succeeded in getting clear results with the formol-gel test, possibly because we have not used carefully standardized tubes. The sedimentation rate will probably prove of value in studying the progress of the disease (Hollins and Lewis-Faning, 1947), but it is hardly of diagnostic value.

General Procedures

When we suspect trypanosomiasis we take a blood film, do a gland puncture, centrifuge the blood, and inoculate an animal in succession, and then carry out lumbar puncture. If necessary, the blood film is repeated daily. It is better to take the blood while the patient has fever. Dr. H. Bermingham, of Kumasi, diagnosed a case by taking blood films at hourly intervals over twenty-four hours. The gland puncture and centrifugation are repeated, if necessary, at intervals of a few days. Lumbar puncture is sometimes repeated, after a month because a case in which fluid is usually abnormal may have normal intervals (Barlovatz, 1934; Van Slype, 1935).

Summary

The simple routine laboratory methods used by the Gold Coast medical field units in the diagnosis of trypanosomiasis are described in detail.

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Medical Memoranda

Two Unusual Cases of Intraperitoneal Foreign Bodies

Cases of peritoneal foreign bodies are reported from time to time in the literature. The following two patients were treated at the Cardiff Royal Infirmary recently.

CASE 1

A man aged 64 was seen in the out-patient department in January, 1949, and admitted a few days later. Six weeks previously he had first had pain in the right lower abdomen. This had been of gradual onset, but within 48 hours had become severe enough to cause him to cease work. It was severe for a period of four to five days, during which he was constipated. When he had his bowels opened at the end of this time the pain gradually lessened. Since then he had constantly been aware of "soreness," with occasional painful exacerbations. During the initial attack he had noticed a swelling in the right iliac fossa at the site of the pain. This had, he thought, decreased in size as the pain diminished, but since then it had remained constant. Except at the onset of the illness, his appetite had been normal and his bowels regular. There were no other symptoms or any past history of significance.

He was an obese man whose general condition was good apart from chronic bronchitis. There was no evidence of anaemia or any enlarged lymph nodes. His tongue was clean and moist. There was a fairly well defined, slightly tender swelling in the right iliac fossa measuring 6 by 8 cm. It appeared to be involving the abdominal wall and moved with it on respiration. With the abdominal wall relaxed the swelling was mobile. No other abnormality was found in the abdomen or on rectal examination.

During the week after admission the patient had evening pyrexia of 99–100° F. (37.2–37.8° C.) on three or four occasions. Blood count and stool examination were normal. A barium enema showed a filling defect proximal to the hepatic flexure which was suggestive of a neoplasm.

He was thought to have an appendix abscess or possibly a carcinoma of the caecum. A laparotomy was performed 10 days after admission. The caecum, ascending colon, and appendix were normal. The palpable mass was found to consist of oedematous indurated omentum adherent to the anterior abdominal wall. On freeing the omentum from the abdominal wall the latter was felt to contain a craggy