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The Haematocrit Centrifuge Technique for the Diagnosis of African Trypanosomiasis

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The haematocrit centrifuge technique for the detection of small numbers of trypanosomes in the blood (WOO, 1969) was used routinely on the blood and cerebrospinal fluid of 21 trypanosomiasis suspects coming to the hospital at the East African Trypanosomiasis Research Organisation, Tororo, Uganda. The peripheral blood, cerebrospinal fluid and gland juice were also examined by experienced technicians at the hospital using wet preparations and thick smears. The protein content and cell count of the cerebrospinal fluid of each patient was also determined.

Two heparinised capillary tubes (each containing approximately 0.06 ml) of peripheral blood or cerebrospinal fluid from each suspect were flamed sealed at one end and centrifuged in a Hawksley Haematocrit Centrifuge at 12,000 rpm for 4 minutes. These tubes were then placed in a capillary tube holder and examined under a microscope using a ×10 objective. In a positive diagnosis, trypanosomes were found at the junction of the plasma and buffy layer in the centrifuged blood and, in centrifuged cerebrospinal fluid, the trypanosomes were usually located at or near the sealed end of the capillary tube.

In this study four criteria were used to differentiate the two types of human trypanosomiasis (Rhodesian and Gambian types): 1) clinical evidence; 2) approximate duration of infection; 3) mouse inoculation (0.5 ml of patient blood or cerebrospinal fluid was inoculated intraperitoneally into each of two mice, tail blood was examined every day post inoculation and negative mice were destroyed after 60 days); and, 4) locality from which the patient originated i.e. whether the patient came from a known Rhodesian or Gambian sleeping sickness area.

The results of this preliminary study are given in Table 1. The haematocrit centrifuge technique is found to be more sensitive than the usual laboratory techniques used in the hospital for detecting trypanosomes in the blood and cerebrospinal fluid. This is especially true in the Gambian type sleeping sickness where trypanosomes are often very scanty in the blood and the cerebrospinal fluid, and mouse or rat inoculations are not reliable because the trypanosomes are often non-infective to these animals. Of the 8 Gambian type infections encountered in this preliminary survey 2 were positive by thick blood smear and gland juice and another positive by wet preparation of cerebrospinal fluid. Trypanosomes were, however, detected in both the blood and or cerebrospinal fluid of all 8 Gambian and 5 Rhodesian type patients by the haematocrit centrifuge technique. In the 8 patients where no trypanosomes were detected by centrifugation, only one patient (No. A153) had an abnormally high cell count and protein content in the cerebrospinal fluid; no trypanosomes were seen in the blood and cerebrospinal fluid when the patient was re-examined three days later. The blood and cerebrospinal fluid of the patient was also examined by the Indirect Fluorescent Antibody Technique (BAILEY, CUNNINGHAM & KIMBER, 1967) and found to be negative. It would seem that the high cell count and protein level in the spinal

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Table 1. Patients examined at E.A.T.R.O. Hospital between May and August, 1970

Hospital patient number	Infection (type)	Locality	Approximate duration of infection	Blood			Gland juice	Cerebrospinal fluid				Number of days mouse became positive after inoculation with	
				W.S.	T.S.	H.C.T.		W.S.	H.C.T.	Cell count	Protein (mg%)	Blood	Cerebrospinal fluid
953	Rhodesian	Lugala	3 weeks	+	+	++++	+	+	++	81	25	5	Not done
954	Gambian	Ayugi	6 months	–	–	+	–	+	++	336	30	*	*
955	Rhodesian	Kagwara	4 months	+	+	+++	+	–	+	488	18	5	6
956	Gambian	Layibi	7 months	–	–	+	–	–	+	126	75	*	*
967	Gambian	Bibia	?	–	–	+	–	–	+	41	15	*	*
958	Rhodesian	Budecho	4 months	+	+	++++	+	+	+++	10	50	6	10
A138	None	Lugala		–	–	–	–	–	–	2	20	*	*
857	None	Sabadu		–	–	–	–	–	–	2	20	*	*
A141	None	Isenda		–	–	–	–	–	–	1	15	*	*
A142	None	Butenge		–	–	–	–	–	–	1	15	*	*
A147	None	Uhembo		–	–	–	–	–	–	1	10	*	*
959	Gambian	Bibia	3 months	–	–	+	–	–	+	131	55	*	*
A148	None	Alego		–	–	–	–	–	–	2	23	*	*
960	Gambian	Bibia	5 months	–	+	+	+	–	+	66	71	*	*
A152	None	Sirawongo		–	–	–	–	–	–	1	10	*	*
961	Gambian	?	6 months	–	–	+	–	–	+	56	56	*	*
A153	None	Busembe		–	–	–	–	–	–	105	40	*	*
962	Rhodesian	Bukeda	4 months	+	+	++++	+	+	++	135	45	3	4
963	Gambian	Kasubi	1 year	–	–	–	–	+	+	152	60	Not done	Not done
964	Gambian	Congo	3 years	–	+	++	+	–	+	26	20	Not done	Not done
965	Rhodesian	Lugala	5 months	+	+	+++	+	+	++	12	25	5	7

Abbreviations: W.S., wet smear; T.S., thick smear; H.C.T., haematocrit centrifuge technique; +, 1–5 trypanosomes; ++, 6–15 trypanosomes; +++, 16–25 trypanosomes; ++++, more than 26 trypanosomes; –, no trypanosomes; *, mice showing no infection, destroyed after 60 days.

fluid was probably due to other causes. None of the mice inoculated with blood and cerebrospinal fluid from negative patients had a trypanosome infection at the end of 60 days.

The technique has also been used successfully for detecting *T. brucei, T. vivax* and *T. congolense* infections in experimentally infected cattle 6–10 days before the infections can be detected by either thick smear or wet preparations. Conceivably, this technique could also be used for the parasitological diagnosis of American trypanosomiasis.

The advantages of the haematocrit centrifuge technique are: 1) Simplicity and hence could be used as a field technique in survey work. 2) Rapidity, it takes approximately 20–30 minutes to process and examine the blood from 12 patients. 3) A parasitological diagnosis of the disease and hence patients could be treated immediately. 4) It is more sensitive than the usual techniques of blood and cerebrospinal fluid examinations for trypanosomes. 5) If no trypanosomes are observed in patients exhibiting the usual signs and symptoms of the disease, the blood in the capillary tube could be quick frozen by dropping it in liquid nitrogen, this frozen blood could be brought back to the laboratory for the Indirect Fluorescent Antibody Technique or for other serological tests.

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