STUDIES ON HOST-PARASITE RELATIONSHIP BETWEEN THE PUERTO RICAN STRAIN OF *SCHISTOSOMA MANSONI* AND *BIOMPHALARIA* SNAILS

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Abstract. Immunoelectrophoretic studies on common antigenicities were carried out by using rabbits sera immunized with the Puerto Rican strain of *Schistosoma mansoni* adult worms or eggs and antigens of several adult *Biomphalaria* snails and *vice versa*. As the result, *S. mansoni* adult worm extracts produced 8 bands both with extracts of *Biomphalaria glabrata* pigmentation and *B. glabrata* pigmentado, 3 to 4 bands with those of *B. glabrata* albino and 1 to 2 bands with those of *B. straminea*. On the other hand, *S. mansoni* egg extracts produced 5 bands with extracts of *B. glabrata* pigmentation, 4 bands with those of *B. glabrata* pigmentado, 2 bands with those of *B. glabrata* albino and 1 band with those of *B. straminea*. In the experimental infection of adult *Biomphalaria* snails with five *S. mansoni* miracidia, the infection rate in *B. glabrata* pigmentation was 78.8%, and 71.2% in *B. glabrata* pigmentado, whereas the infection rate in *B. glabrata* albino was 10.3%, and *B. straminea* was not susceptible to *S. mansoni*. The infectivity of each snail corresponded with the number of bands representing common antigenicities between host and parasite.

Crude antigens of *Biomphalaria* snails were fractionated by Sephadex G-100 column, and each antigen fraction was tested with anti-*S. mansoni* adult worm and egg sera by immunoelectrophoresis. The common antigenicities between fractionated antigens of *Biomphalaria* snails and of ani-*S. mansoni* adult worm or egg sera mostly existed in the first fraction 1 with Mr > 45 kDa.

INTRODUCTION

The Puerto Rican strain of *Schistosoma mansoni* showed different degrees of infectivity to various snail species of *Biomphalaria* (Files and Cram, 1949; Kagan and Geiger, 1965). Saoud (1965) reported that the strain was susceptible to *Biomphalaria glabrata*, but not susceptible to *Biomphalaria alexandria alexandria*. On the other hand, the infectivity of the Chinese strain of *S. japonicum* to *Oncomelania hupensis hupensis* has been reported to be high, while the other *Oncomelania* snails showed less infectivity to the *Schistosome* strain (Iwanaga and Tsuji, 1982 a, b), although there are no distinct differences of morphological features among *Oncomelania* snails. These findings suggest that there exist basic physiological and biochemical differences among these snails. In previous studies with common antigenicities between the Belo Horizonte strain, Brazil of *S. mansoni* and *Biomphalaria* snails (Iwanaga *et al*, 1992; Santana *et al*, 1992), a relationship was found 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*.

The present study deals with common antigenicities between the Puerto Rican strain of *S. mansoni* and several *Biomphalaria* snails and the infectivity among them.

MATERIALS AND METHODS

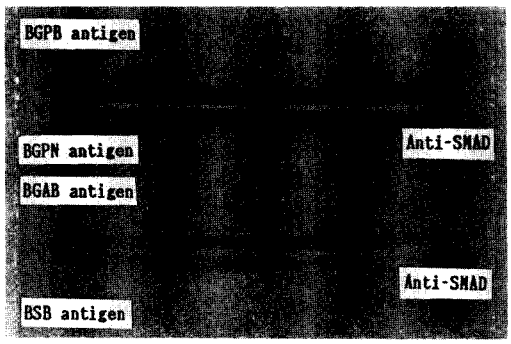
Strains of *Schistosoma mansoni* and *Biomphalaria* snails

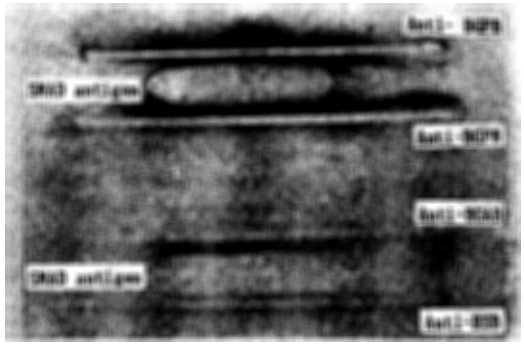
The strain of *Schistosoma mansoni* used in this study originated from Puerto Rico, and the cycle has been maintained by passage through *Biomphalaria glabrata* pigmentation, Puerto Rican strain and Swiss albino mice. Strains of *Biomphalaria* snails were collected from the following areas: *B. glabrata* pigmentado from Jaboatao in Brazil, *B. glabrata* albino

from Belo Horizonte in Brazil, *B. straminea* from São Lourenco da Mata in Brazil, and *B. glabrata* pigmentation from Puerto Rico via NIH in USA. Pigmented *B. glabrata* from Brazil and Puerto Rico were common wild types pigmented with black pigment in body, eyes and mantle collar.

Preparation of antigens and antisera

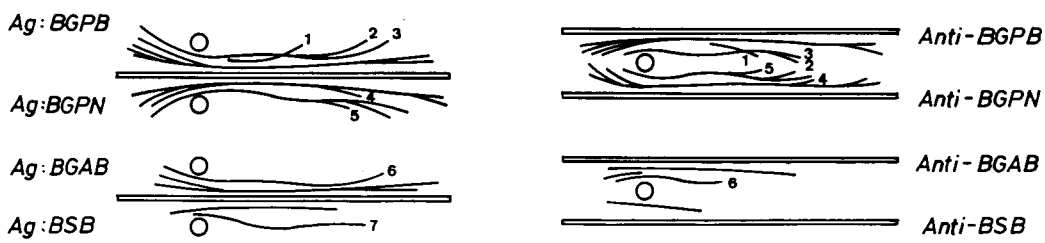
As antigens, 0.1% NaCl extracts of *S. mansoni* adult worms and eggs and whole body of adult *Biomphalaria* snails were prepared as described previously (Tsuji, 1974; Tsuji *et al,* 1978; Iwanaga *et al,* 1988). *Biomphalaria* snails were also fractionated by gel filtration using Sephadex G-100 column chromatography to estimate the molecular weights of molecules which react to anti-*S. mansoni* adult worm or egg sera according to Iwanaga and Tsuji (1985).





SMAD : *Schistosoma mansoni* adult worm
BGPB : *Biomphalaria glabrata* pigmentado (Brazil)
BGPN : *Biomphalaria glabrata* pigmentation (NIH)
BGAB : *Biomphalaria glabrata* albino (Brazil)
BSB : *Biomphalaria straminea* (Brazil)

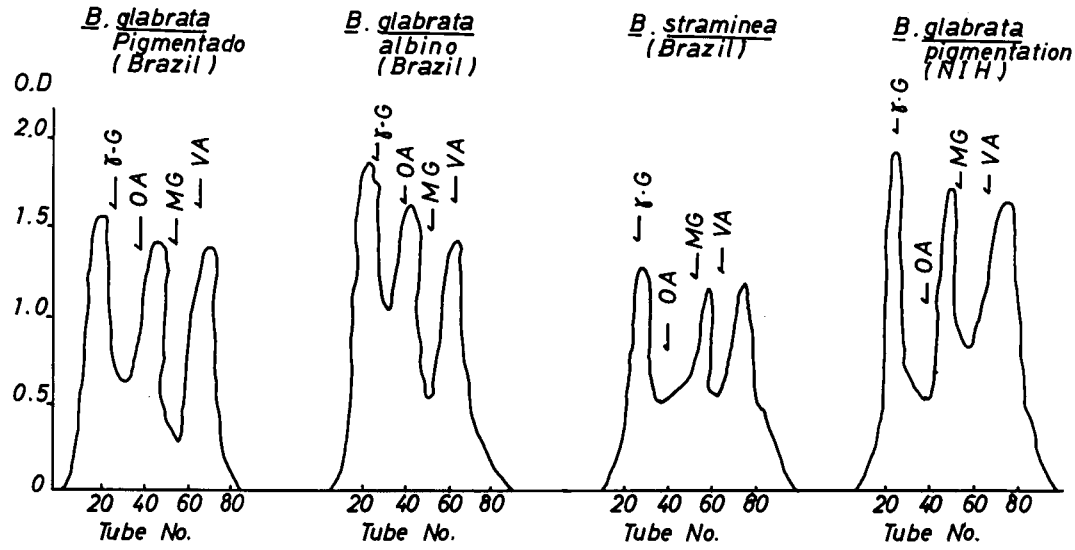
Fig 1–Immunoelectrophoresis between various *Biomphalaria* snails and Puerto Rican strain of *Schistosoma mansoni* adult worms.



Bands 1-7 : Precipitin bands were not recognized between the snail and the Belo Horizonte strain of *Schistosoma mansoni* adult worms.

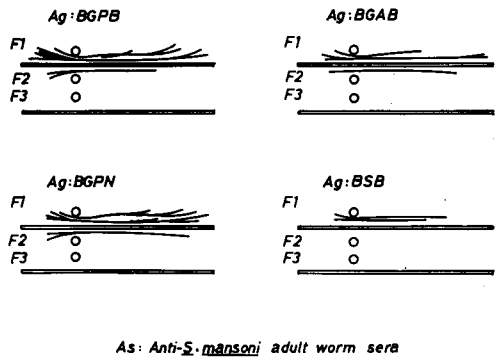
BGPB : *Biomphalaria glabrata* pigmentado (Brazil)
BGPN : *Biomphalaria glabrata* pigmentation (NIH)
BGAB : *Biomphalaria glabrata* albino (Brazil)
BSB : *Biomphalaria straminea* (Brazil)

Fig 2–Immunoelectrophoregrams between various *Biom-phalaria* snails and *Schistosoma mansoni* adult worms.



The column was calibrated with γ-globulin (γ-G MW 158,000), ovalbumin (OA MW 45,00), myoglobine (MG MW 17,000) and vitamin B-12 (VA MW 1,350)

Fig 3–Column chromatograms of various antigens of *Biomphalaria* snails on Sephadex G-100 (OD at 280 nm).



BGPB : *Biomphalaria glabrata* pigmentado (Brazil)

BGPN : *Biomphalaria glabrata* pigmentation (NIH)

BGAB : *Biomphalaria glabrata* albino (Brazil)

BSB : *Biomphalaria straminea* (Brazil)

F1 - F3 : Fraction number

Fig 4–Immunoelectrophoregrams between anti *Schistosoma mansoni* adult worms and fractionated antigens of *Biomphalaria* snails.

Antisera were prepared by the following method; emulsion containing 2 mg of each antigen in Freund's complete adjuvant (Difco Lab, Detroit, USA) were injected into the proximal limbs of rabbits ten times every week, and antisera were obtained from these rabbits ten days after the final injection (Tsuji and Yokogawa, 1974; Iwanaga *et al,* 1988).

Immunoelectrophoresis

Immunoelectrophoresis was done according to the technique of Tsuji (1974) on 0.9% agarose L (Behringwerke, AG, Germany) in veronal buffered saline (pH 8.2) and the electric current was adjusted to 18±2V/8cm length within a gel and applied for 3 hours.

Infectivity of *Biomphalaria* snails to *S. mansoni*

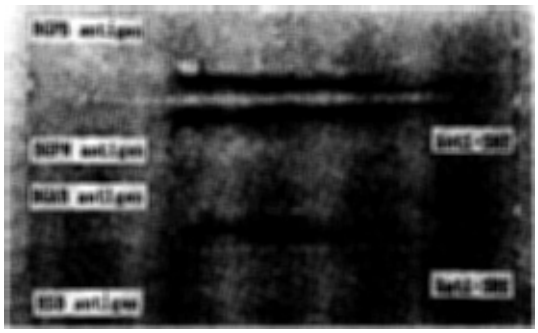
Adult snails were exposed individually to 5 or 10 miracidia in 10 - 15 ml beakers for 24 hours. Exposed snails were maintained in soil-filtrated aquaria (30cm × 20cm × 30 cm) at 26°C. Three weeks after exposure to miracidia, the snails were tested for cercarial emergences. Snails were examined weekly for additional seven weeks. Snails without shedding cercariae were dissected and examined for sporocystsand cercariae at week 10.

Table 1

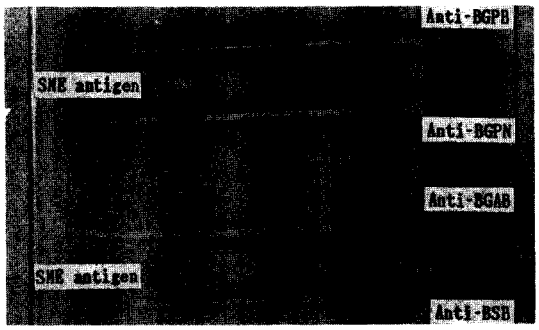
Infection rates of *Biomphalaria* snails exposed to *Schistosoma mansoni* miracidia, Puerto Rican strain.

Adult snails	5 miracidia/snail			10 miracidia/snail		
	A	B(%)	C(%)	A	B(%)	C(%)
B. glabrata pigmentado	125	89 (71.2)	7 (5.6)	95	70 (73.7)	10 (10.0)
B. glabrata albino	87	9 (10.3)	10 (11.5)	65	5 (7.7)	10 (15.4)
B. straminea	111	0 (0.0)	6 (5.4)	50	0 (0.0)	2 (4.0)
B.glabrata pigmentation	245	193 (78.8)	15 (6.1)	189	154 (81.5)	28 (14.8)

A : No. of snails
B : No. of snails infected
C : No. of snails died

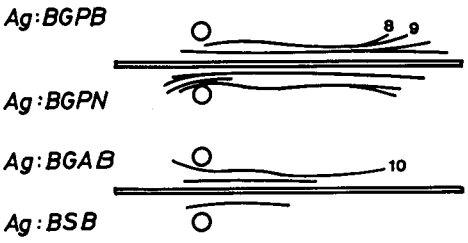


SME: *Schistosoma mansoni* egg
BGPB : *Biomphalaria glabrata* pigmentado (Brazil)
BGPN : *Biomphalaria glabrata* pigmentation (NIH)

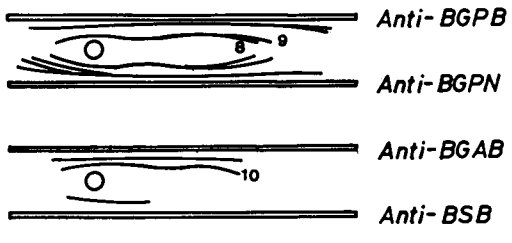


BGAB : *Biomphalaria glabrata* albino (Brazil)
BSB : *Biomphalaria straminea* (Brazil)
SME: *Schistosoma mansoni* egg

Fig 5–Immunoelectrophoresis between various *Biomphalaria* snails and Puerto Rican strain of *Schistosoma mansoni* eggs.

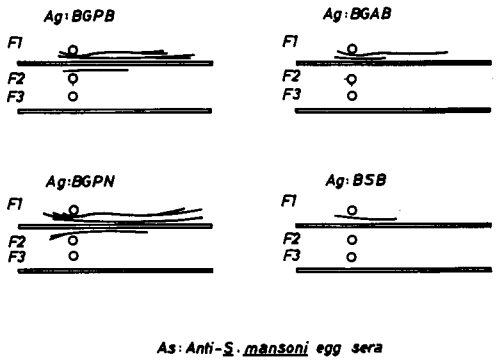


Bands 8 - 10: Precipitin bands were not recognized between the snail and the Belo Horizonte strain of *Schistosoma mansoni* eggs



BGPB : *Biomphalaria glabrata* pigmentado (Brazil)
BGPN : *Biomphalaria glabrata* pigmentation (NIH)
BGAB : *Biomphalaria glabrata* albino (Brazil)
BSB : *Biomphalaria straminea* (Brazil)

Fig 6–Immunoelectrophoregrams between various *Biom-phalaria* snails and *Schistosoma mansoni* eggs.



BGPB : *Biomphalaria glabrata* pigmentado (Brazil)

BGPN : *Biomphalaria glabrata* pigmentation (NIH)

BGAB : *Biomphalaria glabrata* albino (Brazil)

BGS : *Biomphalaria straminea* (Brazil)
F1 - F3: Fraction number

Fig 7–Immunoelectrophoregrams between anti-*Schistosoma mansoni* eggs and fractionated antigens of *Biomphalaria* snails.

RESULTS

Common antigenicities between *S. mansoni* adult worms and *Biomphalaria* snails

As shown in the immunoelectrophoresis and immunoelectrophoretic diagrams in Figs 1 and 2, anti-*S.mansoni* adult worm serum produced 8 bands against both *B.glabrata* pigmentado and *B. glabrata* pigmentation antigens, 4 bands against *B. glabrata* albino antigen and 2 bands against *B. straminea* antigen. In the reverse experiments, *S. mansoni* adult worm antigen showed 8 bands with anti -*B. glabrata* pigmentado and with *B. glabrata* pigmentation serum, 3 bands with anti-*B. glabrata* albino serum and 1 band with anti-*B. straminea* serum.

In column chromatography of 0.1% NaCl extracts antigens of *B. glabrata* pigmentation, three fractions were identified as shown in Fig 3; other *Biomphalaria* snails were also fractionated into three fractions as described previously (Iwanaga *et al,* 1992). With regard to the common antigenicities between anti *S. mansoni* adult worm sera and fractionated antigens of *Biomphalaria* snails, the first fraction of *B. glabrata* pigmentado and *B. glabrata* pigmentation antigens produced 8 bands against adult worm sera, and 2 bands were detected in the second fraction. *B. glabrata* albino antigen produced 4 bands in the first fraction and 1 band in the second fraction. However, the third fraction did not produce any bands for *B. glabrata* pigmentado, *B. glabrata* pigmentation and *B. glabrata* albino. The first fraction of *B. straminea* antigen produced 2 bands as shown in Fig 4, but the precipitin bands in the second fraction seemed to an offshoot on the first fraction. Therefore, most common antigenicities may exist in the first fraction. The first fraction was estimated to have a molecular weight > 45 kDa.

Common antigenicities between *S. mansoni* eggs and *Biomphalaria* snails

The immunoelectrophoresis and immunoelectrophoretic diagrams are shown in Figs 5 and 6. Anti-*S. mansoni* egg serum produced 4 bands against *B. glabrata* pigmentado antigen, 5 bands against *B. glabrata* pigmentation antigen, 2 bands against *B. glabrata* albino antigen and 1 band against *B. straminea* antigen. The reverse experiments, using anti-*Biomphalaria* snails sera against *S. mansoni* egg antigen, showed the same results with those of anti-*S. mansoni* egg serum and antigens of *Biomphalaria* snails. Common antigenicities of anti-*S. mansoni* egg serum and fractionated antigens of *Biomphalaria* snails are shown in Fig 7. *B. glabrata* pigmentado antigen produced 4 bands in the first fraction, 1 band in the second fraction against anti-*S. mansoni* egg sera. *B. glabrata* pigmentation antigen produced 5 bands in the first fraction, 2 bands in the second fraction. *B. glabrata* albino and *B. straminea* antigens produced only 2 and 1 band in the first fraction, respectively.

Infectivity of *Biomphalaria* snails to *S.mansoni*

The results of experimental infection are summarized in Table 1. The infection rate of *S. mansoni* to *B. glabrata* pigmentation exposed individually to 5 miracidia was 78.8%, similarly, 71.2% for *B. glabrata* pigmentado and 10.3% for *B. glabrata* albino, but *B. straminea* were not found to be susceptible to *S. mansoni.* The snails exposed to 10 miracidia showed almost the same infection rates as those exposed to 5 miracidia. The mortalities of *B. glabrata* pigmentation, *B. glabrata* pigmentado and *B. glabrata* albino exposed individually to 5 miracidia showed 6.1%,

5.6% and 11.5%, respectively. Mortalities, however, exposed individually to 10 miracidia were higher than those of 5 miracidia group.

DISCUSSION

Physiological and chemical studies on communities of parasites and intermediate hosts have been done for a long time. Jackson and Moor (1976) reported that sera from *S. haematobium* infected individuals showed higher antibody titers to the antigens of suitable intermediate hosts than non-infected individuals. Marrero and Hiller (1985) found that sera from humans infected with *S. mansoni* cross-reacted with *B. glabrata* soluble antigens. It seems this kind of study can be useful for the diagnosis of parasitic diseases. Several investigators have reported that genetic variation of *Biomphalaria* snails influenced susceptibility of the host snails to *S. mansoni* (Newton, 1953; Paraense and Correa, 1963; Richards and Merritt, 1972; Richards, 1984), but the interaction of parasite and snail host are not yet clearly explained. The immunological approach to studies of hosts and parasites has been carried out by immunoelectrophoresis. Capron *et al* (1965) reported that there were five common antigenic fractions between *S. mansoni* and the livers of infected hamsters. Tsuji and Yokogawa (1972) demonstrated common antigens between several helminths and their suitable intermediate hosts. Therefore, common antigenicities between *S. mansoni* and *Biomphalaria* snails may be basic concepts in studies on host-parasite relationships.

In this study, common antigencities between the Puerto Rican strain of *S. mansoni* and several strains of *Biomphalaria* snails were assessed by immunoelectrophoresis. It was found that both strains of pigmented *B. glabrata* produced more common bands than those of *B. glabrata* albino and *B. straminea* to *S. mansoni* adult worms and eggs. In experimental infections of *Biomphalaria* snails by *S. mansoni,* both strains of pigmented *B. glabrata* showed highly susceptibility, *B. glabrata* albino was much less susceptible, and *B. straminea* was completely refractory. These infection rates are almost parallel with the number of bands representing common antigenicities between hosts and parasites, that is, more bands were seen with increasing infection rates of *S. mansoni.* This observation agreed with the reports that *B. glabrata* (*B. glabrata* pigmentado and *B. glabrata* albino) which showed high infection rates to the Belo Horizonte strain, Brazil of *S. mansoni* produced many common antigencities against *S. mansoni* adult worm and egg (Santana *et al,* 1992; Iwanaga *et al,* 1992). Saoud (1965) reported that pigmented *B. glabrata* from Puerto Rico were highly susceptible (96%) when exposed to the same strain of *S. mansoni,* but pigmented *B. glabrata* from Brazil showed a moderate susceptibility (37.5%) to the Puerto Rican strain of *S. mansoni.* In this study, the pigmented *B. glabrata* from Brazil was highly susceptible to the Puerto Rican strain of *S. mansoni,* and the result did not agree with the report of Saoud (1965).

Compared with precipitin bands, *ie* common antigenicities, between Brazilian *Biomphalaria* snails and the Puerto Rican and/or Belo Horizonte strains of *S. mansoni* adult worms (Santana *et al,* 1992), some precipitin bands showed different patterns, that is, bands 1 to 3 were not recognized as the precipitin bands between *B. glabrata* pigmentado and the Belo Horizonte strain; similiarly, bands 6 and 7 were not identified as precipitin bands between *B. glabrata* albino and *B. straminea* and the Belo Horizonte strain, respectively (Fig 2). Regarding precipitin bands between Brazilian *Biomphalaria* snails and the both strains of *S. mansoni* eggs, as shown in Fig 6, bands 8 to 9 and band 10 were not recognized as precipitin bands between *B. glabrata* pigmentado and *B. glabrata* albino and the Belo Horizonte strain, respectively (Iwanaga *et al,* 1992). On the other hand, precipitin bands between *B. glabrata* pigmentation and the both strains of *S. mansoni* adult worm, bands 4 and 5 as shown in Fig 2, were not recognized as the precipitin bands between the snails and the Belo Horizonte strain (unpublished data). These findings suggest that both Puerto Rican and Belo Horizonte strains of *S. mansoni* are physiologically and/or biochemically different from one another. It is necessary that common antigenicities between *S. mansoni* and *Biomphalaria* snails be characterized using SDS-PAGE and/or Western blotting techniques.

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REFERENCES

Capron A, Biguet J, Rosé F, Vernes A. Les antigéns de *Schistosoma mansoni*. 2. Étude immunoelectrophorétique comparée. De divers stades larvaires et des adultes des deux sexes aspects immunologiques des relations hôte-parasite de la cercarie et de l'adulte de *S. mansoni*. *Ann Inst Pasteur* 1965; 105 : 798-810.

Files VS, Cram EB. A study of the comparative susceptibility of snail vectors to strain of *Schistosoma mansoni*. *J Parasitol* 1949; 35 : 555-60.

Iwanaga Y, Tsuji M. Observation on the infection of *Oncomelania* spp to *Schistosoma japonicum*. (5) The susceptibility of laboratory colonies of *Oncomelania hupensis hupensis* to *S. japonicum*, Chinese strain. *Med J Hiroshima Univ* 1982 a; 30 : 787-90 (Jpn).

Iwanaga Y, Tsuji M. Observation on the infection of *Oncomelania* spp to *Schistosoma japonicum*. (6) The susceptibility of laboratory colonies of *Oncomelania* spp of the different geographical strains to *S. japonicum*, Chinese strain. *Med J Hiroshima Univ* 1982 b; 30 ; 791-6 (Jpn).

Iwanaga Y, Tsuji M. Studies on host-parasite relationship between *Schistosoma japonicum* and *Oncomelania* snails. *Jpn J Parasitol* 1985; 34 : 1-6.

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* 1988; 37 : 151-5.

Iwanaga Y, Santana JV, Goncalves JF. Studies on common antigenicities between the Belo Horizonte strain, Brazil of *Schistosoma mansoni* eggs and *Biomphalaria* snails by immunoelectrophoresis. *Southeast Asian J Trop Med Public Health* 1992; 23 : 98-102.

Jackson TFHG, Moor PP. A demonstration of the presence of anti-snail antibodies in individuals infected with *Schistosoma haematobium*. *J Helminthol* 1976; 50 : 59-63.

Kagan IG, Geiger S. The susceptibility of three strains of *Australorbis glabrata* to *Schistosoma mansoni* from Brazil and Puerto Rico. *J Parasitol* 1965; 51 : 622-7.

Marrero CAR, Hillyer GV. Isolation and partial characterization of shared antigens of *Biomphalaria glabrata* and *Schistosoma mansoni* and their evaluation by the ELISA and the EITB. *J Parasitol* 1985; 71 : 547-55.

Newton WL. The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabrata*. *Exp Parasitol* 1953; 2 : 242-57.

Paraense WL, Correa LR. Variation in susceptibility of populations of *Australorbis glabrata* to a strain of *Schistosoma mansoni*. *Rev Inst Med Trop São Paulo* 1963; 5 : 15-22.

Richards CS. Influence of snail age on genetic variations in susceptibility of *Biomphalaria glabrata* for infection with *Schistosoma mansoni*. *Malacologia* 1984 ; 25 : 493-502.

Richards CS, Merritt JW. Genetic factors in the susceptibility of juvenile *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *Am J Trop Med Hyg* 1972; 21 : 425-34.

Santana JV, Iwanaga Y, Telles AMS, Silva MR, Goncalves JF, Tateno S. Immunoelectrophoretic study on common antigens of São Lourenco da Mata and Belo Horizonte strains of *Schistosoma mansoni* adult worms and *Biomphalaria* snails. *Rev Inst Med Trop São Paulo* 1992; 34 : 49 -54.

Saoud MFA. Susceptibilities of various snail intermediate hosts of *Schistosoma mansoni* to different strains of the parasite. *J Helminthol* 1965; 39 : 365-76.

Tsuji M. On the immunoelectrophoresis for helminthological researches. *Jpn J Parasitol* 1974; 23 : 335-45 (Jpn).

Tsuji M, Yokogawa M. Studies on immuno-diffusion tests of *Schistosoma japonicum*. *Res Filariasis Schistosomiasis* 1972; 2 : 165-77.

Tsuji M, Yokogawa M. Immunological diagnosis of helminthic infection. SEAMEO TROPMED Technological Meeting 1974; 180 : 219.

Tsuji M, Iwanaga Y, Kohno E, Haizuka T, Iwasaki H. Immunoelectrophoretical studies on antigenic communities between *Schistosoma japonicum* and *Oncomelania* snails. *Res Filariasis Schistosomiasis* 1978; 3 : 39-54.