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SEROLOGICAL AND PARASITOLOGICAL DIAGNOSIS OF SLEEPING SICKNESS

by

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This paper does not intend to give a complete and detailed review of all parasitological and serological methods which may lead to the diagnosis of sleeping sickness. Only some procedures involving blood or serum will be discussed.

Detection of trypanosomes in blood

Low grade parasitaemia can easily be missed when only thick blood films are examined. Recently, two more sensitive methods have been introduced : column-separation and haemoculture.

Column-separation

Salivarian trypanosomes may be selectively separated from host blood by an anion-exchange method (Lanham, 1968). By changing the ionic strength of the buffer, the procedure may be adapted to each particular trypanosome-host combination (Lanham and Godfrey, 1970). The trypanosomes present in human blood eluates can be concentrated by centrifugation (Lanham and Godfrey, 1970; Godfrey and Lanham, 1970; Godfrey and Lanham, 1971) or by membrane-filtration (Lanham *et al.*, 1972; Van Meirvenne *et al.*, 1973).

Large volumes of blood can be processed and low grade parasitaemias (only 1 trypanosome per 1000 microscope fields of thick blood films) can be detected.

Haemoculture

In a review of various techniques for the diagnosis of sleeping sickness, (Weinman, 1963) showed the superiority of cultural methods. However,

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difficulties in preparing the media make some excellent cultivation techniques impracticable under field conditions.

Using the liquid monophasic « GLSH » medium (Jadin and Pierreux, 1960; Jadin and Le Ray, 1969), a simple and reliable method has been worked out. All manipulations must be performed under strictly aseptic conditions. The medium is sterilized by filtration and may be stored for several months. Blood is collected using « Liquoid Roche » as anti-complementary anti-coagulant, 1 ml of a 1 per cent solution in distilled water for every 4 ml of blood. About 0.5 ml are inoculated to each of 10 tubes containing 3 ml of medium. Tubes are incubated at 28 °C or at room temperature and examined every 5 days. Preliminary results suggest that this cultural method is even more sensitive than column-separation and animal inoculation.

Serological methods

Sera from 166 parasitologically confirmed cases of sleeping sickness (158 patients with gambian and 8 with rhodesian infection) have been examined by one or more serological tests.

Indirect immunofluorescent antibody test (IFAT)

Details of the methods used have been described by Wéry *et al.* (1970). Tests were routinely performed with serum; on mass surveys, blood samples were collected on filter paper.

The sensitivity of the method proved to be very high. The serum of only 1 out of 158 known gambian sleeping sickness patients gave a doubtful result, all other 157 sera were strongly positive. Sera of 8 patients with rhodesian sleeping sickness, all recent cases, have been tested with the IFAT. For 6 patients, the date of infection was exactly known. Positive results were obtained in all cases where infection had lasted for more than 2 weeks.

Sera from patients with various helminthic and protozoal infections gave negative results. However, strongly positive cross-reactions occurred with sera from patients suffering of visceral leishmaniosis.

During a mass survey of 1171 people showing no signs of trypanosomiasis and chosen at random amongst the inhabitants of 3 villages situated in an hyperendemic area of gambian sleeping sickness, 31 (2,64 per cent) positive IFAT were obtained. Only 24 of the 31 suspected cases were later thoroughly investigated. In 17, trypanosomes could be demonstrated. The positive serological results of the other 7 people remained unexplained although 2 had highly increased serum IgM levels and 1 engorged lymph glands (Wéry *et al.*, 1970).

Serum IgM levels

The relative increase of serum IgM levels was estimated by the double immuno-diffusion technique (Mattern, 1968). In 101 untreated patients with gambian sleeping sickness, only 90 had significantly increased serum

IgM concentrations; 11 had normal or nearly normal levels (table 1). All sera had given positive IFAT results. These data suggest that the determination of serum IgM levels is less sentitive than the IFAT procedure. However, in all the abovementioned 8 patients with recent rhodesian infections, serum IgM levels of at least 8 times normal had developed within 2 to 3 weeks after infection.

TABLE 1

Relative serum IgM levels in 101 untreated patients with gambian sleeping sickness (all sera had given positive IFAT results)

IgM level	Number
Normal	3
2- to 4-fold increase	8
4- to 8-fold increase	34
8- to 16-fold increase	56
Total	101

Immunoelectrophoresis

Comparative immunoelectrophoretic studies with hyperimmune sera from rabbits have shown that culture form and bloodstream form trypanosomes of *T. (T.) brucei brucei* (Le Ray *et al.*, 1973) and of *T. (T.) brucei gambiense* (Afchain *et al.*, 1975) have distinct precipitinogenic characteristics.

In order to compare the diagnostic properties of culture and bloodstream forms of *T. (T.) brucei gambiense*, sera from 89 patients with gambian sleeping sickness have been immunoelectrophoretically tested with both kinds of antigen. Using the antigen of culture forms, only 37 (41 per cent) positive results were obtained, whereas with bloodstream form antigen as much as 82 (92 per cent) positive results were recorded (table 2).

TABLE 2

Immunoelectrophoretic diagnosis of gambian sleeping sickness : comparison of culture and bloodstream form antigens (89 untreated patients)

Antigen	Positive results: Number	%	Localization of precipitation lines
Culture forms	37	41 %	Anode
Bloodstream forms	82	92 %	Anode Cathode

The mean number of precipitation lines obtained with bloodstream form antigen was significantly higher. Moreover, the characteristics of the precipitation patterns obtained with both kinds of antigen were quite distinct. Using bloodstream form antigen, most of the precipitation lines were found on the cathodic side, whereas with culture form antigen, positive results occurred only on the anodic side. Details of this phenomenon are given in table 3. Preliminary experiments with sera from patients with rhodesian sleeping sickness yielded analogous results.

TABLE 3

Immunoelectrophoretic patterns obtained with 19 sera from patients with gambian sleeping sickness when tested with culture and bloodstream form antigens of *T. (T) brucei gambiense* (number and localization of the precipitation lines)

Patient N°	Culture form		Bloodstream form	
	Anode	Cathode	Anode	Cathode
1	0	0	0	1
2	0	0	0	2
3	0	0	0	2
4	0	0	0	3
5	1	0	0	3
6	2	0	2	4
7	0	0	0	2
8	1	0	1	4
9	1	0	0	3
10	0	0	0	3
11	1	0	0	2
12	1	0	0	2
13	0	0	0	3
14	1	0	1	2
15	0	0	0	1
16	0	0	0	1
17	0	0	0	1
18	1	0	0	1
19	0	0	0	2

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