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Comparative Studies for Development of *Schistosoma mansoni* Sporocysts in Puerto Rican and Brazilian *Biomphalaria glabrata*

Yuzuru IWANAGA[1], Masanobu TANABE[2] and Tsutomu TAKEUCHI[2]

1) *Department of Immunology and Parasitology, Hiroshima University School of Medicine, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan*
2) *Department of Tropical Medicine and Parasitology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160–0016, Japan*

ABSTRACT

The development of sporocysts of *Schistosoma mansoni* was monitored in pigmented and albino *Biomphalaria glabrata* from Puerto Rico and Brazil. The snails were exposed individually to 20 miracidia, and sporocysts were allowed to develop for 3 to 12 weeks. Most of the immature sporocysts were found in the seminal receptacle sac and vas deferens during development. In contrast, mature daughter sporocysts were detected everywhere except in the foot at 12 weeks after exposure to the miracidia. It was found that mature daughter sporocysts formed more rapidly in the pigmented than in the albino snails, but no difference was observed in the formative time between the same types of Puerto Rican and Brazilian snails. It seems likely that there is a correlation between the infection rate and the time required for formation of mature daughter sporocysts in *B. glabrata.*

Key words: *Schistosoma mansoni, Sporocysts, Biomphalaria glabrata*

Biomphalaria snails serve as intermediate hosts for *Schistosoma mansoni*. It is well known that the Puerto Rican strain of *S. mansoni* shows different degrees of infectivity to various species of *Biomphalaria*[1,4,6,7,12]. Although the larval development of *S. mansoni* in *Biomphalaria* has been described[7,11,13], few detailed observations exist on the relationship between the location of sporocysts and cercarial maturation time in snails.

The present paper examines this relationship using the Puerto Rican strain of *S. mansoni* and the pigmented and albino types of Puerto Rican and Brazilian snails.

MATERIALS AND METHODS

Parasite and snails

In this study, the Puerto Rican strain of *S. mansoni* was used. The strain was maintained in our laboratory by passage through the most suitable host snails and Swiss albino mice. Pigmented and albino types of Puerto Rican and Brazilian *B. glabrata* snails were used as intermediate hosts. The snails were reared in our laboratory by the modified method of Iwanaga and Tsuji[3]. The Puerto Rican strains of *B. glabrata*, pigmented (BG. P. PR.) and albino (BG. A. PR.), were obtained from Keio University, Japan. The Brazilian strains of snails were from the following areas in Brazil: *B. glabrata* pigmented from Jaboatao, Pernambuco (BG. P. BR.) and *B. glabrata* albino from Belo Horizonte, Minas Gerais (BG. A. BR.). Only adult snails were used.

Distribution of sporocysts

The experimental snails were divided into two groups: the first group consisting of snails in which mature daughter sporocysts, including mature cercariae, developed; the second group consisting of snails in which neither mature daughter sporocysts nor mature cercariae developed. To localize the sites in the snails where the parasite developed, the snails were divided into four regions as shown in Fig. 1: the foot (region 1), seminal receptacle sac and vas deferens (region 2), prostate gland and oviduct (region 3), and ovotestis and digestive gland (region 4). The snails

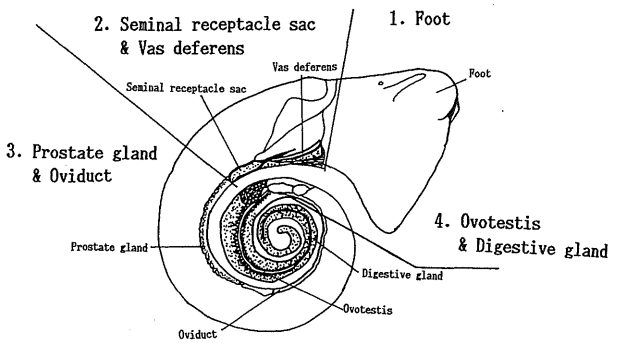

Fig. 1. Diagram showing *Biomphalaria glabrata*, divided into regions

were individually exposed to 20 miracidia, and 3 snails each were examined 3, 5, 7, 9, and 12 weeks later. Examination of some snails was with a dissecting microscope; other snails were fixed in Bouin's, serially sectioned at 8–10μ, and stained with hematoxylin-eosin for histological observation.

Maturation time of cercariae

50 snails were individually exposed to 10 miracidia in 10–15 ml beakers for 24 hours. These snails were maintained in soil-filtered aquaria (30cm × 20cm × 30cm) at 26°C. The shedding of cercariae was monitored daily for 2 to 12 weeks after miracidial exposure. Snails not shedding cercariae were dissected and examined for sporocysts and cercariae at week 13.

RESULTS

Distribution of sporocysts in snails

Fig. 2 shows the distribution of immature and mature daughter sporocysts in the snails. There was a slight difference in distribution between the immature and mature daughter sporocysts. The majority of immature sporocysts were detected at 3 weeks postexposure (*wpe*) in region 2, where they generally remained during the experiment. Only one sporocyst each was found in region 1 at 3 and 7 *wpe*, but no sporocysts reached region 4 until the end of the experiment. The number of sporocysts recovered in each region decreased towards the ninth *wpe*, and appeared to decrease more rapidly in the albino type snails than in the pigmented type. Histological examination of sporocysts was at 3, 7 and 9 *wpe*. The sporocysts, which appeared to be developing normally at 3 *wpe*, consisted of a thin walled sac containing widely-dispersed germinal cells. A host tissue response was not noted (Fig. 3). At 7 *wpe*, a few degenerating sporocysts were observed in regions 2 and 3, and a definite host tissue reaction was seen around the sporocysts (Fig.4). At 9 *wpe* and later, many destroyed sporocysts were found in regions 2 and 3, and they were being resorbed by accumulations of amoebocytes (Fig. 5). Mature daughter sporocysts were detected in all snails at 5 *wpe* (Fig.2). Although sporocysts in the pigmented types of both strains of snail were detected in regions 3 and 4, those in the albino type were found only in region 3 at 5 *wpe*. At 9 *wpe*, mature daughter sporocysts in the pigmented types were recovered from regions 2, 3 and 4, but those in the albino types were not found in region 2. At 12 *wpe*, mature daughter sporocysts were observed in regions 2, 3 and 4 in all snails. However, no mature daughter sporocyst was recovered from region 1 in any snail during the experiment.

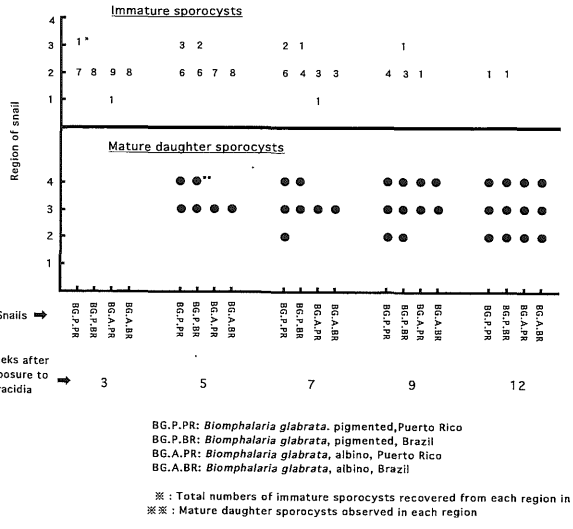

Fig. 2. Distribution of immature and mature daughter sporocysts of *Schistosoma mansoni* in *Biomphalaria*

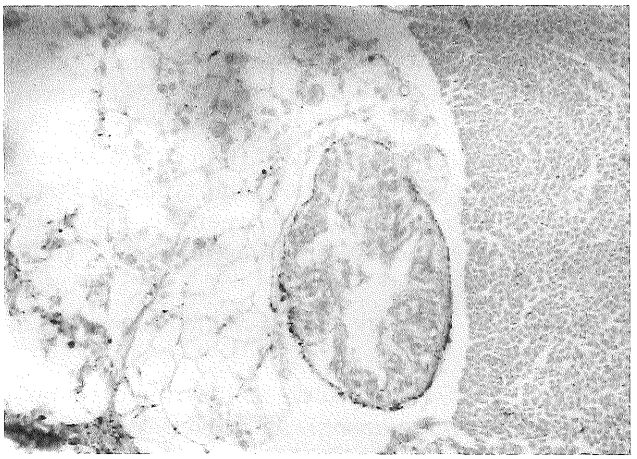

Fig. 3. Section of normally developing immature sporocyst※ in seminal receptacle sac and vas deferens of snail 3 weeks postexposure, hematoxylin-eosin stain, × 400

※ Sporocyst recovered from pigmented type snail, Puerto Rican strain

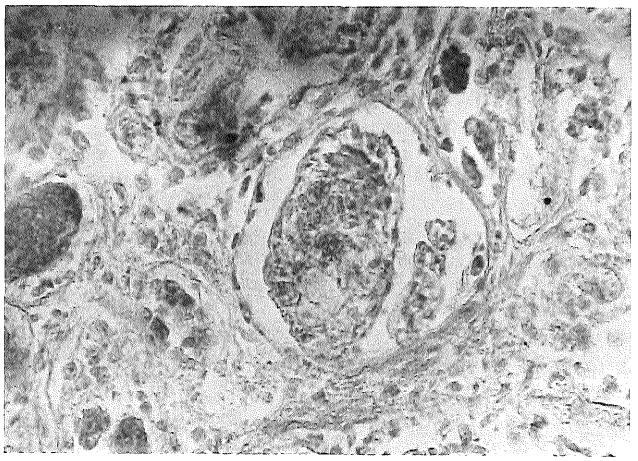

Fig. 4. Section of degenerating sporocyst※ in seminal receptacle sac and vas deferens of snail 7 weeks post-exposure, hematoxylin-eosin stain, × 400

※ Sporocyst recovered from pigmented type snail, Puerto Rican strain

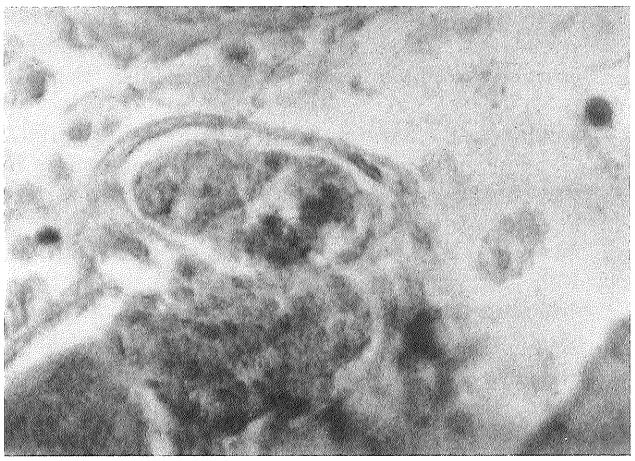

Fig. 5. Section of destroyed immature sporocyst※ in seminal receptacle sac and vas deferens of snail 9 weeks postexposure, hematoxylin-eosin stain, × 500
※ Sporocyst recovered from pigmented type snail, Puerto Rican strain

Table 1. Infection *and mortality rates of *Biomphalaria* snails exposed to Puerto Rican strain of *Schistosoma mansoni* miracidia.

Strain of Snail	Number of snails examined	Number of snails infected	Number of snails died
Puerto Rican			
B.glabrata (pigmented)	189	154 (81.5)	28 (14.8)
B.glabrata (albino)	168	43 (25.6)	19 (11.3)
Brazilian			
B.glabrata (pigmented)	150	111 (74.0)	16 (10.6)
B.glabrata (albino)	115	20 (17.4)	18 (15.7)

*: Infection rates obtained by exposure to 10 miracidia
( ): Infection rates and mortalities(%)
The data are extrapolated from reports of Iwanaga et al (1992, 1997)

Cercarial maturation time

Fig.6 shows the number of snails infected with *S. mansoni* at various times after exposure to miracidia. Cercariae were first detected in BG. P. PR., BG. A. PR., and BG. P. BR. at 4 *wpe*. Mature cercariae had formed by the 7th and 8th *wpe* in BG. P. PR. and BG. P. BR., respectively. The cercarial maturation time was shorter in the pigmented than in the albino type snails.

The infection and mortality rates of *S. mansoni*-infected *Biomphalaria* are summarized in Table 1. Snails suitable as a host for the Puerto Rican strain of *S. mansoni* were BG. P. PR. and BG. P. BR., both of which had a shorter cercarial maturation time and higher infection rate than the albino type snails. On the other hand, the albino type snails generally showed lower levels of infectivity and longer cercarial maturation times than the pigmented snails.

DISCUSSION

Almost all of the immature sporocysts were observed in the seminal receptacle sac and vas deferens (region 2) in the snails at 3 *wpe*, and appeared to be developing normally. This result agrees with the report by Sullivan and Richards[13], who found that sporocysts developed in snails without provoking a tissue response. As described previously by Pan[11], degenerating sporocysts were found 7 *wpe* in this experiment.

In the pigmented snails, most of the mature daughter sporocysts first localized in regions 3 and 4 at 5 *wpe*. During the experiment, the dense population of mature daughter sporocysts in the prostate gland and oviduct (region 3) of the snails was noteworthy. In general, sporocysts in the pigmented snails matured more rapidly in each region than in the albino snails.

The time necessary for formation of cercariae

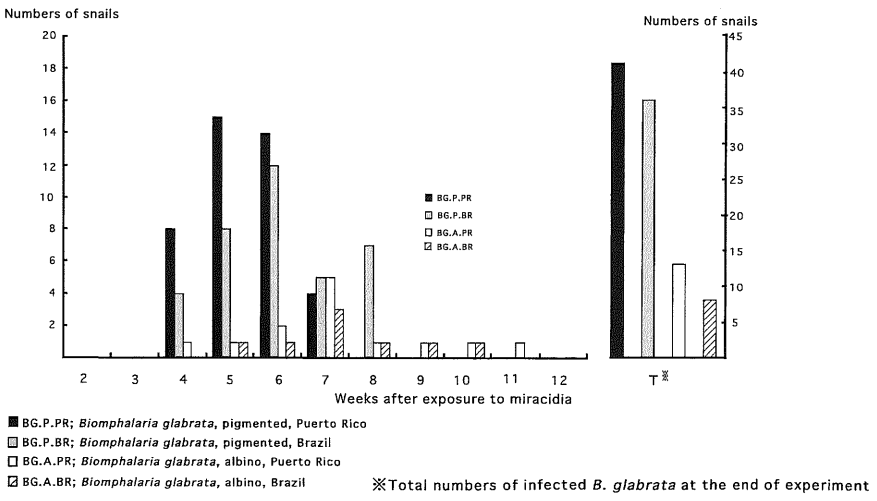

Fig. 6. Numbers of *Biomphalaria glabrata* infected with *Schistosoma mansoni* after exposure to miracidia

was 4 *wpe* in BG. P. PR., BG. A. PR., and BG. P. BR. snails, and 5 *wpe* in BG. A. BR. The results in BG. P. PR. and BG. P. BR. are in accord with the report by Kagan and Geiger[7]. The formation of mature daughter sporocysts was more rapid in the pigmented than in the albino snails.

The results of this investigation are comparable with a recent investigation by Iwanaga et al[5] who studied the correlation between infection rate and maturation time of *S. japonicum* cercariae in *Oncomelania hupensis*. The present results strongly suggest that there is a correlation among infection rate, the maturation time of cercariae, and the distribution of sporocysts in snails infected with *S. mansoni*. Iwanaga[2] reported the existence of different antigenic structures in strains of *Biomphalaria* snails from Brazil. Previously, genetic differences among *Biomphalaria* strains had also been reported[9,10]. For instance, Newton and von Brand[8] showed that physiological differences existed between *Australorbis glabrata* from Brazil and Puerto Rico. These physiological differences might account for the differences noted in parasite development.

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REFERENCCES

1. **Files, V.S. and Cram, E.S.** 1949. A study on the comparative susceptibility of snail vectors to strains of *Schistosoma mansoni*. J. Parasitol. **35:** 555–560.
2. **Iwanaga, Y.** 1992. Comparative studies of Brazilian species of *Biomphalaria* snails by immunoelectrophoresis. Jap. J. Malac. **4:** 315–321.
3. **Iwanaga, Y. and Tsuji, M.** 1972. Fundamental studies on laboratory breeding of *Oncomelania hupensis nosophora*. Hiroshima J. Med. Sci. **20:** 1–12.
4. **Iwanaga, Y., Tanabe, M. and Takeuchi, T.** 1997. Development of *Schistosoma mansoni* miracidia in Puerto Rican and Brazilian *Biomphalaria glabrata*. Parasitol. Int. **46 (Suppl):** 90.
5. **Iwanaga, Y., Lee, K.M., Fan, P.C. and Tsuji, M.** 1998. Comparative studies for development of three different geographic strains of *Schistosoma japonicum* cercariae in five subspecies of *Oncomelania hupensis*. Jap. J. Trop. Med. Hyg. **3:** 167–171.
6. **Iwanaga, Y., Tsuji, M., Tanabe, M. and Takeuchi, T.** 1992. Studies on host-parasite relationship between *Biomphalaria* snails and the Puerto Rican strain of *Schistosoma mansoni*. Jpn. J. Parasitol. **41 (Suppl):** 48.
7. **Kagan, I.G. and Geiger, S.J.** 1965. The susceptibility of three strains of *Australorbis glabrata* to *Schistosoma mansoni* from Brazil and Puerto Rico. J. Parasitol. **51:** 622–627.
8. **Newton, W.L. and von Brand, T.** 1955. Comparative physiological studies on two geographic strains of *Australorbis glabrata*. Exp. Parasitol. **4:** 244–255.
9. **Mulvey, M., Newman, M.C. and Woodruff, D.S.** 1988. Genetic differentiation among West India population of the schistosome-transmitting snails *Biomphalaria glabrata*. Malacologia. **29:** 309–317.
10. **Mulvey, M. and Vrijenhock, R.C.** 1984. Genetics of *Biomphalaria glabrata*: Linkage analysis and crossing compatibilities among laboratory strains. Malacologia. **25:** 513–524.
11. **Pan, C.T.** 1965. Studies on the host-parasite relationship between *Schistosoma mansoni* and the snails *Australorbis glabrata*. Am. J. Trop. Med. Hyg. **14:** 931–976.
12. **Saoud, M.F.A.** 1965. Susceptibilities of various snails intermediate hosts of *Schistosoma mansoni* to different strains of the parasite. J. Helminthology **4:** 363–376.
13. **Sullivan, J.T. and Richards, C.S.** 1981. *Schistosoma mansoni*, NIH-SM-PR-2 strain, in susceptible and nonsusceptible stocks of *Biomphalaria glabrata*; Comparative histology. J. Parasitol. **67:** 702–708.