

## Schistosomiasis *Mansoni* in Brazil: Intradermal Tests with Antigen of Proteolytic Enzyme and Veronal Buffered Saline Extract of *Schistosoma mansoni*

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### Introduction

The immediate type intradermal test (IDT) is widely used in the diagnosis of schistosomiasis. However, there are several reports suggesting that the IDT for schistosomiasis is less sensitive and less specific in children than in adults (McMahon, 1967; Velimirovic *et al.*, 1969; McKay *et al.*, 1973). In 1976, we have also obtained similar results of the IDT in children in São Lorenzo, Pernambuco, an endemic area of schistosomiasis mansoni in Brazil. In the present study, we examined again the sensitivity and specificity of the test at the same place, using two different types of antigens. The results presented here confirm previous observations of others and ourselves that false negative reactions occur more frequently in children than in adult subjects.

### Materials and Methods

*Subjects.* Among children examined for

the IDT, 92 children including 40 males, 45 females and 7 of undescribed of either sex were the subjects for final analyses. Their age ranged from 7 to 14 years old with an average of 10.5 years. Twenty-three hospitalized adult patients (16 to 64 years old) in Bahia University Hospital in Salvador were also tested with the same methods as in children in São Lorenzo, Pernambuco.

*Antigens.* Veronal buffered saline extract (VBS antigen) was prepared from adult worms of *Schistosoma mansoni* by the modified method of Chaffee *et al.* (1954) as described (Yokogawa and Awano, 1956). A batch of proteolytic enzyme antigen (Senft antigen) was a generous gift of Dr. Alfred W. Senft. Another batch of the enzyme antigen was prepared from the schistosomes at Juntendo University as described (Senft and Maddison, 1975) (Senft-J antigen).

*Intradermal tests (IDT) and other immunological tests.* As for the Senft antigen and Senft-J antigen, 0.05 ml of each antigen containing 1  $\mu$ g N/site and 1  $\mu$ g P/site, respectively, was injected intradermally into the forearm and 15 minutes later wheal reactions were outlined with a ball-point pen and transferred to a sheet of absorbent paper moistened with alcohol. The area of the reaction was measured and a lesion size of 100 mm<sup>2</sup> or larger was considered as positive. In addition, 0.02 ml of VBS antigen containing 1  $\mu$ g P/site was injected intradermally into the fore-

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Table 1 Results of stool examinations in schoolchildren\* in São Lorencó, Pernambuco, Brazil

Eggs of	No. of positive (%)		
	Kato-Katz	MGL	Combined
<i>Schistosoma mansoni</i>	28 (29.7)	31 (32.9)	38 (40.4)
<i>Ascaris lumbricoides</i>	71 (75.5)	69 (73.4)	74 (78.2)
<i>Trichuris trichiura</i>	88 (93.6)	88 (93.6)	91 (96.8)
Hookworm	71 (75.5)	75 (79.7)	82 (87.2)

\* Ninety-four children, 7 to 14 years old, were examined.

Table 2 Results of the IDT with VBS, Senft and Senft-J antigens in schoolchildren in São Lorenço, Pernambuco, Brazil

<i>S. mansoni</i> eggs in stools		Results of the IDT with					
		VBS antigen		Senft antigen		Senft-J antigen	
No.		+	-	+	-	+	-
Positive	37	9	28 (75.6%)	2	35 (94.5%)	5	32 (86.4%)
Negative	55	17 (30.9%)	38	2 (3.6%)	53	6 (10.9%)	49
Total	92	26	66	4	88	11	81

arm; this was enough to raise a wheal 3 mm in diameter. A wheal reaction exceeding 8 mm in diameter at 15 minutes after the injection was considered to be positive.

At the time of the final reading, blood samples were obtained from the antecubital vein of each subject, and sera were stored at  $-20^{\circ}\text{C}$ . Immunological tests such as circum oval precipitation test (COPT), complement fixation test (CFT) and double diffusion test in agar (DDT) were performed on these samples. Serum IgE levels were determined by using immunodiffusion plates (Behring Institute) according to a modified method (Kojima *et al.*, 1972) of Rowe (1969).

**Stool examinations.** Stool examinations were performed on all subjects by both of formalin-ether centrifugation (MGL) (Ritchie, 1948) and modified Kato-Katz cellophane thick smear techniques (Komiya and Kobayashi, 1966; Katz *et al.*, 1972).

### Results

Results of stool examinations are summa-

rized in Table 1. *S. mansoni* eggs were detected in a total of 38 (40.4%) of 94 schoolchildren by the combination of Kato-Katz technique and MGL. A high prevalence was found for *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm infections. Any significant difference in recovery rates of these helminth eggs was not observed between both techniques.

With respect to the sensitivity of the IDT, a large number of false negative reactions were observed in children even though a relatively purified antigen such as Senft antigen was used for the test. As shown in Table 2, when VBS antigen was employed, 28 cases (75.6%) out of 37 children were negative in spite of the fact that all of them had proven infection with *S. mansoni*. When Senft-J antigen was used, 32 cases (86.4%) showed false negative reactions. The worst result was obtained when Senft antigen was used; 35 (94.5%) were false negative.

On the other hand, there were 17 (30.9%) positives with VBS antigen among 55 child-

Table 3 Results of immunological tests in schoolchildren in São Lorenzo, Pernambuco, Brazil

<i>S. mansoni</i> eggs in stools	No.	COPT		CFT		DDT	
		+	-	+	-	+	-
Positive	19	12	7 (36.8%)	2	17 (89.4%)	4	15 (78.9%)
Negative	21	13 (61.9%)	8	2 (9.5%)	19	3 (14.3%)	18
Total	40	25	15	4	36	7	33

Table 4 Results of the IDT and immunological tests in adult patients in Salvador, Brazil

<i>S. mansoni</i> eggs in stools	No.	IDT				COPT		CFT		DDT	
		VBS		Senft		+	-	+	-	+	-
		+	-	+	-						
Positive	8	8	0	4	4	6	2	6	2	5	3
Negative	12	7	5	4	8	6	6	2	10	8	4
Total	20	15	5	8	12	12	8	8	12	13	7

ren who were negative for *S. mansoni* eggs but only 2 (3.6 %) or 6 (10.9 %) were positive with Senft antigen or Senft-J antigen, respectively.

A large number of false negative reactions were also found in other immunological tests among children infected with *S. mansoni* (Table 3). The false negative reactions in COPT, CFT and DDT among 19 children who were positive for *S. mansoni* eggs in stools were 7 (36.8 %), 17 (89.4 %), and 15 (78.9 %), respectively, indicating that the CFT and DDT were far less sensitive than the COPT.

In adult subjects, 15 out of 20 patients were positive for the IDT with VBS antigen, while 7 patients showed positive reactions to both of Senft antigen and VBS antigen. All of 8 patients having proven *S. mansoni* infection showed positive skin reactions to VBS antigen, whereas only half of them were positive with Senft antigen (Table 4). Among these 8 patients, however, 6 were positive for COPT and CFT, and 5 were positive for DDT (Table 4). On the other hand, 7 out of

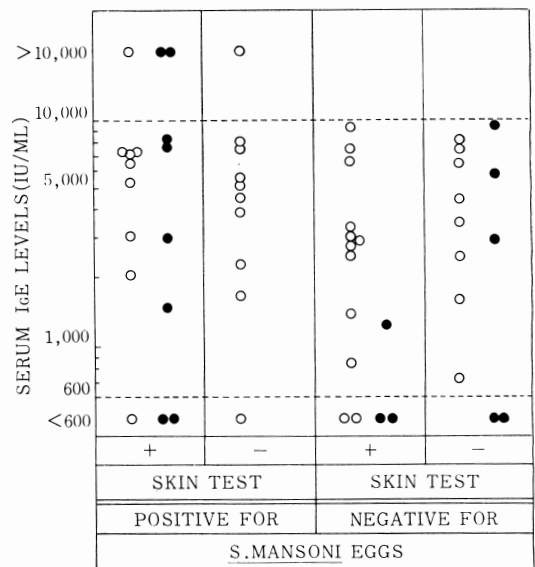


Fig. 1 Serum IgE levels of schoolchildren (○) and adults (●) with or without proven schistosomiasis mansoni.

12 individuals negative for *S. mansoni* eggs were positive for IDT with VBS antigen

Table 5 Serum levels of schoolchildren and adults positive for helminth eggs

Helminth eggs		IgE levels (IU/ml)		
in stools	No.	> 3,000	3,000-1,000	<1,000
Positive	35* (16)	23 (5)	10 (5)	2 (6)
Negative	0 (9)	0 (3)	0 (2)	0 (4)
Total	35 (25)	23 (8)	10 (7)	2 (10)

\* Number of schoolchildren examined.

( ) Number of adults examined (including patients hospitalized in Bahia University Hospital).

(Table 4).

In order to examine a possibility that IgE synthesis may be inherently inhibited among individuals who showed false negative reactions for the IDT, serum IgE concentration was determined. It was found that there was no statistical difference between IDT-positive and -negative groups, although several cases positive for *S. mansoni* eggs in the stool showed very high levels of serum IgE (>10,000 IU/ml) (Fig. 1). Fourteen (73.7%) of 19 children positive for *S. mansoni* eggs showed more than 3,000 IU/ml serum IgE levels, whereas 9 (45.0%) of 20 cases of egg negative children were found to exceed this level (Fig. 1).

Since all of the schoolchildren were infected with at least one of helminths listed in Table 1, it was hard to eliminate another possibility that the IDT might be inhibited by saturation of the receptors on mast cells with IgE nonspecifically synthesized by the potentiating effect of parasitic infections. In fact, IgE levels were found to be more than 1,000 IU/ml in 94.3% of the schoolchildren positive for helminth eggs (Table 5).

### Discussion

The results obtained in the present study revealed the occurrence of a large number of false negative reactions in the IDT and the other three immunological tests among schoolchildren infected with *S. mansoni*, whereas the results in adult patients correlated with those of stool examinations rather well. This is in agreement with earlier observations of other investigators (McMahon, 1967; Ve-

limirovic *et al.*, 1969; McKay *et al.*, 1973). Thus, it seems likely that the aging factor is of considerable importance concerning the sensitivity and specificity of these immunological tests for schistosomiasis. However, this is quite different from our previous observations on paragonimiasis in which there was no difference of immune responses between children and adults (Yokogawa and Tsuji, 1962).

The reason why such false negative reactions occur in schistosomiasis but not in paragonimiasis is not yet clear. From the results of measurement of serum IgE levels, it seems unlikely that Brazilian children have any inheritance of low ability of IgE synthesis. Instead, a very high IgE concentration of more than 1,000 IU/ml was found in 94.3% of schoolchildren positive for helminth eggs in the stool. Thus, a possibility can not be excluded by the present study that the receptors for IgE antibody on mast cells may be saturated with IgE antibodies produced against any of helminths other than *S. mansoni*, or with IgE potentiated nonspecifically by parasitic infections (Kojima *et al.*, 1972) so as to inhibit binding of IgE antibody specific for *S. mansoni*, resulting in false negative skin reactions. Alternatively, it is possible that suppression of IgE as well as other classes of antibodies to schistosome antigen(s) might occur in childhood due to repeated infections with *S. mansoni*.

As for the IDT antigens for schistosomiasis, a crude preparation such as VBS antigen appeared to be better in the sensitivity than a purified antigen such as proteolytic enzymes,

although VBS antigen induced at a consistently higher frequency both of false negative and false positive reactions.

It has been reported by McKay *et al.* (1973) that a striking and highly significant relationship was observed between the intensity of the infection and the IDT response in children infected with *S. mansoni*. However, we could not demonstrate such a relationship in the present study (data not shown). Further studies will be needed to clarify this point.

### Summary

Sensitivity and specificity of the IDT in schistosomiasis mansoni were examined on schoolchildren and adults in São Lorenzo and Salvador, the endemic areas of the disease in Brazil where soil-transmitted helminthiases were also endemic. Two different types of antigens, VBS antigen and proteolytic enzyme antigens (Senft antigen and Senft-J antigen), prepared from the adult worms of *S. mansoni* were used for the IDT. A large number of false negative reactions were observed in children with both of the antigens. Among 37 children 28 (75.6 %) with VBS antigen and 35 (94.5 %) with Senft antigen were found to be negative for the IDT, in spite of the fact that all of these children had proven infection with *S. mansoni*. On the other hand, there were 17 (30.9 %) positives with VBS antigen for the IDT among 55 children negative for *S. mansoni* eggs in stools but only 2 (3.6 %) of them were positive with Senft antigen. Immunological tests such as COPT, CFT and DDT also revealed the presence of false negative reactions among these children. However, all of 8 adult patients with schistosomiasis showed positive skin reaction with VBS antigen, although half of them were positive with Senft antigen. Increase of serum IgE concentration was found in more than 90 % of schoolchildren who discharged helminth eggs in the stool. Based on these observations, several possibilities involved in the occurrence of false negative reactions were discussed.

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## ブラジルにおけるマンソン住血吸虫症，特に蛋白分解酵素抗原 および VBS 抗原による皮内反応について

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ブラジルのマンソン住血吸虫症流行地において，マンソン住血吸虫成虫より作製した VBS 抗原および蛋白分解酵素抗原 (Senft 抗原) を用い，学童並びに成人患者に対し皮内反応を行い虫卵検査成績と比較した。その結果，マンソン住血吸虫卵陽性の学童のうち，VBS 抗原で75.6%，Senft 抗原で94.5%が皮内反応陰性であった。これに対し，成人では虫卵陽性者の全員が VBS 抗原で，またその半数が Senft 抗原で，皮内反

応陽性であった。学童の全員が回虫・鞭虫・鉤虫の少なくともいずれか1種の土壌媒介線虫に感染しており，かつその94.3%に血清 IgE 値の上昇が認められたことから，他種蠕虫に対する IgE 抗体あるいは非特異的に増量した IgE によるマスト細胞レセプターの飽和により，住血吸虫抗原に対する皮内反応が陰性を示す可能性のあることが示唆された。