

IMMUNOELECTROPHORETIC STUDY ON COMMON ANTIGENS OF SÃO LOURENÇO DA MATA AND BELO HORIZONTE STRAINS OF *SCHISTOSOMA MANSONI* ADULT WORMS AND *BIOMPHALARIA* SNAILS.

José Valfrido de SANTANA (1,2), Yuzuru IWANAGA (2,3), Adriana Maria da Silva TELLES (2), Maria Risoleta da SILVA (2), José Felipe GONÇALVES (4) & Seiki TATENO (2,5).

SUMMARY

Immunoelectrophoretic studies on common antigens were carried out by using rabbits sera immunized against São Lourenço da Mata and Belo Horizonte strains of *Schistosoma mansoni* adult worms and antigens of *Biomphalaria glabrata* pigmented (Jaboatão - PE); *B. glabrata* albino (Belo Horizonte - MG) and *B. straminea* (São Lourenço da Mata, PE). Furthermore, the reverse approach was proceeded, namely, sera anti *Biomphalaria* snails produced in rabbits were tested against both strains of *Schistosoma* adult worm antigens.

The analysis of the common antigens between the SLM strains of *S. mansoni* adult worm and *B. glabrata* pigmented showed 8 to 9 precipitin bands, 3 bands with *B. glabrata* albino and only 1 band with *B. straminea* crude extracts. On the other hand, the BH strain of *S. mansoni* adult worm antiserum produced 6 to 7 bands with *B. glabrata* pigmented, 5 bands with *B. glabrata* albino and 1 band with *B. straminea* antigenic extract.

Biomphalaria snails crude extracts were fractionated by Sephadex G-100 column and three fractions were collected from each snail strain. The fractions were tested with anti SLM and BH strains of *S. mansoni* adult worm sera by immunoelectrophoresis. The common antigens fractionated from *Biomphalaria* snails crude extracts and those found for both strains of *S. mansoni* adult worm mostly existed in the first fraction and they were estimated to have molecular weight over 158,000 daltons.

In our laboratory, it was found a relationship between the antigenic similarities and experimental infection rates of *S. mansoni* towards *Biomphalaria* snails so that more bands were seen with increasing infection rates of *S. mansoni*.

KEY WORDS: *Schistosoma mansoni*; Physiology; Schistosomiasis mansoni; Immunology; *Biomphalaria*; Parasitology; Immunoelectrophoresis.

INTRODUCTION

It is well known that strains of *S. mansoni* originated from different geographical regions show different infectivities to various species of *Biomphalaria* snails^{3,4,5,10,11,12}. The Belo Horizonte (BH) strain of *S. mansoni* has been reported to infect *B. glabrata* from Belo Horizonte, Brazil, but

does not infect *Biomphalaria tenagophila* from the same area³. These findings support the hypothesis that common antigens are one of the factors defining that different susceptibilities of various species of *Biomphalaria* snails to geographical strains of *S. mansoni*.

(1) Depto. de Biofísica e Radiobiologia, Centro de Ciências Biológicas, UFPE, Recife, PE, Brasil.

(2) Laboratório de Imunopatologia Keizo Asami (LIKA), Universidade Federal de Pernambuco, Recife, PE, Brasil.

(3) Department of Parasitology, School of Medicine, Hiroshima University, Hiroshima, Japan.

(4) Centro de Pesquisas Aggeu Magalhães, FIOCRUZ, Cidade Universitária, Recife, PE, Brasil.

(5) Department of Parasitology, School of Medicine, Keio University, Tokyo, Japan.

Address for correspondence: José Valfrido de Santana - Depto. de Biofísica e Radiobiologia da Universidade Federal de Pernambuco. Cidade Universitária. 50730 Recife, PE, Brasil.

Recently, immunoelectrophoresis has been employed to study the common antigenicities between parasite and intermediate hosts^{7,9}. IWANAGA & TSUJI⁸ reported that several common bands were seen between the Chinese strain of *S. japonicum* adult worm and *Oncomelania hupensis hupensis*, which were most suitable host for *S. japonicum*, Chinese strain.

This paper describes common antigens between *Biomphalaria* strains and *S. mansoni* adult worm, as detected by immunoelectrophoresis.

MATERIAL AND METHODS

Strains of *S. mansoni* SLM were isolated from patients in São Lourenço da Mata (Pernambuco, Brazil) and BH from patient in Belo Horizonte (Minas Gerais, Brazil)¹¹. Both strains have been maintained in the laboratory by passage through *B. glabrata* pigmented and Swiss albino mice. The laboratory colonies were originated from the followings areas of Brazil: *B. glabrata* pigmented (P) from Jaboatão, PE; *B. glabrata* albino (A) from Belo Horizonte, MG and *B. straminea* from São Lourenço da Mata, PE.

Immunized rabbits sera against *Biomphalaria* snails and *S. mansoni* adult worm crude antigens were produced by using crude extracts of these animals prepared in 0.1% w/v NaCl¹⁷. Immunoelectrophoresis was proceeded according to TSUJI¹³ on 0.9% agarose in veronal buffered saline, pH 8.2, and the current was adjusted to 18 ± 2 V/8 cm length/gel and applied for 3 h.

Biomphalaria antigens were also fractionated by gel filtration on Sephadex G-100 column chromatography to estimate the molecular weight of the substances responsible for the common antigenicities against anti-*S. mansoni* adult worm sera according to IWANAGA & TSUJI⁸. The chromatography column was calibrated with gamma globulin (MW=158,000); ovalbumin (MW=45,000); myoglobulin (MW=17,000) and Vitamin B-12 (MW=1,350)

RESULTS

1. Common antigens between SLM strain of *S. mansoni* adult worm and *Biomphalaria* snails.

As shown in Fig. 1a, anti-*S. mansoni* adult

worm serum produced 9 bands against *B. glabrata* (P) antigen; 3 bands against *B. glabrata* (A) antigen, and 1 band against *B. straminea* antigen. The reverse, using anti-*Biomphalaria* snail sera against *S. mansoni* adult worm antigen showed 8 bands with anti-*B. glabrata* (P) serum, 3 bands with anti *B. glabrata* (A) serum and 1 band with anti *B. straminea* serum.

2. Common antigen between the BH strain of *S. mansoni* adult worm and *Biomphalaria* snails.

As shown in the immunoelectrophoretics diagrams in Fig. 1b, anti *S. mansoni* adult worm serum produced 6 bands against *B. glabrata* (P) antigen, 5 bands against *B. glabrata* (A) and 1 band against *B. straminea* antigen. On the other hand, the *S. mansoni* adult worm antigens were tested against anti *Biomphalaria* snails sera. As a result, *S. mansoni* adult worm antigens showed 7 bands with anti *B. glabrata* (P) serum, 5 bands with anti *B. glabrata* (A) serum and 1 band with *B. straminea* serum.

3. Common antigen between SLM and BH strains of *S. mansoni* adult worm and fractionated antigens of *Biomphalaria* snails.

Biomphalaria snails crude antigens were collected in 3 fractions by Sephadex G-100 gel filtration (Fig. 2.). Immunoelectrophoretic studies of the anti-SLM strain of *S. mansoni* sera and fractions of the antigens of *Biomphalaria* snails are shown in Fig. 3. *B. glabrata* (P) antigen produced 9 bands using the first fraction, 5 bands using the second fraction. Furthermore, *B. glabrata* (A) antigen produced 3 bands with the first fraction, 1 band with the second fraction. However, the third fraction did not produced any band for both *B. glabrata*, pigmented and albino. *B. straminea* antigen produced only 1 band using the first fraction.

Immunoprecipitation reactions between BH strain of *S. mansoni* adult worm sera and antigens of *Biomphalaria* snails resulted in: *B. glabrata* (P) antigen: 6 bands with the first fraction, 4 bands with the second fraction and no band with the third fraction; *B. glabrata* (A) antigen produced: 5 bands with the first fraction, 2 bands with the second fraction and no band with the third fraction; *B. straminea* antigen produced 1 band each with the first and the second fractions.

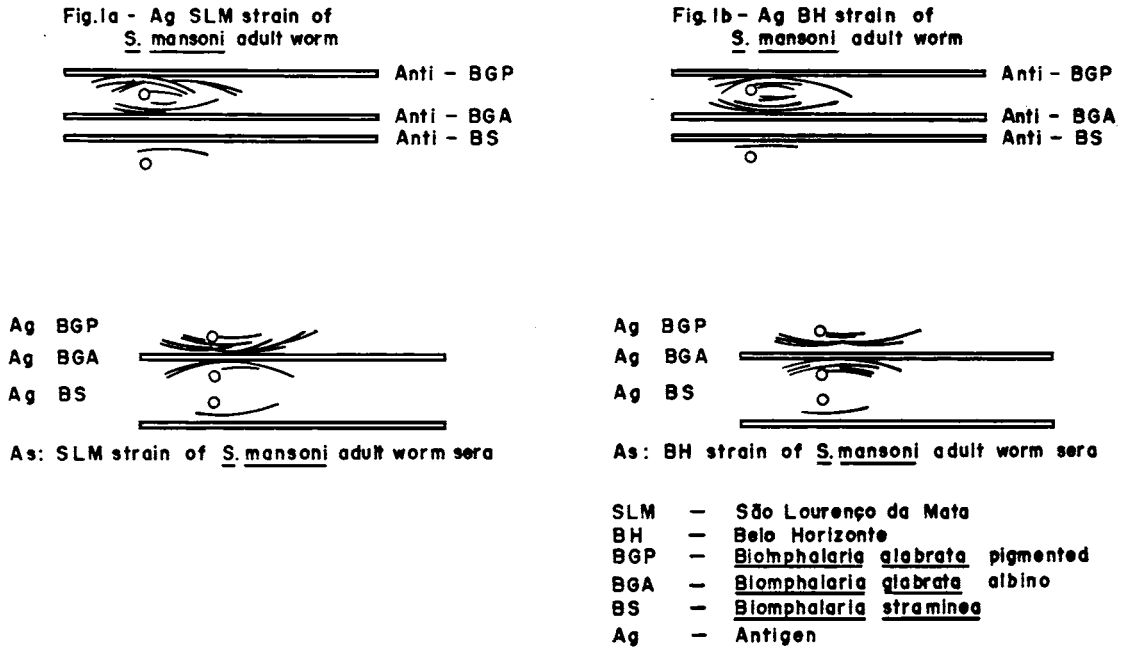


Fig 1 - Immunoelectrophoregrams between various *Biomphalaria* snails and *Schistosoma mansoni* adult worms

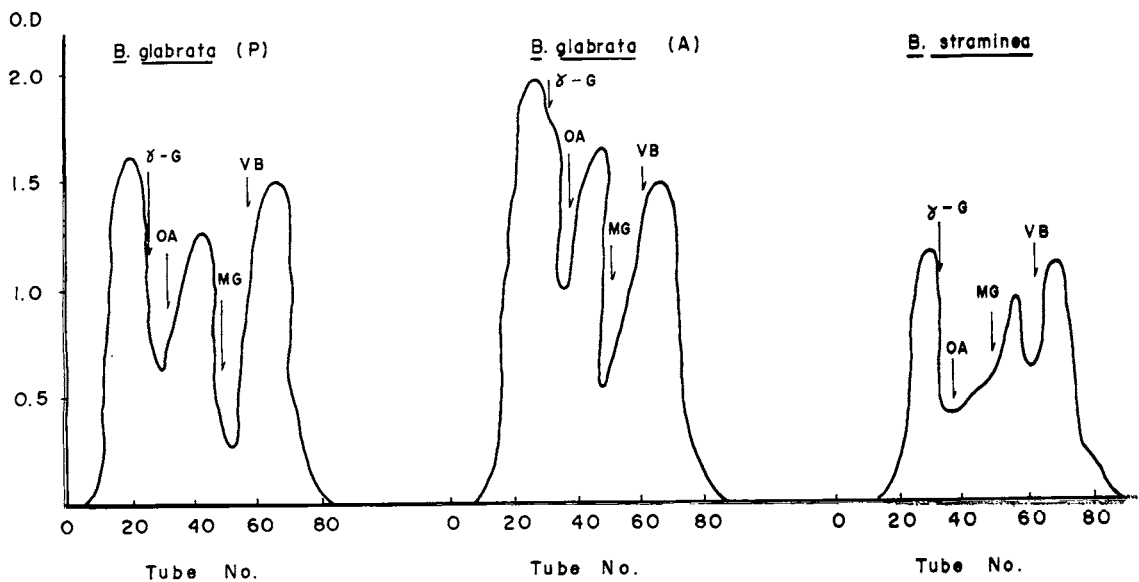


Fig 2 - Column chromatography for various antigens of *Biomphalaria* snails on Sephadex G - 100 (OD at 280 nm)

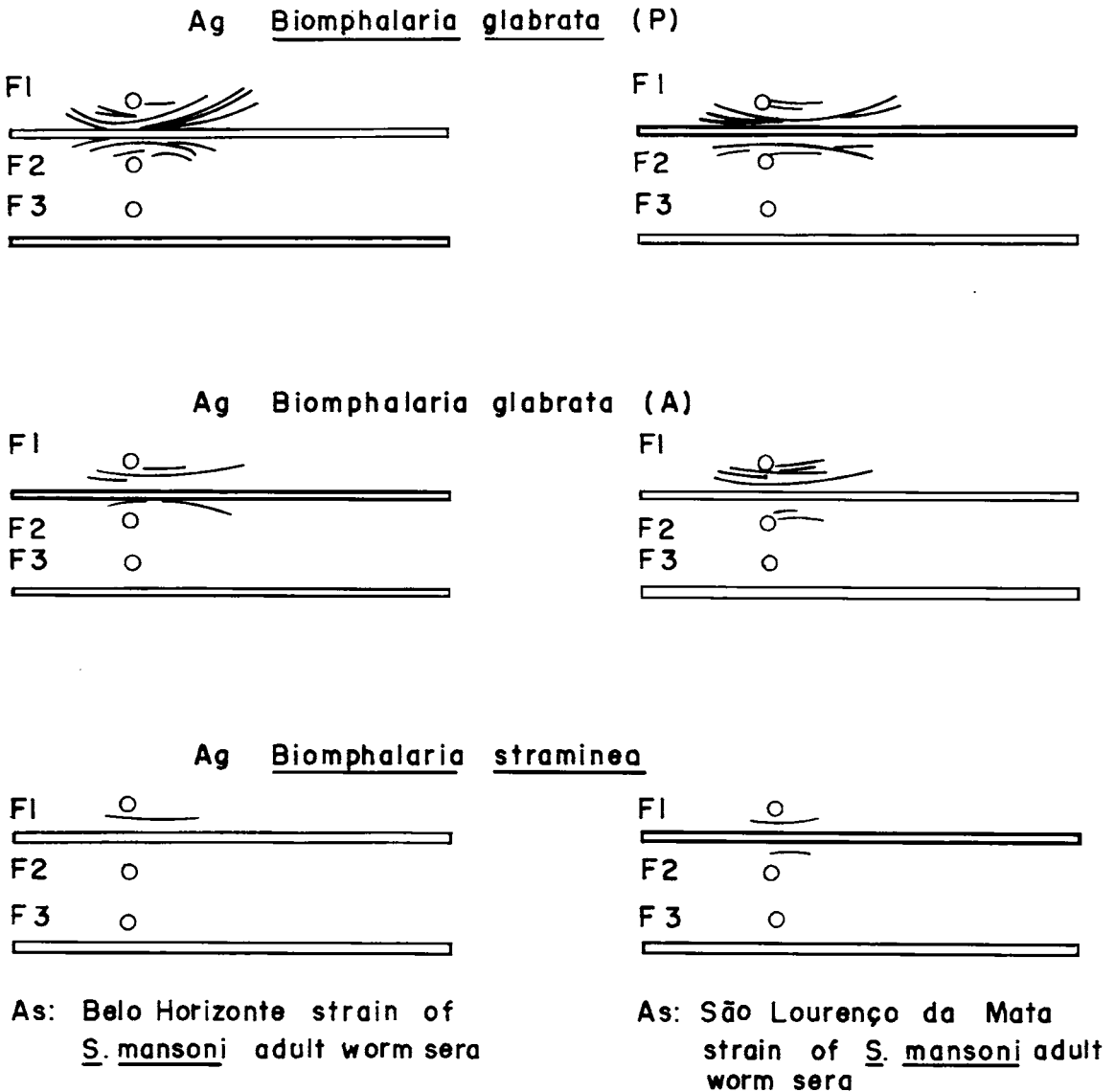


Fig 3 - Immunoelectrophoregrams between Anti-*Schistosoma mansoni* adult worms and fractionated antigens of *Biomphalaria* snails

In our laboratory, experimental infection rates of *S. mansoni* miracidia, SLM strain, to *Biomphalaria* snails, showed that *B. glabrata* pigmented, after exposure to SLM strain miracidia, presented the highest infection rate (78%) of the two species of *Biomphalaria* snails (Table 1 and 2).

DISCUSSION

Since the establishment of the immunoelectrophoresis by GRABAR & WILLIAMS⁶, this

method has been extensively employed for immunological studies of physiology and morphological features of parasites¹. Furthermore, this method has been used for studying common antigens between adult and larval parasite^{2,14}.

In this study, common antigens between *Biomphalaria* snails and *S. mansoni* adult worms were evaluated by immunoelectrophoresis. As results, it was found that *B. glabrata* (P) produced more bands (8 to 9) with SLM strain of *S. mansoni* adult worm.

TABLE 1

Infection rates of *Biomphalaria* snails exposed to *Schistosoma mansoni* miracidia SLM strain (5 miracidia/snail)

Snail strain	Number of snail	Number of snails (%)	
		Infected	Dead
<i>Biomphalaria glabrata</i> pigmented	50	37 (74.0)	4 (8.0)
<i>Biomphalaria glabrata</i> albino	55	5 (9.1)	3 (5.4)
<i>Biomphalaria straminea</i>	85	0 (0)	4 (4.7)

TABLE 2

Infection rates of *Biomphalaria* snails exposed to *Schistosoma mansoni* miracidia BH strain (5 miracidia/snail)

Snail strain	Number of snail	Number of snails (%)	
		Infected	Dead
<i>Biomphalaria glabrata</i> pigmented	75	47(64.3)	7(9.6)
<i>Biomphalaria glabrata</i> albino	60	33(55.0)	4(6.7)
<i>Biomphalaria straminea</i>	75	0(0)	3(4.0)

On the other hand, *B. glabrata* (P) also produced more bands (6 to 7) with BH strain of *S. mansoni* adult worm. These findings suggested that more precipitation correlates bands with the highest infection rate and this observation agrees with that reported by IWANAGA et alii⁷. These authors reported that *Oncomelania hupensis nosophora* obtained the highest infection rate common antigens against Yamanashi strain and presented numerous common antigens with Yamanashi strain of *S. japonicum* adult worm by immunoelectrophoresis.

GONÇALVES et alii⁵ reported that *B. glabrata* (A) presented low infectivity to *S. mansoni* SLM strain whereas the pigmented variety showed high infectivity to both SLM and BH strains of *S. mansoni*. These observations are in accordance with the results observed in our laboratory and displayed in Table 1 and 2.

Finally, the study using the fractions collected by Sephadex G-100 gel filtration of the *Biomphalaria* snails antigens to produce precipi-

tation of the anti SLM and BH *S. mansoni* adult worms sera showed that most of the common antigens are present in the first fraction and that the precipitin bands of the second fraction seemed to be offshoot of the first one. Therefore, most common antigens in the first fraction were estimated to have molecular weight over 158,000 daltons.

RESUMO

Estudos imunoelétricos sobre antígenos comuns entre vermes adultos de *Schistosoma mansoni*, cepas de São Lourenço da Mata e de Belo Horizonte, e moluscos *Biomphalaria*.

Estudos sobre antígenos comuns entre vermes adultos do *S. mansoni*, das linhagens SLM (São Lourenço da Mata, Pernambuco, Brasil) e BH (Belo Horizonte, Minas Gerais, Brasil), e moluscos das espécies *Biomphalaria glabrata* albino (Belo Horizonte) pigmentado (Pernambuco), e *Biomphalaria straminea* (Pernambuco) foram realizados mediante o emprego da técnica de imunoelétrforese.

Com relação à antigenicidade comum entre o verme adulto da linhagem SLM do *S. mansoni* e vários caramujos *Biomphalaria*, verificou-se que o verme adulto produziu 8 a 9 bandas de precipitação contra o *B. glabrata* pigmentado, 3 bandas contra *B. glabrata* albino e 1 banda contra o *B. straminea*. A linhagem BH por sua vez produziu 6 a 7 bandas contra o *B. glabrata* pigmentado, 5 bandas contra *B. glabrata* albino e 1 banda contra o *B. straminea*.

Extratos brutos de moluscos *Biomphalaria* foram fracionados por filtração em gel Sephadex G-100 e as três frações coletadas foram testadas por imunoelétrforese contra soros antivermes adultos das linhagens SLM e BH produzidos em coelhos. A antigenicidade comum entre antígeno de vermes adultos foi demonstrada principalmente na primeira fração revelando massa molecular acima de 158.000 daltons.

Em nosso laboratório, a relação entre a antigenicidade comum e o índice de infecção experimental para moluscos *Biomphalaria* tem demonstrado haver uma relação entre as bandas observadas com a maior suscetibilidade.

ACKNOWLEDGMENTS

The authors are greatly indebted to Prof. Tsutomu Takeuchi, Keio University, for his cooperation throughout this research and Prof. Moriyashi Tsuji and Luiz Carvalho for their comments. This study was supported by Japan International Cooperation Agency (JICA).

REFERENCES

1. BIGUET, J.; CAPRON, A. & TRAN VAY KY, P.- Les antigènes de *Schistosoma mansoni*. *Ann. Inst. Pasteur*, **103**: 763-777, 1962.
2. CAPRON, A.; BIGUET, J.; ROSE, F. & VERNES, A. - Les antigènes de *Schistosoma mansoni*. *Ann. Inst. Pasteur*, **105**: 789-810, 1965.
3. CORREA, M.C.; COELHO, P.M. & FREITAS, J.R. - Susceptibilidade de linhagens de *Biomphalaria tenagophila* e *Biomphalaria glabrata* a duas cepas de *Schistosoma mansoni*. *Rev. Inst. Med. trop. S. Paulo*, **21**: 72-76, 1979.
4. DIAS, L.C.S.; UETA, M.T. & GUARALDO, A.M.A. - Susceptibilidade de *Biomphalaria glabrata*, *B. straminea* e *B. tenagophila* a diferentes cepas de *Schistosoma mansoni*. *Rev. Inst. Med. trop. S. Paulo*, **29**: 205-212, 1987.
5. GONÇALVES, J.F.; NASCIMENTO, L.M.; AMÂNCIO, F.F. & SANTANA, J.V. - Susceptibility of *Biomphalaria glabrata* to São Lourenço da Mata and Belo Horizonte strains of *Schistosoma mansoni*. *Mem. Inst. Oswaldo Cruz*, **84** (Suppl): 250, 1989.
6. GRABAR, P. & WILLIAMS, C.A. - Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immuno-chimiques d'un mélange de protéines. Application au sérum sanguin. *Biochim. Biophys. Acta (Amst.)*, **10**: 193-194, 1953.
7. IWANAGA, Y.; KATAYAMA, S. & TSUJI, M. - Studies on host parasite relationship between *Schistosoma japonicum* and *Oncomelania* snails. *Jap. J. Parasit.*, **35**: 243-248, 1986.
8. IWANAGA, Y. & TSUJI, M. - Studies on host parasite relationship between *Schistosoma japonicum* and *Oncomelania* snails. *Jap. J. Parasit.*, **34**: 1-6, 1985.
9. IWANAGA, Y.; TSUJI, M. & TANAKA, N. - Studies on antigenic communities between the Yamanashi and Chinese strains of *Schistosoma japonicum* eggs and *Oncomelania* snails by immunoelectrophoresis. *Hiroshima J. med. Sci.*, **37**: 151-155, 1988.
10. MAGALHÃES, L.A. - Estudo dos dados obtidos de uma população de *Biomphalaria glabrata* de Belo Horizonte infectada por *Schistosoma mansoni* da mesma cidade, e de uma população de *B. tenagophila* de Campinas, infectada por *S. mansoni* de São José dos Campos. *Rev. Soc. bras. Med. trop.*, **3**: 195-196, 1966.
11. PARAENSE, W.L. & CORREA, L.R. - Susceptibility of *Australorbis tenagophilus* to infection with *Schistosoma mansoni*. *Rev. Inst. Med. trop. S. Paulo*, **5**: 23-29, 1963.
12. POLLOM, T.E.V.; MACHADO, F.R. & COELHO, P.M.Z. - Qualitative studies on the evolutive process of LE and SJ *Schistosoma mansoni* strains in *Biomphalaria glabrata*. *Mem. Inst. Oswaldo Cruz*, **84** (suppl): 249, 1989.
13. TSUJI, M. - On the immunoelectrophoresis for helminthological researches. *Jap. J. Parasit.*, **23**: 335-345, 1974.
14. TSUJI, M. - Comparative studies on the antigenic structure of several helminths by immunoelectrophoresis. *Jap. J. Parasit.*, **24**: 227-236, 1975.

Recebido para publicação em 18/1/1991
Aceito para publicação em 29/11/1991