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Evaluation of Some Laboratory Procedures in Diagnosing Infections with *Schistosoma mansoni* *

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This paper reports on a comparative evaluation carried out in Puerto Rico on the following procedures used in diagnosing bilharziasis: recovery of S. mansoni *ova from stools; serological tests (complement-fixation tests with adult worm and cercarial antigens, slide flocculation test with cercarial antigens, cercarial agglutination test, and circumoval precipitin test) and intradermal tests with adult worm, cercarial and egg antigens.*

Stool examinations revealed infections in only 74% of 485 patients hospitalized for bilharziasis, but most of the serological and intradermal tests gave results which, when corrected for false negative reactions, suggested infections in 89%-94% of patients. Cercarial agglutination results were discounted because of weak reactions and low specificity; the intradermal test with egg antigen lacked specificity.

From the results of their comparative studies, the authors suggest particular uses for the various serological and stool tests, but consider that the intradermal tests do not appear to have a definable role in the diagnosis or detection of bilharziasis.

Continuous studies on the laboratory diagnosis of *Schistosoma mansoni* infections were conducted at the US Army Tropical Research Medical Laboratory from 1955 through 1960. Collaboration with various clinics in the metropolitan area of San Juan, Puerto Rico, provided nearly 2000 patients for various phases of the investigations.

Some aspects of these studies have been published. Anderson (1960) has described a slide flocculation test using cercarial antigens, and this was followed by a report by Anderson & Naimark (1960) on the sensitivity of intradermal and serological tests on human patients with an unequivocal diagnosis of bilharziasis.

Research involving the US Army Tropical Research Medical Laboratory prior to 1955 has provided some of the techniques used in this programme. The procedure for refining schistosome antigens for complement-fixation and intradermal tests was developed by Chaffee et al. (1954). Chaffee & Nieves (1957) reported on the specificity of the complement-fixation test using antigen prepared from adult schistosomes. Horstman et al. (1954) used this test and intradermal tests to detect schistosomal infections in Puerto Rican soldiers.

The circumoval precipitin test was developed by Oliver-Gonzalez (1954) and its species specificity determined by Oliver-Gonzalez, Bauman & Benenson (1955b). Specific reactions elicited by the host to the cercarial, adult, and egg stages were investigated by Oliver-Gonzalez, Bauman & Benenson (1955a).

Although data on patients with proven infections have already been extracted (Anderson & Naimark, 1960), results of serological and intradermal tests on patients whose infections were clinically suspected but parasitologically unproven have not been considered. We hope to reach a decision on the infection status of these patients, and then turn our attention to more basic problems, i.e., the usefulness and limitations of these laboratory procedures in clinical and in public health studies of bilharziasis.

The clinician, concerned with individual patients, requires procedures which will confirm his diagnosis

* The opinions or assertions contained in this article are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

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and which will evaluate the effectiveness of specific chemotherapy. For these problems, the procedures must possess high sensitivity and specificity. Complexity of the tests is not a serious limitation.

Public health officials face somewhat similar problems. They need procedures which will establish the prevalence of bilharziasis in the population and which will aid in the evaluation of control measures. While a high degree of sensitivity and specificity of the tests is desired, these requirements can be compromised for simplicity and standardization. To evaluate control measures, the tests should be especially sensitive in the younger segment of the population, should be specific, and should become non-reactive when the infections are eliminated.

MATERIALS AND METHODS

Plans to complete six serological tests, three intradermal tests and repeated stool examinations on each patient were not fulfilled for all patients. Only 485 patients, whose serological studies were completed, have been considered so that the various serological tests could be compared directly.

Of the 485 patients 70% were males and 40% were 9-13 years old (Table 1 and Fig. 1). This peculiar age and sex distribution of the patients has complicated analysis of the data.

Intradermal tests were performed, a blood specimen was taken for serum, and arrangements were completed for multiple stool specimens when the patient first reported.

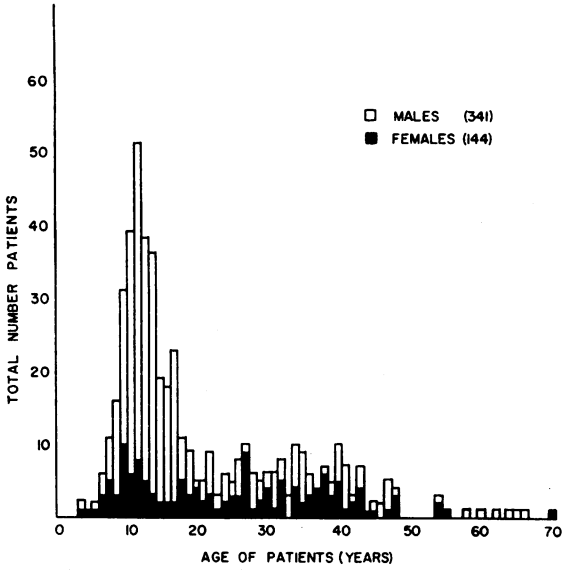
TABLE 1

DISTRIBUTION OF PATIENTS BY AGE AND SEX

Age-group (years)	Females		Males		Both	
	No.	%	No.	%	No.	%
<11	24	16.7	45	13.2	69	14.2
11-20	40	27.8	203	59.5	243	50.1
21-30	30	20.8	33	9.7	63	13.0
31-40	33	22.9	35	10.3	68	14.0
>40	17	11.8	25	7.3	42	8.7
Total :						
No.	144	100.0	341	100.0	485	100.0
%	29.7	—	70.3	—	100.0	—

FIG. 1

AGE AND SEX DISTRIBUTION OF THE 485 PATIENTS



Each person was injected with three intradermal antigens (saline extracts of adult, cercarial, and egg stages of *S. mansoni*) and the phenol-preservative saline control. Egg antigen was prepared according to the method described by Oliver-Gonzalez, Bauman & Benenson (1955b). The preparation of cercarial and adult antigens and the testing procedure followed the methods of Horstman et al. (1954).

Six serological tests were performed on each serum. Five tests were for bilharziasis. Egg antigen was used in the circumoval precipitin (COP) test (Oliver-Gonzalez, 1954). Many of the COP tests were performed for us by technicians in the laboratory of Dr José Oliver-Gonzalez, at the School of Medicine, University of Puerto Rico. Cercarial antigens were employed in the cercarial agglutination (CA) test of Liu & Bang (1950), in the complement-fixation (CFC) test, and the slide flocculation (SFC) test of Anderson (1960). Antigens from adult worms were used in the complement-fixation (CFA) test of Chaffee et al. (1954). The complement-fixation (CFS) test for syphilis using cardiolipin as antigen was routinely performed as control. The procedures for these tests have been described by Anderson & Naimark (1960).

Stool specimens were usually examined on the day of arrival. However, storage under refrigeration for

a few days produced no apparent adverse effects. To recover schistosome eggs, the entire stool specimen was submitted to the AMS III ether sedimentation technique of Hunter et al.(1948).

Supplementary information on the specificity of the serological tests was obtained by testing sera from 96 healthy naval personnel of both sexes aged 18-25 years who had no record of duty outside of the continental USA. These sera were provided by the Laboratory Officer, US Naval Air Station, Pensacola, Fla.

Additional sera, representing patients with a variety of infections, were tested as they became available. Most were provided by the Walter Reed Army Institute of Research, Washington, D.C., and the Public Health Service, Communicable Disease Center, Atlanta, Ga.

TABLE 2

RECOVERY OF *S. MANSONI* EGGS IN CONCURRENT STOOL EXAMINATIONS ACCORDING TO AGE OF PATIENTS

Age-group (years)	No. of patients			Patients infected	
	Total	Not examined	Examined	No.	%
<11	69	8	61	46	75.4
11-20	243	27	216	185	85.6
21-30	63	13	50	32	64.0
31-40	68	4	64	38	59.4
>40	42	3	39	15	38.5
Total	485	55	430	316	73.5

RESULTS

Stool examinations

The number of stool specimens each patient provided varied from zero to 12. Some of the patients contributed consecutive specimens; others delivered them at irregular intervals of time.

One or more specimens were received from 430 patients (89%) concurrently with serological studies. Eggs of *S. mansoni* were recovered from faeces of 316 persons (74%).

Ages of the patients seemed to influence the infection rate. Infections were confirmed for 87% of the patients in the 11-20-year age-group. Both younger and older patients had lower infection rates (Table 2).

Infections were proven in a significantly greater proportion of male than female patients. However, these sex differences were not significant when specific age-groups were compared (Table 3).

The number of stools examined was important in detecting the infection. Significantly more patients were found infected when four or more stools per patient were examined than when fewer were provided (Table 4).

Three consecutive stools were provided by 300 patients. Schistosome eggs were recovered from all

TABLE 3

DIFFERENCES IN RECOVERY OF *S. MANSONI* EGGS IN CONCURRENT STOOL EXAMINATIONS ACCORDING TO SEX OF PATIENTS

Age-group (years)	Male patients		Female patients		Sex difference
	No. examined	Infected (%)	No. examined	Infected (%)	% ± S. E.
< 11	39	76.9	22	72.7	4.2 ± 11.5
11-20	181	84.5	35	91.4	6.9 ± 6.5
21-30	26	61.5	24	66.7	5.2 ± 13.6
31-40	33	69.7	31	48.4	21.3 ± 12.3
> 40	24	41.7	15	33.3	8.4 ± 16.0
Total	303	76.6	127	61.4	15.2 ± 2.9

TABLE 4

RECOVERY OF *S. MANSONI* EGGS IN CONCURRENT STOOL EXAMINATIONS ACCORDING TO NUMBER OF STOOLS PER PATIENT EXAMINED

No. of stools examined	No. of patients	Patient infected	
		No.	%
1	30	19	63.3
2	61	43	70.5
3	157	107	68.2
Total	248	169	68.1
4	64	54	84.4
5	52	41	78.8
6	66	52	78.9
Total	182	147	80.8

three stools of 201 persons, and were absent from all specimens of 45 persons. Stools from the remaining 54 patients inconsistently contained eggs (Table 5).

Additional information was obtained from the records of previous stool examinations at other laboratories, as shown in the medical histories of the patients. These data were considered only if the examinations were performed within six months of the serological tests and if the patients had not received intervening chemotherapy for bilharziasis. Such supplementary information was available for 338 patients, including 34 who did not provide stool specimens in the concurrent studies. The number of specimens examined and the methods of examination were not recorded.

Most of the data from concurrent and previous stool examinations were in agreement (Table 6). By combining the results into a composite of stool data, the number of patients lacking parasitological examinations was reduced to 21 and the number of patients with confirmed infections was increased to 379 (Table 7). Differences between the proportions

TABLE 5

PATTERNS IN RECOVERY OF *S. MANSONI* EGGS FROM THREE CONSECUTIVE STOOLS IN CONCURRENT STOOL EXAMINATIONS

Egg recovery			Patients	
Stool sample number			No.	%
1	2	3		
+	+	+	201	67
—	—	—	45	15
+	+	—	3	1
+	—	+	15	5
—	+	+	15	5
+	—	—	6	2
—	+	—	6	2
—	—	+	9	3
Total			300	100

TABLE 6

COMPARISON OF RESULTS OF CONCURRENT AND PREVIOUS STOOL EXAMINATIONS FOR EGGS OF *S. MANSONI*

Results of concurrent examinations [a]	Results of previous examinations [a]				
	+	—	Total + & —	No data	Grand total
+	228	6	234	82	316
—	32	38	70	44	114
Total + & —	260	44	304	126	430
No data	31	3	34	21	55
Grand total	291	47	338	147	485

[a] + = Eggs present; — = eggs absent.

TABLE 7

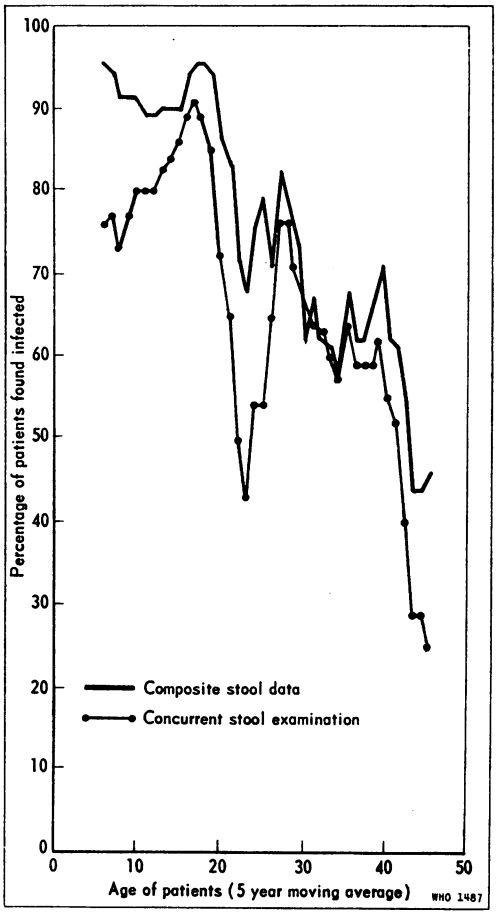
RECOVERY OF EGGS OF *S. MANSONI* IN COMPOSITE STOOL DATA ACCORDING TO AGE OF PATIENTS

Age-group (years)	No. of patients			Patients infected	
	Total	Not examined	Examined	No.	%
< 11	69	1	68	62	91.1
11-20	243	8	235	215	91.5
21-30	63	7	56	42	75.0
31-40	68	3	65	41	63.1
> 40	42	2	40	19	47.5
Total	485	21	464	379	81.7

of patients found infected on concurrent examinations and those found infected according to composite stool data were greatest for children less than 11 years of age, and for adults 21-24 and over 42 years of age (Fig. 2).

FIG. 2

PROPORTIONS OF PATIENTS PROVED INFECTED WITH *S. MANSONI* BY CONCURRENT STOOL EXAMINATIONS AND BY COMPOSITE STOOL DATA



Serological tests

The serological data have been considered first without regard for parasitological findings because all patients were clinically suspected of bilharziasis and because negative parasite data were considered inconclusive.

A large proportion (70%) of the sera reacted in all five serological tests for bilharziasis. Of the remainder, only seven sera were non-reactive in all tests while 139 reacted in one or more tests (Table 8). Nearly 87% of the sera reacted in at least four of the tests..

Comparison of the results of the serological tests showed fewest disagreements when the CFA was reactive and most disagreements when the COP was non-reactive (Table 9). Total disagreement between tests, expressed as the proportion of 485 sera, varied from 4.3% between the CFA and CFC tests to 21.4% between the CA and COP tests (Table 10).

When the serological results were arranged according to the ages of the patients, a high proportion of sera reacted in the CA test regardless of the patient's age (Table 11). The proportion of sera reacting in the other four tests tended to decrease as the ages of the patients increased. This tendency was most notable in the COP test. All five serological tests were reactive in a high proportion of sera from patients less than 20 years of age. In older age-groups considerable variation was noted in the

TABLE 8

NUMBERS OF SEROLOGICAL TESTS TO WHICH SERA REACTED (ALL PATIENTS)

No. of tests reactive	Sera		Individual tests reactive				
	No.	%	CFA	CFC	SFC	CA	COP
5	339	69.9	339	339	339	339	339
4	81	16.7	80	75	72	67	30
3	29	6.0	21	15	18	21	12
2	14	2.9	4	2	4	13	5
1	15	3.1	0	0	2	11	2
0	7	1.4	0	0	0	0	0
Total:							
No.	485	00.0	444	431	435	451	388
%	100.0	—	91.6	88.9	89.7	93.0	80.0

TABLE 9

NUMBERS OF SERA SHOWING DISAGREEMENTS AMONG VARIOUS TESTS

No. of tests reactive \ No. of tests non-reactive	CFA	CFC	SFC	CA	COP
CFA		17	24	23	70
CFC	4		20	22	65
SFC	15	24		21	68
CA	30	42	37		83
COP	14	22	21	20	

results of the tests (Fig. 3). (These patterns may reflect the duration of infection rather than patients' ages.)

When serological data were compared with the composite stool examinations four patterns emerged: parasitological examinations were positive or negative, and serological tests were reactive or non-reactive. Twenty-one patients had to be excluded because they lacked stool examinations.

TABLE 10

PROPORTIONS OF SERA SHOWING DISAGREEMENTS IN THE RESULTS OF VARIOUS TESTS

Percentage of sera \ No. of sera	CFA	CFC	SFC	CA	COP
CFA		21	39	53	84
CFC	4.3		44	64	87
SFC	8.0	9.1		58	89
CA	10.0	13.2	12.0		103
COP	17.3	17.9	18.4	21.4	

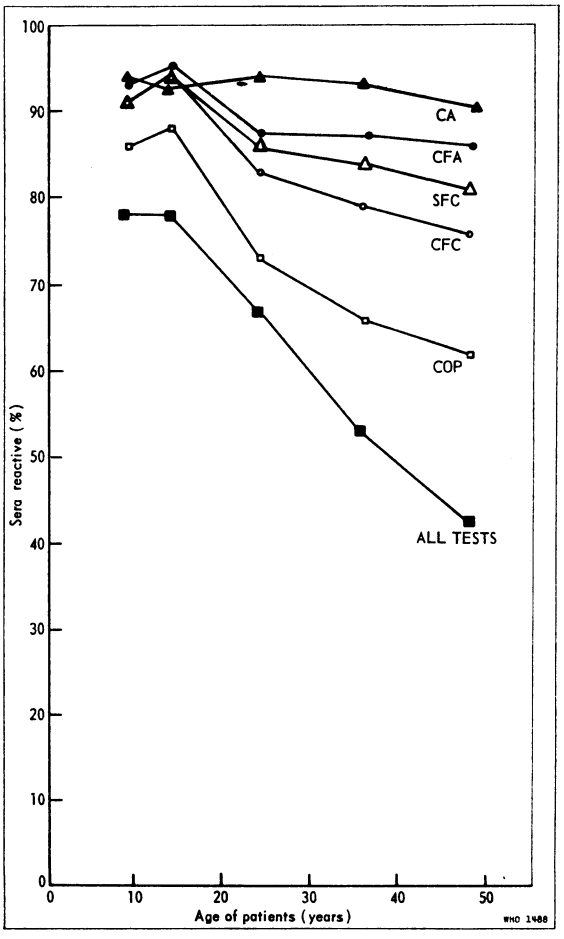
TABLE 11

NUMBERS OF SERA REACTING IN INDIVIDUAL TESTS

Age-group (years)	Total sera	Sera reactive				
		CFA	CFC	SFC	CA	COP
< 11	69	64	63	63	65	59
11-20	243	231	229	228	226	212
21-30	63	54	53	53	59	46
31-40	68	59	54	57	63	45
> 40	42	36	32	34	38	26
Total	485	444	431	435	451	388

FIG. 3

PROPORTIONS OF SERA REACTIVE IN VARIOUS SEROLOGICAL TESTS ACCORDING TO AGE OF PATIENTS



Of the serologically reactive sera, approximately 87% came from patients with parasitologically confirmed infections. Non-reactive sera came from persons with proven infections as well as those apparently uninfected. The former must be considered false negative serological reactions. The proportion of false negative reactions was smallest for the CFA test and greatest for the CA and COP tests (Table 12).

Comparison of the results of serological tests and stool examinations according to the ages of the patients provided many interesting patterns (Fig. 4). The proportions of false negative reactions in CFA tests were greatest among patients aged 21-25 years; in CFC among those 20-25 and over 41 years; in SFC among those 22-26 and 36-45 years; in CA among those 21-25 years; and they were consistently high in COP tests.

The proportions of patients under 21 years of age who were both serologically non-reactive and stool negative were small. Except in the COP test, high proportions of patients with negative stool data were serologically reactive. Thus, except for the COP test, serological tests have suggested that a far greater proportion of patients were infected than indicated by parasitological data (Table 13).

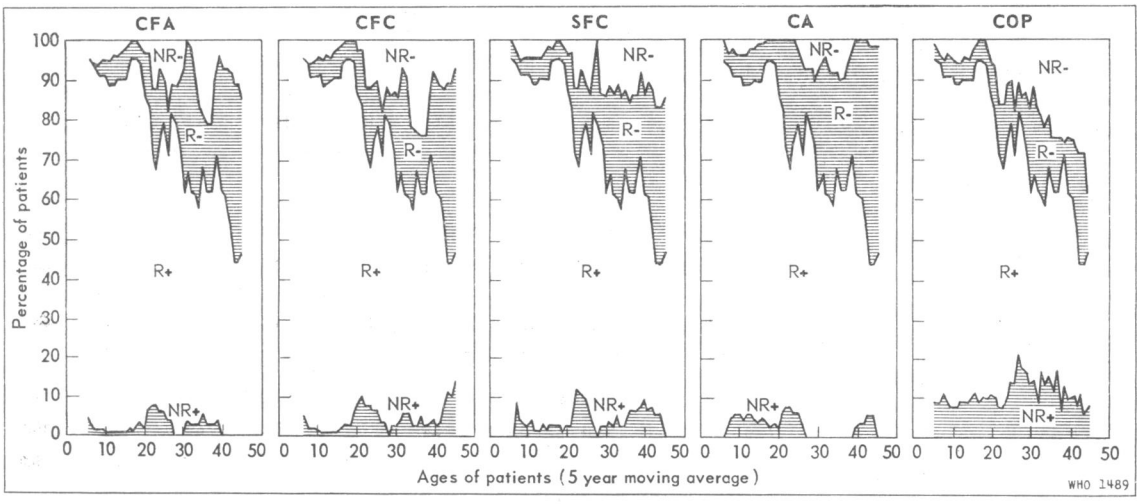
TABLE 12

RESULTS OF STOOL EXAMINATIONS OF PATIENTS ACCORDING TO REACTIVITY OR NON-REACTIVITY IN SEROLOGICAL TESTS

Serological test	No. of sera tested	Sera from patients with positive stools	
		No.	% ± S.E.
Reactive sera			
CFA	426	369	86.6 ± 1.6
CFC	416	363	87.3 ± 1.6
SFC	419	363	86.6 ± 1.7
CA	433	361	83.4 ± 1.9
COP	375	332	88.5 ± 1.6
Non-reactive sera			
CFA	38	10	26.3 ± 7.1
CFC	48	16	33.3 ± 6.9
SFC	45	16	35.6 ± 7.1
CA	31	18	58.1 ± 8.9
COP	89	47	52.8 ± 5.3

FIG. 4

COMPARISON OF RESULTS OF SEROLOGICAL TESTS AND OF STOOL EXAMINATIONS ACCORDING TO AGE OF PATIENTS



Serological tests:
R = reactive;
NR = non-reactive.

Stool examinations:
+ = *S. mansoni* ova present;
− = *S. mansoni* ova absent.

TABLE 13

COMPARISON OF THE RESULTS OF STOOL EXAMINATIONS WITH THOSE OF SEROLOGICAL TESTS ACCORDING TO AGE OF PATIENTS

Stool examination			Percentage of sera									
Results	Patients		CFA [a]		CFC [a]		SFC [a]		CA [a]		COP [a]	
	No.	%	R	NR	R	NR	R	NR	R	NR	R	NR
Age <11 years												
+	62	91.2	89.7	1.5	88.2	2.9	86.8	4.4	86.8	4.4	80.9	10.3
—	6	8.8	2.9	5.9	2.9	5.9	4.4	4.4	7.3	1.5	4.4	4.4
Total	68	100.0	92.6	7.4	91.1	8.8	91.2	8.8	94.1	5.9	85.3	14.7
Age 11-20 years												
+	215	91.5	89.8	1.7	89.4	2.1	88.9	2.5	86.4	5.1	82.6	8.9
—	20	8.5	6.0	2.5	6.0	2.5	6.0	2.5	6.8	1.7	5.5	3.0
Total	235	100.0	95.8	4.2	95.4	4.6	94.9	5.0	93.2	6.8	88.1	11.9
Age 21-30 years												
+	42	75.0	71.4	3.6	71.4	3.6	69.6	5.4	71.4	3.6	62.5	12.5
—	14	25.0	14.3	10.7	12.5	12.5	14.3	10.7	21.4	3.6	10.7	14.3
Total	56	100.0	85.7	14.3	83.9	16.1	83.9	16.1	92.8	7.2	73.2	26.8
Age 31-40 years												
+	41	63.1	60.0	3.1	58.5	4.6	58.5	4.6	63.1	0	50.8	12.3
—	24	36.9	26.1	10.8	21.5	15.4	24.6	12.3	30.8	6.2	15.4	21.5
Total	65	100.0	86.1	13.9	80.0	20.0	83.1	16.9	93.9	6.2	66.2	33.8
Age > 40 years												
+	19	47.5	45.0	2.5	37.5	10.0	42.5	5.0	40.0	2.5	37.5	10.0
—	21	52.5	40.0	12.5	40.0	12.5	37.5	15.0	42.5	5.0	27.5	25.0
Total	40	100.0	85.0	15.0	77.5	22.5	80.0	20.0	82.5	7.5	65.0	35.0
All ages												
+	379	81.7	79.6	2.1	78.3	3.4	78.0	3.7	77.8	3.9	71.6	10.1
—	85	18.3	12.3	6.0	11.4	6.9	12.1	6.2	15.5	2.8	9.3	9.0
Total	464	100.0	91.9	8.1	89.7	10.3	90.1	9.9	93.3	6.7	80.9	19.1

[a] N = reactive; NR = non-reactive.

TABLE 14

SEX DIFFERENCES IN THE PROPORTIONS OF PATIENTS REACTING TO VARIOUS ANTIGENS IN INTRADERMAL TEST

Sex	Patients: No. tested	Reactive: No.	Reactive: %
SMA			
Both	480	296	61.7
Males	336	219	65.2
Females	144	77	53.5
(Sex diff.)		(11.7 ± 15.3%)	
SMC			
Both	483	360	74.5
Males	340	262	77.1
Females	143	89	68.5
(Sex diff.)		8.6 ± 13.8%	
SME			
Both	450	126	28.0
Males	317	98	30.9
Females	133	28	21.1
(Sex diff.)		(9.8 ± 14.7%)	

Intradermal tests

Results of intradermal tests have been considered first without regard for either parasitological or serological data on the patients. Cercarial antigen (SMC) stimulated more intradermal reactions than the adult (SMA) or egg (SME) antigens (Table 14).

Both the age and the sex of the patients appeared to influence the results of intradermal tests. The proportion of male patients reacting was greater than that of female patients. Moreover, reactions were experienced more frequently by adults than by children, especially girls under 11 years of age (Table 15).

When compared with parasitological data, the intradermal tests with SME showed low diagnostic value. Moreover, both SMC and SMA failed to detect high proportions of infected patients in the younger age-groups. Finally, SMC yielded the fewest false negative reactions (Fig. 5).

Results of SMC and SMA intradermal tests have been compared with four standards: three serological tests (CFA, SFC, and COP) in addition to the parasitological examinations. Regardless of the standard, a rather constant, high percentage of the patients had

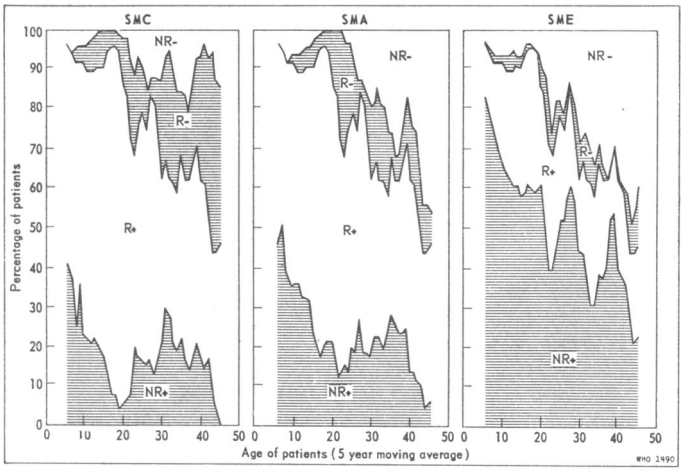
TABLE 15

AGE DIFFERENCES IN THE PROPORTIONS OF PATIENTS REACTING TO VARIOUS ANTIGENS IN INTRADERMAL TESTS

Age-group (years)	Males: No. tested	Males: Reactive No.	Males: Reactive %	Females: No. tested	Females: Reactive No.	Females: Reactive %	Both: No. tested	Both: Reactive No.	Both: Reactive %
SMA									
< 11	45	25	55.6	24	11	45.8	69	36	52.2
11-20	198	130	65.7	40	26	65.0	238	156	65.5
21-30	33	25	75.8	30	16	53.3	63	41	65.1
31-40	35	23	65.7	33	14	42.4	68	37	54.4
> 40	25	16	64.0	17	10	58.8	42	26	61.9
SMC									
< 11	45	35	77.8	24	14	58.3	69	49	71.0
11-20	202	158	78.2	39	32	82.1	241	190	78.8
21-30	33	25	75.8	30	20	66.7	63	45	71.4
31-40	35	26	74.3	33	22	66.7	68	48	70.6
> 40	25	19	76.0	17	10	58.8	42	29	69.0
SME									
< 11	36	3	8.3	19	3	15.8	55	6	10.9
11-20	188	63	33.5	38	10	26.3	226	73	32.3
21-30	33	10	30.3	29	5	17.2	62	15	24.2
31-40	35	14	40.0	33	6	24.8	68	20	29.4
> 40	24	8	33.3	17	4	23.5	41	12	29.3

FIG. 5

COMPARISON OF RESULTS OF INTRADERMAL TESTS AND OF STOOL EXAMINATIONS ACCORDING TO AGE OF PATIENTS



Intradermal tests:
R = reactive;
NR = non-reactive.

Stool examinations:
+ = *S. mansoni* ova present;
— = *S. mansoni* ova absent.

TABLE 16

COMPARISON OF RESULTS OF INTRADERMAL TESTS WITH THOSE OF SOME SEROLOGICAL TESTS AND OF STOOL EXAMINATIONS ON THE SAME PATIENTS

Results of serological and stool tests [a]		Intradermal tests [a]					
		SMA			SMC		
		No. of patients	R	NR	No. of patients	R	NR
CFA	R	480	59.2	32.3	483	71.6	20.3
	NR		2.5	6.0		3.1	5.0
SFC	R	480	58.8	31.0	483	69.6	20.1
	NR		2.9	7.3		5.2	5.2
COP	R	480	52.1	28.3	483	62.1	19.9
	NR		9.6	10.0		12.6	5.4
Stool	+	459	52.3	29.4	460	63.3	18.3
	—		7.4	10.9		11.5	7.0

[a] R = reactive ; NR = non-reactive.

false negative reactions with intradermal antigens. With SMA antigen the proportion of false negative reactions varied from 28.3% (COP) to 32.3% (CFA); with SMC antigen from 18.3% (stool) to 20.3% (CFA). Best agreement was with the CFA test for SMC antigen and with the SFC test for SMA antigen (Table 16).

DISCUSSION

Parasitological examination

Parasitologically, all the patients were unknowns when referred to us. Their infections were suspected, but not confirmed, prior to stool examinations. The patients' medical records, when provided, were not available until long after the laboratory studies had been completed.

Stool examinations provided a definitive diagnosis when eggs of *S. mansoni* were found. We have shown, as have others, that chances of recovering eggs were enhanced by examination of multiple stool specimens, and that eggs were not consistently detected in stools from infected persons. The latter point was emphasized when the concurrent stool data were compared with records of previous examina-

tions. The disagreements in the results may have been caused by differences in laboratory techniques or by changes in the parasitological status of the 38 patients. Since variations occurred, even with the same technique, the changes are probably not real.

We established that 316 patients were infected at the time of the serological studies (concurrent stools positive) and that an additional 53 patients were probably infected (previous stools positive). The infection status of 85 patients with negative stool findings and 21 patients without stool examinations has remained unsettled.

In comparative studies, Hernandez-Moralez & Maldonado (1946) showed that in contrast to rectal biopsies examination of three stools per person with the acid-ether concentration technique was relatively ineffective in detecting infections. Of 50 patients found infected by rectal biopsies, only 41% were positive by stool examinations. Thus, examination of three stools per patient did not detect eggs even when they are being produced.

Other patients may be infected, but are not producing eggs. In early infections and those in which only one sex of the parasite is present, eggs are not produced. Moreover, Diaz-Rivera et al. (1957) have concluded that the main effect of early stibophen therapy was the transitory or prolonged suppression of oviposition by the parasite. Courses of 40-60 ml of stibophen failed to eradicate the parasite or to suppress oviposition. Repeated treatment with the same doses was of no apparent additional benefit. Larger initial total doses (80-100 ml) suppressed oviposition for as long as five months to one year, but failed to eradicate the parasite.

Although infections have been demonstrated in 379 (78%) of the patients in this series, additional infections must be suspected among the remaining 106 patients because of vagaries of stool examinations.

Serological tests

Sensitivity. Anderson & Naimark (1960) have established the relative sensitivity of the five serological tests on patients with unequivocal diagnosis of *S. mansoni* infection (concurrent stools positive). Nearly all sera from their patients were reactive in the CFA (97%), CFC (96%), SFC (98%), and the CA (98%). However, 21% of the reactions in the CA test were weak. Such weak reactions defy interpretation. Other studies using the same tests and techniques have been summarized (Appendix Table 1).

From all available information, the CFA, CFC, and SFC tests all appeared to possess great sensitivity. Results with the CA test have been variable. Some authors have reported a high proportion of weak reactions; others have observed a high production of false negative reactions. In either case, the proportion of sera with decisive reactions was relatively small.

The COP test seemed to be considerably less sensitive than the CFA, CFC, or SFC tests.

Specificity. Compilations of original data plus those available in the literature provide information on the specificity of the serological tests. The CFA, SFC, and COP tests have been studied with limited numbers of sera from other schistosome infections (Appendix Table 2). Since reactions occurred in sera from patients with *S. japonicum* and with *S. haematobium*, none of these tests appears to be species specific.

From the few tests reported on sera representing other human trematode infections only two weak reactions in the SFC test and a single reaction in the COP test have been observed.

Intestinal nematodes do not stimulate non-specific reactions in the CFA, SFC, or COP tests. Trichinosis sera, on the other hand, freely cross-reacted with all *S. mansoni* antigens. Reactions were weak in the CFA test and strong in the CFC, SFC, COP, and CA tests (Appendix Table 3).

All of the tests have been performed on syphilitic sera. These sera were usually non-reactive. However, when the titre was very high in tests for syphilis some non-specific reactions occurred in the CFA test (Sleeman, 1960). In our laboratory the CF test for syphilis (using cardiolipin antigen) has been routinely performed on all sera. Only two patients in the 485 considered in this report reacted to cardiolipin antigen. Both patients were stool positive for *S. mansoni* eggs and were serologically reactive in all five serological tests for bilharziasis.

Sera from patients with a variety of other diseases have been used in the SFC test by Sadun et al. (1961). Weak non-specific reactions occurred in some tuberculous sera. A reaction and two weak reactions were observed in five sera from patients with lupus erythematosus (Appendix Table 4).

Sera from 96 healthy naval personnel with no overseas experience were non-reactive in the CFA, CFC, SFC, and COP tests. One serum reacted in the CA test. Studies by Kagan & Levine (1956)

suggest that false CA reactions occurred far more frequently than our data have suggested. They reported reactions in 43% of the "normal" human sera tested. Other authors have found most "normal" sera were non-reactive in the other tests (Appendix Table 5).

From the available information, the CFA test showed the greatest specificity, although weak reactions occasionally occurred in trichinosis sera. Tests using egg (COP) antigen and cercarial antigens (SFC, CFC, and CA) readily cross-reacted with *Trichinella* antibodies. The CA test was the least specific procedure since normal sera also reacted. In the SFC test, weak reactions may be to schistosome antibodies, or may be non-specific.

Since trichinosis has not been found in Puerto Rico and since all sera were tested for syphilis, reactions observed in our studies probably cannot be attributed to these diseases.

From the available information, both original and that published elsewhere, the CFA appeared to be the most specific test for bilharziasis. The COP was only slightly less specific, followed by the CFC and SFC. If weak reactions were excluded, the SFC test would approximate the CFA in specificity. The CA test has lacked specificity.

Intradermal tests

Extensive literature on intradermal testing for bilharziasis has been reviewed by Mayer & Pifano (1946), by Pellegrino (1958) and by Kagan & Pellegrino.[1] Except for studies by Anderson & Naimark (1960) and Horstman et al. (1954), comparison of our data on intradermal tests with those of other authors is virtually impossible. Nearly every investigator has employed different methods of preparing antigens and of performing and interpreting the tests. Moreover, the age and sex compositions of the populations under study have varied.

Anderson & Naimark (1960), testing persons with confirmed bilharziasis, found that 75% reacted to cercarial antigens (SMC), 66% to adult worm antigen (SMA), and only 16% to egg antigen (SME). They found all intradermal tests were considerably less sensitive than serological tests. Their patients represented both sexes and a wide age distribution.

Our results were comparable, with 78% of the patients reacting to SMC, 69% to SMA and 34% to SME.

Horstman et al. (1954) performed intradermal tests on male military personnel. In their study of 276 unselected Puerto Rican soldiers, the intradermal test with SMA antigen was reactive in 45%, the CFA test was reactive in 44%, and eggs of *S. mansoni* were found in stools of 19%. No reactions to SMA antigen occurred among 158 persons who had never been exposed to schistosome infections.

Observations that cutaneous reactions were stronger in adult patients than in children have been reported by Mayer & Pifano (1946), Martins (1949), Pessôa & Barros (1953), and Pellegrino et al. (1957). Martins (1949) also noted that men responded to intradermal tests more frequently than women. In our studies, intradermal tests failed to detect many of the infections in children. Unless the population being tested is limited to young adults (approximately 18-25 years of age), a high proportion of false negative reactions will be experienced in intradermal testing. Thus, in reporting and interpreting the results of intradermal tests, the age and sex of the patients must be considered.

Diagnosis of patients in this study

Patients passing eggs were obviously infected with *S. mansoni*. Concurrent stool examinations revealed infections in 73.5% of 430 patients. When these data were supplemented with the information from previous examinations, infections were confirmed in 81.7% of 464 patients.

In contrast, the serological tests suggested that of the 485 patients, infections occurred in 91.9% (CFA), 89.7% (CFC), 90.1% (SFC), 93.3% (CA), and 80.9% (COP). However, the prevalence of infection is obviously higher than indicated because of known false negative reactions. Consequently, these figures have been corrected by the addition of false negative reactions in the serological tests. The proportion of patients believed infected was increased to 94.0% (CFA), 93.1% (CFC), 93.8% (SFC), 97.2% (CA), and 91.0% (COP).

The high prevalence figure suggested by the CA test must be disregarded because of the non-specific reactions and the frequent weak reactions experienced with this test. The remaining tests have indicated infections in 443 to 456 of the patients. Even the intradermal tests, when corrected for false negative reactions, indicated that 89.1% (SMA) to 93.0% (SMC) of the patients were infected.

The most important difference between the various tests was the frequency of false negative reactions. These were fewest in the CFA test (2.1%). Using this test as a standard, the proportion of patients missed by the various procedures were:

[1] See the article on page 611 of this issue.

Concurrent stool examinations:	20.5%
Composite stool data:	12.3%
Complement-fixation test (CFA):	2.1%
Complement-fixation test (CFC):	3.3%
Slide flocculation test (SFC):	3.9%
Circumoval precipitin test (COP):	13.1%
Intradermal test (SMA):	32.3%
Intradermal test (SMC):	19.5%
Intradermal test (SME):	66.0%

Some of the serological and intradermal reactions could represent infections which have been eliminated either spontaneously or by chemotherapy. However, proof of parasitological cure in human patients is much more difficult to obtain than proof of clinical cure.

Oliver-Gonzalez, Ramos & Coker (1955) have shown that the COP test fails to react six months after successful therapy with stibophen. If their conclusions are accepted, our comparative data suggest that the various serological tests (except CA) must become non-reactive at approximately the same time. Differences in the corrected proportions of patients found infected varied by only 3%—from 94.0% (CFA) to 91.0% (COP).

Suggested uses of the tests included in this study

As mentioned earlier in this report we hope to indicate the usefulness and limitations of these laboratory procedures in clinical and public health studies of bilharziasis. The opinions must be limited to the techniques as we used them and to bilharziasis as it occurs in Puerto Rico.

Stool examinations seem to provide a technique of last resort or of ultimate decision. Alone, this procedure does not afford a reliable index of infection in a population or an accurate assessment of the effectiveness of chemotherapy in an individual patient. Its greatest value appears to be that of establishing the infection status of a patient when other procedures have failed. When used for this purpose, a series of stool specimens may have to be examined before eggs are found. If all stool specimens are negative, rectal biopsies should be examined. The ultimate value of parasitological examinations is to corroborate results of other diagnostic tests.

Of the five serological tests we have considered, two do not appear to have important roles in the diagnosis of bilharziasis. The cercarial agglutination (CA) test is frequently doubtfully reactive and lacks specificity. Consequently, CA reactions may or may not represent *S. mansoni* infections. The complement-fixation test with cercarial antigen (CFC) depends on the same techniques and the same serological system as the CF test with adult worm antigen (CFA). However, the CFC is slightly less specific and less sensitive than the CFA test. If a complement-fixation test is to be used, the CFA test is to be preferred. Each of the remaining serological tests (CFA, SFC, and COP) has merit.

Circumoval precipitin reactions apparently indicate schistosome infections. Cross-reactions with trichinosis can occur. If this infection is suspected, slide flocculation tests with antigen prepared from *Trichinella spiralis* should be performed. Schistosome antibodies do not cross-react with *Trichinella* antigen in this test.

The COP test does provide a procedure by which clinicians can screen patients. If this test is non-reactive, further studies are needed to exclude a diagnosis of bilharziasis. However, the COP test will detect as many schistosome infections as a series of stool examinations. Moreover, as indicated by Oliver-Gonzalez, Bauman & Benenson (1955b), the COP test also has prognostic value since the test (if reactive prior to treatment) becomes negative a few months after successful therapy. Rectal biopsies, repeated stools examinations, or both, should detect most of the infections missed by the COP tests.

When large numbers of persons have to be screened, two serological tests are available—the CFA and the SFC tests. Each has certain limitations. The complement-fixation test with adult worm antigen is more sensitive and specific, but requires experienced technicians and a well-equipped laboratory for the rather exacting techniques. The slide flocculation test with cercarial antigen sacrifices a little sensitivity and specificity, but adequately compensates for this by its simplicity.

Intradermal tests, with the antigens we have used, do not appear to have a definable role in either clinical or public health studies. Their apparent simplicity does not compensate for their lack of sensitivity. Studies on other intradermal antigens will be reported separately.

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RÉSUMÉ

L'étude comparée de méthodes de diagnostic de la bilharziose, effectuée à Porto Rico, portait sur la recherche des œufs de *Schistosoma mansoni* dans les fèces, et les tests sérologiques suivants: fixation du complément avec antigènes provenant des vers adultes ou des cercaires; floculation sur lame avec antigènes de cercaires; agglutination avec antigène de cercaires; test circumoval de précipitation; test intradermique avec antigènes de vers adultes, de cercaires ou d'œufs. Les malades provenaient de divers dispensaires bilharziens de la zone métropolitaine de San Juan, Porto Rico. On n'a tenu compte que des malades pour lesquels on avait effectué tous les tests sérologiques, soit 485 personnes.

L'examen des fèces révéla l'infection chez 74% des malades; mais les tests sérologiques, compte tenu des réactions faussement négatives, indiquèrent 89-94% d'infections. Les résultats des tests d'agglutination des cercaires ont été éliminés, les réactions étant peu nettes et peu spécifiques. Le test intradermique avec l'antigène des œufs était aussi trop peu sensible.

Le test de fixation du complément avec l'antigène de ver adulte se révéla excellent, sensible et spécifique, au point qu'il fut adopté comme de test de référence pour l'évaluation des autres. La proportion des infections qui échappèrent aux tests était de 2,1% pour la fixation du complément (ver adulte), 3,3% pour la fixation du complément (cercaires), 3,9% pour la floculation sur lame (cercaires), 13,1% pour le test circumoval, 32,3% pour le test intradermique (ver adulte), 19,5% pour le test intradermique (cercaires) et 20,5% pour la recherche des œufs dans les fèces.

On a proposé, sur la base de ces résultats l'emploi des tests suivants: Dans les études cliniques, le test circumoval peut être utile pour le dépistage des malades. L'examen des fèces, complété par des biopsies rectales, assure une sécurité supplémentaire au diagnostic et confirme d'autres tests. Dans les enquêtes, s'il s'agit d'établir quelle est la fréquence globale de la maladie dans une population, le test de fixation du complément (ver adulte) peut être utilisé si les sérums sont examinés dans un laboratoire bien équipé; on recourra au test de floculation sur lame pour le diagnostic sur le terrain. Les tests intradermiques, de l'avis des auteurs, ne trouvent guère emploi pour la recherche des infections à *S. mansoni*.

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APPENDIX TABLE 1

SENSITIVITY OF *S. MANSONI* ANTIGENS IN SEROLOGICAL TESTS USING SERA FROM CONFIRMED HUMAN CASES OF BILHARZIASIS

Serological test	No. of sera				Reference
	Total	Reactive	Weakly-reactive	Non-reactive	
CFA	379	354	15	10	Original
	540	507	23	10	Anderson & Naimark (1960)
	53	49	?	4	Horstman et al. (1954)
	98	95	1	2	Chaffee et al. (1954)
	126	112	6	8	Sleeman (1960)
	60	60	?	0	Senterfit (1958)
CFC	379	353	10	16	Original
	544	505	16	23	Anderson & Naimark (1960)
SFC	379	332	30	17	Original
	435	396	32	7	Anderson (1960)
	519	470	40	9	Anderson & Naimark (1960)
	70	57	9	6	Sadun et al. (1960)
CA	379	295	66	18	Original
	405	314	84	7	Anderson & Naimark (1960)
	60	60	?	0	Sleeman (1960)
	56	10	?	46	Oliver-Gonzalez, Bauman & Benenson (1955a)
COP	379	330	—	49	Original
	506	445	—	61	Anderson & Naimark (1960)
	34	34	—	0	Oliver-Gonzalez (1954)
	56	56	—	0	Oliver-Gonzalez, Bauman & Benenson (1955a)
	6	6	—	0	Oliver-Gonzalez, Bauman & Benenson (1955b)
	15	15	—	0	Oliver-Gonzalez, Ramos & Coker (1955)

APPENDIX TABLE 2

SPECIFICITY OF SEROLOGICAL TESTS WITH *S. MANSONI* ANTIGENS APPLIED TO HUMAN SERA FROM BILHARZIAL INFECTIONS

Serological test	No. of sera				Reference
	Total	Reactive	Weakly-reactive	Non-reactive	
			S. haemotobium		
CFA	9	7	1	1	Sleeman (1960)
SFC	59	42	16	1	Hernandez-Moralez & Maldonado (1946)
	10	6	4	0	Anderson (1960)
	18	11	4	3	Sadun et al. (1960)
COP	5	2	—	3	Oliver-Gonzalez, Bauman & Benenson (1955b)
	9	3	—	6	Original
			S. japonicum		
CFA	6	2	3	1	Chaffee et al. (1954)
	3	2	1	0	Sleeman (1960)
SFC	11	10	0	1	Anderson (1960)
	1	1	0	0	Sadun et al. (1960)
COP	3	3	—	0	Oliver-Gonzalez, Bauman & Benenson (1955b)
	2	0	—	2	Original

APPENDIX TABLE 3

SPECIFICITY OF SEROLOGICAL TESTS WITH *S. MANSONI* ANTIGENS APPLIED TO HUMAN SERA FROM OTHER HELMINTHIC INFECTIONS

Serological tests	No. of sera				Reference
	Total	Reactive	Weakly-reactive	Non-reactive	
			Clonorchis sinensis		
SFC	1	0	0	1	Sadun et al. (1960)
COP	1	0	0	1	Original
			Fasciola hepatica		
CFA	1	0	0	1	Sleeman (1960)
SFC	2	0	2	0	Sadun et al. (1960)
COP	?	0	—	all	Oliver-Gonzalez, Bauman & Benenson (1955b)
			Opisthorchis felineus		
SFC	1	0	0	1	Sadun et al. (1960)
COP	2	1	—	1	Original
			Fasciolopsis buski		
SFC	2	0	2	0	Original
COP	2	0	0	2	Original
			Paragonimus westermani		
CFA	1	0	0	1	Sleeman (1960)
	2	2[a]	0	0	Chaffee & Nieves (1957)
SFC	1	0	0	1	Anderson (1960)
	10	0	0	10	Sadun et al. (1960)
COP	?	0	—	all	Oliver-Gonzalez, Bauman & Benenson (1955b)
	1	0	—	1	Original
			Intestinal nematodes		
CFA	108	2[a]	0	106	Chaffee & Nieves (1957)
	6	0	1	5	Sleeman (1960)
SFC	33	0	0	33	Anderson (1960)
COP	9	0	—	9	Oliver-Gonzalez (1954)
			Trichinella spiralis		
CFA	10	0	2	8	Sleeman (1960)
	1	0	1	0	Chaffee et al. (1954)
CFC	8	7	1	0	Original
SFC	8	6	2	0	Original
	11	8	2	1	Sadun et al. (1960)
	20	11	9	0	Anderson (1960)
CA	8	7	0	1	Original
COP	21	13	—	8	Original
			Echinococcus granulosus		
SFC	11	0	0	11	Original
COP	11	0	0	11	Orignal

[a] Possibly also infected with schistosomes.

APPENDIX TABLE 4

SPECIFICITY OF SEROLOGICAL TESTS WITH *S. MANSONI* ANTIGENS APPLIED TO HUMAN SERA REPRESENTING NON-HELMINTHIC INFECTIONS

Serological test	No. of sera				Reference
	Total	Reactive	Weakly-reactive	Non-reactive	
			Syphilis		
CFA	28	0	0	28	Chaffee et al. (1954)
	35	9	2	24	Sleeman (1960)
CFC	20	0	0	20	Original
SFC	12	0	0	12	Anderson (1960)
	12	0	4	8	Sadun et al. (1960)
CA	?	0	0	all	Liu & Bang (1950)
COP	32	0	—	32	Original
			Tuberculosis		
SFC	11	0	3	8	Sadun et al. (1960)
COP	11	0	—	11	Original
			Leishmaniasis		
SFC	9	0	0	9	Sadun et al. (1960)
COP	8	0	—	8	Original
			Histoplasmosis		
SFC	3	0	0	3	Sadun et al. (1960)
			Coccidiomycosis		
SFC	3	0	0	3	Sadun et al. (1960)
			Blastomycosis		
SFC	2	0	0	2	Sadun et al. (1960)
			Liver cirrhosis		
SFC	2	0	0	2	Sadun et al. (1960)
			Hepatitis		
SFC	3	0	0	3	Sadun et al. (1960)
			Lupus erythematosus		
SFC	5	1	2	2	Sadun et al. (1960)
			Infectious mononucleosis		
SFC	2	0	0	2	Original
COP	2	0	—	2	Original

APPENDIX TABLE 5

SPECIFICITY OF SEROLOGICAL TESTS WITH *S. MANSONI* ANTIGENS APPLIED TO NORMAL HUMAN SERA

Serological tests	No. of sera				Reference
	Total	Reactive	Weakly-reactive	Non-reactive	
CFA	158	0	0	158	Horstmann et al. (1954)
	46	0	0	46	Chaffee et al. (1954)
	50	0	1	49	Sleeman (1960)
	96	0	0	96	
CFC	96	0	0	96	Original
SFC	56	0	0	56	Anderson (1960)
	34	0	2	32	Sadun et al. (1960)
	96	0	0	96	Original
CA	86	37	?	49	Kagan & Levine (1956)
	96	1	0	95	Original
COP	15	2 [a]	—	13	Oliver-Gonzalez (1954)
	1	0	—	1	Oliver-Gonzalez, Bauman & Benenson (1955b)
	96	0	—	96	Original

[a] Possibly infected with *S. mansoni*.