Use of the Bentonite Flocculation Test for the Diagnosis of Schistosomiasis

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NUMEROUS immunological methods have been used for the diagnosis of schistosomiasis (*1*). In the diagnostic serology laboratory of the Center for Disease Control (CDC), one or preferably two tests are selected for use as reference standard methods. The cholesterol-lecithin (C-L) slide flocculation test described by Anderson (*2*) is used as the standard serologic procedure for the diagnosis of schistosomiasis because the results obtained with the indirect hemagglutination (IHA) test, the fluorescent antibody (FA) test, the complement fixation (CF) test, and the charcoal card test were not as satisfactory (*3*).

In a laboratory receiving serum specimens from all sections of the United States, contaminated or chylous serums are common. Distinguishing the particulate matter from the floccules produced by an antigen-antibody reaction is difficult, and we have found that results of the C-L test with these serums cannot be interpreted easily. The bentonite flocculation (BF) technique is used routinely in this laboratory to test for other parasitic diseases; therefore, we have evaluated it with *Schistosoma* antigens.

Other workers (*4, 5*) have obtained variable results in their efforts to sensitize bentonite particles with antigens of *Schistosoma japonicum*. The antigen used in this study was prepared from cercariae of *Schistosoma mansoni* and adsorbed onto bentonite particles. Its reactivity with selected serums in the BF test was compared with reactivities obtained in the C-L, IHA, CF, FA, and charcoal card tests.

Materials and Methods

Antiserums. The antiserum specimens tested included 278 serums from patients having a variety of parasitic and nonparasitic diseases, with 20 specimens from proved infections of schistosomiasis, and 95 human serums collected in Rhodesia from patients whose stools and urine contained eggs of either *S. mansoni* or *Schistosoma haematobium*, or both.

Antigens. Two types of antigens, one from cercariae and another from adult *S. mansoni*, were evaluated. The antigen used in the BF, C-L, and charcoal card tests, a triethanolamine buffered salt extract of dried *S. mansoni* cercariae that had been delipidized with anhydrous ether before extraction, was prepared essentially according to the method described by Anderson (*2*). This

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antigen was lyophilized in 1-ml. amounts for storage. The antigen used for the CF and IHA tests, a veronal buffer extract of lyophilized adult schistosomes that had been delipidized with anhydrous ether, was prepared according to the directions of Chaffee and associates (*6*).

Tests. The BF test described by Bozicevich and associates (*7*) for trichinosis was used except that the *Schistosoma* antigens were substituted. A vial of lyophilized cercarial antigen was resuspended in 1 ml. of distilled water and diluted with an equal volume of 0.85 percent saline for coating the bentonite particles.

The C-L flocculation test was used as the reference procedure. The technique of the test is similar to that standardized for use in the serology of syphilis.

The charcoal card test for schistosomiasis (*8*), with an antigen-cholesterol-lecithin-charcoal complex, was performed according to the procedure developed for the plasma reagin card test for syphilis by Portnoy and associates (*9*). Eighteen-millimeter circle cards were used for the tests.

The CF test was performed according to the Laboratory Branch complement fixation method, standardized for the CDC Laboratory Division and adapted to the microtechnique (*10*). Chaffee's adult antigen was used at an optimal dilution of 1:32.

The IHA test for schistosomiasis was described by Kagan (*11*) and by Kagan and Oliver-Gonzalez (*12, 13*). It was patterned after the method of Boyden (*14*) and based on the principle of the adsorption of protein antigens onto sheep erythrocytes treated with tannic acid.

The FA test procedure was essentially that described by Sadun and associates (*15*) as modified by Kagan and co-workers (*16*). The antigen was made from living cercariae, which were treated and preserved according to the technique of Anderson and associates (*17*).

Results

Several attempts to sensitize bentonite particles with antigen prepared from adult *S. mansoni* were unsuccessful; therefore efforts to use this antigen were discontinued. The cercarial antigen, however, was adsorbed onto bentonite particles, which were easily adjusted for use in the test. The reactions of antigen and antiserums were typical and easily read.

The sensitivity of the various serologic tests was evaluated against serums from Rhodesian patients with proved *Schistosoma* infections (table 1). The lowest degree of sensitivity with the test procedures was observed in the 17 patients whose stools were positive for eggs of *S. mansoni.*

The reactivity of the serums ranged from only 29 percent with the FA test to 59 percent with the C-L test. In the 41 patients whose urines were positive for eggs of *S. haematobium,* the degree of sensitivity obtained with the BF, C-L, and FA test procedures was greater than that obtained with the *S. mansoni* infections. The charcoal card, CF, and IHA tests were about the same. All the test procedures were more sensitive, however, in detecting antibody with the 37 serums from patients having eggs of both *S. mansoni* and *S. haematobium* in their stools and urine. The reactivity of the serums ranged from 53 percent with the CF test to 74 percent with the C-L test.

The overall sensitivity of the test procedures ranged from 47 percent in the IHA test to 68 percent in the C-L test. The BF and FA tests ranked

Table 1. Results of serologic tests with serums from schistosomiasis patients in Rhodesia

Tests	Total number of serums tested	Percent reactive, stool or urine positive for—			
		Schistosoma mansoni[1]	*Schistosoma haematobium*[2]	*Schistosoma mansoni* and *Schistosoma haematobium*[3]	All specimens
Flocculation:					
Bentonite	90	44	51	69	57
Cholesterol-lecithin slide	93	59	66	74	68
Charcoal card	93	47	46	69	55
Complement fixation	84	44	44	53	48
Fluorescent antibody	95	29	59	69	57
Indirect hemagglutination	93	35	39	63	47

[1] 17 specimens. [2] 41 specimens. [3] 36 specimens.

between them in sensitivity. Titers in the BF test ranged from 1:5 to 1:40. Of the 51 serums that were reactive in the BF test, 49 were also positive in the C-L test, 39 in the FA test, 31 in the CF test, 31 in the IHA test, and 41 in the charcoal card test.

To determine the sensitivity and specificity of the BF procedure, 278 serum specimens from patients with parasitic and nonparasitic diseases were evaluated (table 2). Twenty serums from patients with confirmed and serologically positive cases of schistosomiasis and 95 serums from patients with proved cases of swimmer's itch, caused by bird schistosomes, were included in the group.

Eighty percent (16 of 20) of the human schistosomiasis serums were positive, and only 2 percent of the swimmer's itch serums (2 of 95) were reactive. In addition, the serums included those that had produced positive serologic reactions with antigens of *Trichina, Toxocara, Ascaris, Echinococcus, Filaria, Cysticercus,* and *Protozoa.* There also were a limited number of positive syphilitic serums and other microbic antiserums.

Reactivity in tests with antiserums of trichinosis and visceral larva migrans was high, and several cross-reactions were obtained with serums of four other patients with parasitic infections. Of 57 normal serums tested, only one was reactive.

Because of the high percentage of cross-reactions in tests with serums from patients with visceral larva migrans, serums from 44 American Indian children in Cherokee, N.C., a population with a high rate of infection from *Ascaris lumbricoides,* were tested. Ten (23 percent) were positive with the BF test, which confirmed the high proportion of cross-reactivity. Twenty-six (59 percent) of these serums were positive with the C-L test, and none was reactive with the CF test.

Table 2. Sensitivity and specificity of the bentonite flocculation test for schistosomiasis

Human serums tested (diagnosis)	Number tested	Number reactive	Percent reactive
Schistosomiasis	20	16	80
Swimmer's itch	95	2	2
Trichinosis	24	15	62
Visceral larva migrans	18	7	39
Syphilis	8	1	12
Echinococcosis	19	2	11
Filariasis	14	1	7
Toxoplasmosis	7	0	0
Amebiasis	3	0	0
Cysticercosis	1	0	0
Kala-azar	3	0	0
Viral infections	9	0	0
Normal	57	1	2

Table 3. Sensitivity of serologic tests with serums from schistosomiasis patients in Rhodesia, by age group

Age group (years)	Number of serums tested	Percent reactive					
		BF	C-L	Charcoal card	CF	FA	IHA
1–10	24	79	83	71	63	79	54
11–20	22	59	82	64	[1] 57	82	64
21–30	31	[2] 50	61	48	[3] 30	26	61
31–40 and over	18	[4] 41	44	44	[5] 43	50	17

[1] 21 specimens tested. [2] 28 specimens tested. [3] 30 specimens tested. [4] 17 specimens tested. [5] 14 specimens tested.

Table 4. Sensitivity of serologic tests with serums from schistosomiasis patients in Rhodesia, by sex

Sex	Number of serums tested	Percent reactive					
		BF	C-L	Charcoal card	CF	FA	IHA
Females	53	64	79	57	[1] 78	64	51
Males	42	[2] 50	55	57	[3] 17	48	52

[1] 48 specimens tested. [2] 38 specimens tested. [3] 41 specimens tested.

Reactivity obtained in tests with serums of the Rhodesian patients was analyzed by age of donor (table 3). The greatest sensitivity for all techniques was obtained with serums from patients in the 1- to 20-year age group. Sensitivity decreased in all tests with serums from patients over 30 years old, except in CF and FA tests. Sensitivity of the serologic tests, by sex of donor, is shown in table 4. Reactivities in the BF, C-L, CF, and FA tests were higher for females than males.

Discussion

Selected groups of serums reacted differently in the BF test. With serums from patients who passed schistosome eggs and also exhibited other clinical symptoms, the BF test had a sensitivity of 80 percent. With serums from randomly selected patients living in a hyperendemic area in Rhodesia, the sensitivity of the BF test was lower.

For patients passing eggs of *S. mansoni,* reac-

tivity of the test was 44 percent; for patients passing eggs of *S. haematobium,* reactivity was 51 percent; and for those passing eggs of both species, reactivity was 69 percent. Total reactivity for all serums in this area was 57 percent.

Gelfand (*18*) found that in this region of Africa, 73 percent of a group of natives passing eggs of *S. mansoni* showed no symptoms of clinical grade. In natives passing eggs of *S. haematobium,* he found that 51 percent showed no localizing symptoms. Schistosomiasis in Rhodesian natives is relatively benign, which may explain why the serologic response is lower than in some other regions of Africa though the prevalence of infection is high.

Although both *S. mansoni* and *S. haematobium* occur in this area, the antigen employed exhibits group reactivity; in fact, in all serologic procedures used, the *S. mansoni* antigen was slightly more reactive with the serums from patients infected with *S. haematobium.* Reactivity was highest with serums from patients with double infections. Patients having double infections may have an enhanced antibody response or may be more severely infected.

The specificity of the BF test was lower than we prefer to accept. Wright and associates (*4*) reported cross-reactions with *Ascaris* antiserums. Trichinosis antiserums cross-reacted freely with the schistosome cercarial antigen in the BF test, a nonspecific result that several authors had reported (*2, 15, 19*). *S. mansoni* antiserums, however, did not always react with the antigen of *Trichinella spiralis.* The BF test appeared to be more specific than the C-L test. Until more purified antigens are available, nonspecific cross-reactions can be expected.

In the routine diagnosis of schistosomiasis, a number of difficulties have been encountered. Many serums are sent by mail, and anticomplementary activity precludes the exclusive use of the CF procedure. The IHA test does not have the sensitivity required to complete a routine diagnosis for persons who have not lived long periods in endemic areas. We found the FA test with serums from Peace Corps volunteers too reactive for routine diagnoses. Because of the age and physical state of the antigen used, it may be difficult occasionally to interpret the charcoal card test; moreover, the antigen is not commercially available to the laboratory doing routine diagnoses. Exclusive use of the C-L test was ruled out because serums from too many missionaries with eggs of *S. mansoni* and *S. haematobium* in their stools and urine were negative (*20*).

For the diagnosis of schistosomiasis, we recommend using more than one serologic method. Although the BF test is not an ideal serologic technique for schistosomiasis, it is an excellent companion test to the C-L procedure because it can be used with serums that are not suitable for the C-L test. In this capacity the BF test, despite its shortcomings, has a place in the diagnostic laboratory.

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To overcome some deficiencies in the cholesterol-lecithin slide flocculation test used in the diagnostic serology laboratory, Center for Disease Control, during the routine diagnosis of schistosomiasis, a bentonite flocculation (BF) test procedure was evaluated. A total of 278 serum specimens from patients with proved cases of parasitic and nonparasitic diseases were tested. In addition, 95 serum specimens from Rhodesian natives infected with schistosomes were evaluated.

Sensitivity was 80 percent in the BF test with serums from patients having clinical schistosomiasis and 57 percent with serums from Rhodesians, living in an endemic area, who passed eggs of schistosomes but without clinical symptoms. Results of the BF test were evaluated by comparing them with results of the cholesterol-lecithin flocculation, charcoal card, complement-fixation, fluorescent antibody, and indirect hemagglutination tests.

The greatest sensitivity for all techniques was obtained with serums from patients in the 1- to 20-year age group. Reactivities were higher for females than for males. Evaluation of these data suggests that the BF test for schistosomiasis can be used in the laboratory that does routine diagnoses with specimens which are not suitable for testing by other flocculation procedures.