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Fluorescent Antibody Technic for Sero-diagnosis of Schistosomiasis in Humans.* (26087)

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The diagnostic value of fluorescent antibody procedures has been adequately demonstrated in a wide variety of infectious diseases (1,2,3,4,5,6). Except for a study by Jackson (7,8), conducted to locate antibody binding sites in or about *Trichinella spiralis* and *Nippostrongylus muris* in infected animals, the fluorescent antibody technics have not been applied heretofore to the field of helminthology.

This report describes a fluorescent antibody test developed for laboratory diagnosis of schistosomiasis. The procedure was evaluated with human sera and results compared with those obtained by the slide flocculation test described recently by one of us(9).

Materials and methods. During preliminary investigations, eggs, miracidia, cercariae and adults of *Schistosoma mansoni* were stained by fluorescein tagged antisera. Brightest fluorescence and greatest uniformity of results were obtained with cercariae. Therefore, *S. mansoni* cercariae, freshly emerged from laboratory infected *Australorbis glabratus* snails, were used throughout these studies.

Human sera from 227 well-documented cases were tested by the fluorescent antibody test. Of these, 206 were also tested by the slide flocculation test. All schistosomiasis sera were obtained from persons in whom the diagnosis had been established by demonstrating the eggs in stools or urine. Some of the sera had been treated with merthiolate and others showed obvious signs of bacterial contamination. However, neither merthiolate in the amounts used for serum preservation nor the contamination seemed to interfere greatly with the reactions with either test. To determine the specificity of the reaction, sera from individuals with proven trematode, cestode, nematode, protozoan, mycotic and bacterial infections were tested. To test whether the presence of auto-antibodies would give rise to false positives, sera of patients with lupus erytemathosus were included. To test whether the positive reactions obtained in schistosomiasis patients might be due to nonspecific factors deriving from liver damage, a few sera from persons with other diseases of the liver were also included. Normal sera were obtained from healthy donors undergoing physical examination as candidates for appointment to the U. S. Military Academy. All sera were heated at 56°C for 30 minutes before being tested.

The indirect technic using a labelled goat antihuman globulin was employed. Dilution of sera and globulins were prepared in 0.01 M phosphate buffered saline at a pH of 7.2 (PBS). Labelled antihuman globulin was prepared by conjugation with fluorescein-iso-

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thiocyanate(10). Prior to use, each batch of labelled antibody was absorbed twice with powdered liver and was titrated with known immune and normal sera. The highest dilution of conjugate giving brilliant fluroescence of cercariae with *S. mansoni* antiserum and essentially no fluorescence with normal serum was used. In most instances a dilution of 1 to 2 satisfied these conditions. Fluorescent microscopy was accomplished by use of a Zeiss ultra-violet light assembly and microscope with dark field condenser and appropriate filters.

Test procedure. All reactions were carried out in 13 x 100 mm test tubes to prevent drying of the cercariae. For each test, 1 ml of cercarial suspension containing between 200 and 400 cercariae was added to 1 ml of 20% formalin. After 5 minutes the cercariae were washed twice in PBS by centrifugation at approximately 100 × G. One-tenth ml of test serum was added to the sedimented cercariae, and incubated for 30 minutes at room temperature (22°-24°C) while being gently agitated on a slide rotator (110 rpm). The cercariae were then washed twice with PBS and treated with 0.1 ml of diluted fluorescein-labelled antihuman globulin for 15 minutes on the slide rotator. The cercariae were again washed twice in PBS and suspended in 0.1 ml of 90% buffered glycerine. A small drop was placed on a micro-slide, mounted with a glass coverslip and examined under the ultraviolet light. The following controls were used: 1) Cercariae treated with 10% formalin, 2) Cercariae treated with antisera only, 3) Cercariae treated with normal serum followed by treatment with fluorescein-labelled antiglobulin, 4) Cercariae treated with normal serum only, 5) Cercariae treated with known positive serum followed by treatment with fluorescein labelled antiglobulin, and 6) Cercariae treated with fluorescein labelled antiglobulin only. Only cercariae which were treated with sera from known schistosomiasis patients revealed fluorescence following application of antihuman globulins. Positive reactions varied from strong to weak fluorescence. Degree of fluorescence was estimated according to criteria described previously (6).

To determine whether the fluorescence of cercariae treated with sera of schistosomiasis patients was due to a true antigen-antibody reaction, absorption of the antibodies from 3 sera which had produced bright fluorescence and positive flocculation reactions were attempted by treating these sera with an excess of cercarial antigen adsorbed onto cholesterol-lecithin crystals. Precipitates which were produced were removed by centrifugation and the supernatant was retested by the fluorescent antibody reaction. When retested, these sera were negative for presence of fluorescent antibodies.

Preparation of the antigen emulsion for the slide flocculation test and the performance of the test have been described(9).

Results. The results obtained with the 2 serological tests are summarized in Table I. The findings with sera from schistosomiasis patients illustrate the relative sensitivity of the 2 procedures. The findings with sera from patients with other conditions and from healthy individuals provide an index of the relative specificity of the 2 tests. Extensive cross reactions were obtained in both tests with sera from trichinosis patients. No significant difference in sensitivity and specificity of the 2 tests was observed.

Discussion. It is evident from our results that use of intact *S. mansoni* cercariae as an antigen and fluorescein-labelled antiglobulin as an indicator of cercaria-antibody coupling is a wholly feasible procedure. Drying of cercariae renders the test unsatisfactory since nonspecific staining is associated with desiccation of the cercariae.

The fact that sera reactive to the fluorescent antibody technic became nonreactive after absorption with soluble cercarial antigen indicates that this is a true antigen-antibody reaction. When the same absorbed sera were retested by the slide flocculation test, one was negative and two gave a doubtful reaction.

The occurrence of cross-reactions with sera from patients with *S. japonicum* and *S. hematobium* infections is consistent with findings with other tests and emphasize the close antigenic relationship of the schistosomes. The cross-reaction with sera from individuals with

TABLE I. Comparison of Results Obtained with fluorescent Antibody and Slide Flocculation Tests in Human Sera.

	Fluorescent antibody tests				Slide flocculation tests			
		Reaction				Reaction		
Diagnostic status	No. tested	Positive	Doubtful	Negative	No. tested	Positive	Doubtful	Negative
Schistosomiasis mansoni	88	67	13	8	72	59	9	4
Schistosomiasis japonicum	1	1	0	0	1	1	0	0
Schistosomiasis haematobium	18	12	5	1	18	11	4	3
Total schistosomiasis	107	80(75)	18(17)	9(8)	91	71(78)	13(14)	7 (8)
Clonorchiasis	1	0	0	1	1	0	0	1
Opisthorchiasis	1	0	1	0	1	0	0	1
Fascioliasis	2	0	1	1	2	0	2	0
Paragonimiasis	1	0	0	1	1	0	0	1
Echinococcosis	15	2	2	11	15	1	1	13
Trichinosis	11	9	0	2	11	8	2	1
Leishmaniasis	9	0	0	9	9	0	0	9
Histoplasmosis	3	0	0	3	3	0	0	3
Coccidioidomycosis	3	0	0	3	3	0	0	3
Blastomycosis	2	0	0	2	2	0	0	2
Syphilis	12	0	2	10	12	0	4	8
Tuberculosis	11	0	0	11	11	0	3	8
Liver cirrhosis	2	0	0	2	2	0	0	2
Hepatitis	3	0	0	3	3	0	0	3
Lupus erytematosus	5	0	0	5	5	1	2	2
Healthy controls	39	1(3)	3(8)	35 (90)	34	0(—)	2 (6)	32(94)

The figures in parentheses indicate %.

trichinosis has been observed before(9,11) and does not seriously handicap the test as a diagnostic tool because geographical distribution of the two diseases is fairly distinct.

The results suggest that the fluorescent antibody test may provide a relatively simple and reliable procedure for the diagnosis of schistosomiasis of particular value to small laboratories, since the procedure is not expensive, materials can be made available from commercial sources, the actual reading of the tests is very rapid and results can be obtained within a few hours after collection of blood. Increasing experience with the observation of cercariae by the use of fluorescent microscopy should reduce the number of doubtful reactions. However, the reliability of the test must await more complete documentation. Therefore, it is too early to speculate on the immunological significance of the antibodies detected by these means and whether the same antibodies are responsible for the flocculation and the fluorescent antibody reactions.

Summary. Use of fluorescein-labelled antiglobulin as an indicator of cercarial antibody is described. Sensitivity of the procedure appears to be great for all human schistosome infections. While non-specific staining occurs regularly with sera from trichinosis patients, the specificity with other sera suggests that this procedure may be useful in serological diagnosis of human schistosomiasis.

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