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A quantitative study of *Schistosoma japonicum* egg production in ddY mice

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Abstract

This is a statistical study of the number of schistosome eggs produced by a female *Schistosoma japonicum* worm.

Each 4-week-old female ddY mouse was exposed percutaneously to 20 cercariae of *S. japonicum* (the Japanese strain). The number of eggs in the feces and in the tissues was counted up to 15 weeks postinfection (PI).

Female worms began to produce eggs in the liver at 4 weeks PI and schistosome eggs in the feces were first found between 36 and 42 days PI. The number of eggs increased in the liver dramatically until 6 weeks PI, but leveled off after that. On the other hand, the number of schistosome eggs in the intestine increased constantly until 15 weeks PI. At 15 weeks PI, about 83% of the deposited eggs were found in the small intestine, mainly in the proximal portion thereof. The mean cumulative number of eggs in the feces was about 250,000 at 15 weeks PI, 28.2% of all the produced eggs. About 650 eggs per female worm per day were found in the feces. The cumulative number of eggs in the feces and the total number of eggs in the organs was about 870,000 and 5.6 pairs of adult worms were recovered at 15 weeks PI.

In ddY mice, a female *S. japonicum* worm was suggested to produce at least 2,100 eggs a day.

Key words: ddY mouse, *Schistosoma japonicum*, schistosome eggs, schistosomiasis japonica,

Introduction

Schistosoma japonicum infection takes place by penetration of the wet skin by the cercariae. The schistosomula, which are immature worms, travel around in the host body through the circulatory system. In the portal vein of the liver they grow and develop and, when adults or near adulthood, migrate against the portal flow to the mesenteric venules of the small or large intestine. A female worm, clasped in the male's gynecophoral canal, produces many eggs for many years. It is believed that the main symptoms of schistosomiasis japonica are induced not by the adult worms, but by the granulomas which form around schistosome eggs.

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Mice are often used as an experimental model for schistosomiasis japonica, for the reason that the symptoms in the mouse, *i.e.* hepatosplenomegaly, portal hypertension and esophageal varices, resemble those in the human. In previous papers (Amano and Oshima, 1984a, 1984b, 1985), we investigated the pathophysiology of mice infected with *S. japonicum*. In these studies, the schistosome eggs began to be found from 4 weeks postinfection (PI). The number of eggs in the liver increased rapidly until 7 weeks PI, but after that the number of eggs did not significantly increase. However, we did not investigate the number of schistosome eggs that were deposited in other organs and subsequently passed into the feces. The number and distribution of schistosome eggs in the host organs may be related to the severity of those symptoms. There are only a few papers dealing with the number of eggs that pass into the feces and are found in the tissues of mice and other animals during infection.

In this study, we counted the number of

schistosome eggs in the liver, intestines and feces of ddY mice during infection.

Materials and Methods

Four-week-old female ddY mice were purchased from the Shizuoka Laboratory Animal Center (Hamamatsu). A Yamanashi strain of *S. japonicum* was maintained in our laboratory. Each of mice was anesthetized by an intraperitoneal injection of sodium pentobarbital (Dainippon Pharmaceutical Co., Ltd., Osaka), followed by exposure percutaneously to 20 cercariae of *S. japonicum* (Amano and Oshima, 1984a).

To collect the schistosome eggs in the feces, six experimental animals were individually maintained in a plastic cage fitted with a wire mesh floor with filter paper under the floor. These cages were changed once a week and the number of schistosome eggs was counted from the feces on the filter paper. At first the fecal pellets were separated from indigestible flour food through a steal sieve and were then incubated in 100 ml of Oshima sedimentation solution (Oshima *et al.*, 1965). The fecal suspension was allowed to stand for 3 hours and then the supernatant was siphoned off to a level of 50 ml. The number of schistosome eggs was counted from three 0.15 ml samples and the total number of schistosome eggs in the feces was correlated.

Five or 6 mice were killed at 4, 6, 9, 12 and 15 weeks PI by an intraperitoneal injection of sodium pentobarbital including 5 U/ml of Novo heparin (Kodama Co., Tokyo). To make it easy to count the harvested adult worms in the portal and mesenteric vein, experimental animals were perfused by the Duvall and Dewitt method (1967). We also examined the remaining worms in the mesenteric vein, because sometimes adult pair worms were observed in the mesenteric vein. Then liver and intestine were removed. Those intestines were opened longitudinally and the contents in the digestive tract were washed out before the intestine was divided into three parts. The proximal part consisted of the duodenum to half portion of the small intestine and middle part consisted of the half portion of the small intestine to the terminal ileum. The distal part consisted of the complete large intestine from coecum to rectum. Each of isolated organs and the contents was stocked in 50 ml of 5% W/V potassium hydroxide at 37°C for 4 hours. The solutions of the contents were concentrated by centrifugation and the supernatant was siphoned off to 10 ml. The number of schistosome eggs in the liver, isolated intestines and the contents was counted from three 0.15 ml samples and the total number of eggs was correlated. Early after infection, all solutions were concentrated by centrifugation and the supernatant was discarded, and all sediments were used in the egg count. All egg shells counted are referred to as eggs. The schistosome eggs found in the lungs and spleen were not counted, because they were small in number.

Results

All of the experimental animals survived until 15 weeks PI, but lost body weight and ruffled coats after 12 weeks PI.

In this study, 4 to 7 pairs of adult worms were recovered from infected mice and the mean was 5.6 pairs at 15 weeks PI. The mean number of adult worms recovered was almost the same in the course of infection (data not shown).

1) Number of schistosome eggs in the feces

Each bar in Fig. 1 shows the mean number of schistosome eggs in the feces during each week after infection. The schistosome eggs first appeared in the feces between 36 and 42 days PI. The number of schistosome eggs in the feces increased until 11 weeks PI (42,100 ±10,900 eggs in a week at 11 weeks PI), but then declined. The points in Fig. 1 show the cumulative number of schistosome eggs that passed in the feces after infection. The cumulative number of schistosome eggs in the feces was 253,000 ±49,300 (S.E.) while 15 weeks PI. Four to 7 pairs of adult worms were recovered

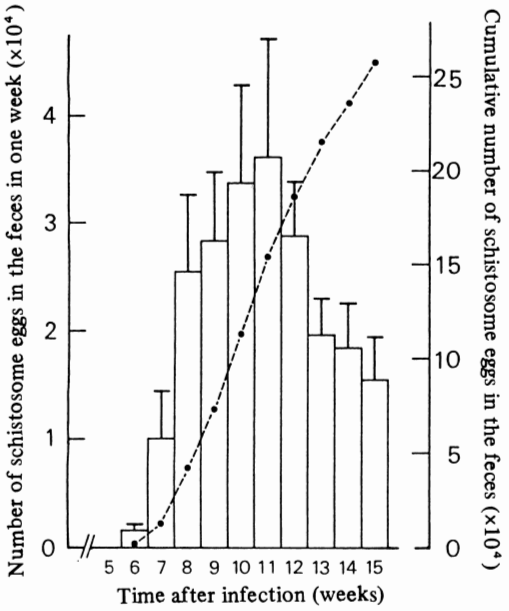

Fig. 1 Number of schistosome eggs in the feces in one week and the cumulative number of eggs. Each open bar shows the mean number of schistosome eggs in the feces at one week and each bar represents the limited standard error. Each point shows the mean cumulative number of eggs that passed in the feces.

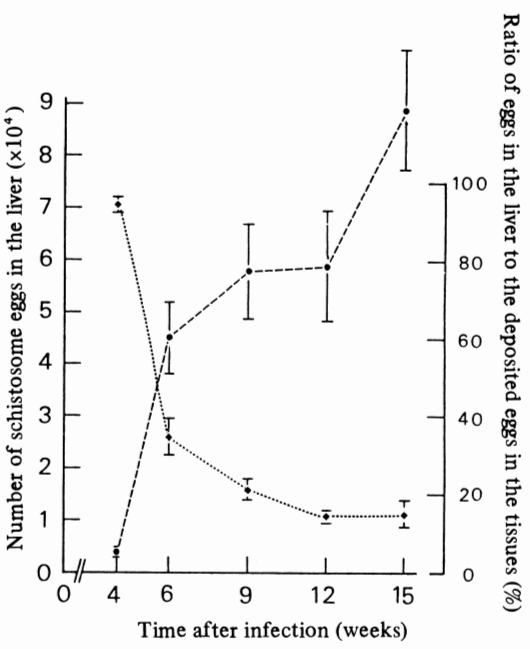

Fig. 2 Number of schistosome eggs deposited in the liver and the ratio to all deposited eggs. Each point (•) shows the mean number of schistosome eggs in the liver and the bar represents the limited standard error. Each (♦) shows the mean ratio to all deposited eggs and the bar represents the limited standard error.

at 15 weeks PI. Overall a mean of 653 ±110 (S.E.) eggs per female worm per day were found in the feces.

2) Number of schistosome eggs in the liver

As shown in Fig. 2, the schistosome eggs were already recognized in the liver of all infected mice at 4 weeks PI (4,100 ±1,300). The number of the eggs increased dramatically up until 6 weeks PI (45,200 ±6,800) at which time the tendency to increase weakened. For this reason, the ratio of schistosome eggs in the liver to the total number of eggs deposited in the tissues decreased. At 15 weeks PI, an average of 15.5 ±3.4% of the deposited eggs were found in the liver.

3) Number of schistosome eggs in the intestine (Fig. 3)

In 4 out of 6 infected mice, schistosome eggs were observed in the intestine and the number of eggs was smaller than in the liver at 4 weeks PI. There were more schistosome eggs in the large intestine than in the small intestine at 6 weeks PI. However, after that schistosome eggs were mainly deposited in the proximal portion of the small intestine and the ratio of schistosome eggs in the large intestine to the total number of eggs in the intestine had declined by more or less 5%. At 15 weeks PI, the ratio of schistosome eggs in the middle portion of the intestine was relatively high (21.3 ± 13.8%), because in 2 cases out of 6 the thickened nodules, induced by the granulomas around schistosome eggs, were formed at the border between the proximal and middle parts of the small intestine. An average of 83.2 ±3.6% of the deposited eggs were found in the small intestine. At 15 weeks PI, the total cumulative number of schistosome eggs in the feces was about 50% of the deposited schistosome eggs in the intestine.

4) The total number of schistosome eggs

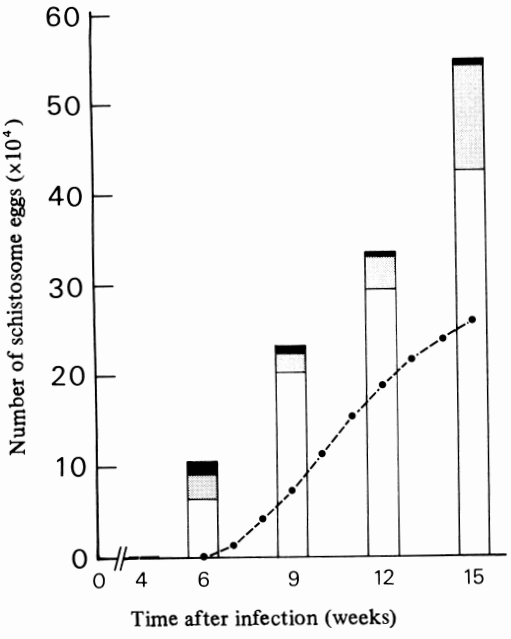

Fig. 3 Number of schistosome eggs in the intestine and cumulative number of eggs in the feces.
Each bar represents the number of schistosome eggs in the intestine. The white bar shows the number of eggs in the proximal small intestine. The light gray bar shows the number of eggs in the distal small intestine. The dark gray bar shows the number of eggs in the large intestine.
Each point shows the cumulative number of eggs that passed in the feces.

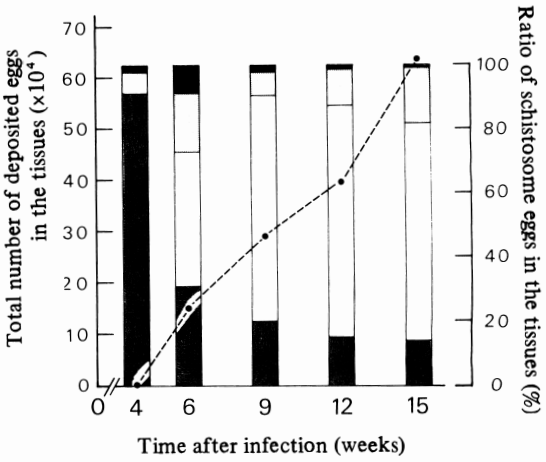

Fig. 4 Total number of schistosome eggs deposited in the tissues and the ratio of eggs in the organs.
Each point shows the mean number of eggs deposited in the organs.
Each bar shows the ratio of eggs in the organs. The dark gray bar shows the ratio of eggs in the liver. The open bar shows the ratio of eggs in the proximal small intestine. The light gray bar shows the ratio of eggs in the distal small intestine. The dark bar shows the ratio of eggs in the large intestine.

in the infected mice (Fig. 4)

At 4 weeks PI, many schistosome eggs were found in the liver, but after that most schistosome eggs were found in the intestine. At 15 weeks PI, the cumulative number of schistosome eggs in the feces and the total number of eggs in the organs was 866,100 ±114,400. An average of 28.2% of the *S. japonicum* eggs produced passed in the feces. In this study, 4 to 7 pairs of adult worms per mouse were recovered. It might be possible to presume that the adult female worms began to produce schistosome eggs on 28 days PI and the schistosome eggs were not destroyed in the organs. Hence each female worm would have produced from 1,500 to 3,200 eggs per day in their individual hosts. The mean number of egg production per female worm per day was 2,100 ±300.

Discussion

In schistosomiasis, the main pathological change is considered to be induced by the gradual accumulation of eggs in the tissues. Adult worms persist mainly in the portal or mesenteric veins and produce eggs. About 50% of the eggs are trapped in the branches of intrahepatic portal vein and the other half are mainly trapped on the mesenteric vein. Most schistosome eggs pass through the intestinal wall into the intestinal lumen. However, there are few studies done on mice, in which the production and distribution of the *S. japonicum* eggs are definitively discussed.

In this study, at 4 weeks PI all of the infected mice had schistosome eggs in the liver, but in the intestine the eggs were detected in 4 out of 6 mice. The number of eggs, also, was

more predominant in the liver than in the intestine. Faust and Meleney (1924) also calculated that the minimum elapsed time from invasion of the host to maturity of the worm was approximately 28 days. Vogel (1942) reported that on the 26th day PI all of the infected mice had eggs in the liver and on the 27th day PI schistosome eggs were first found in the wall of the intestine. From the above examinations, it is possible to conclude that in mice the *S. japonicum* worm needs about 28 days to mature after percutaneous invasion.

In our previous papers (Amano and Oshima, 1984a, 1984b, 1985), the number of schistosome eggs in the liver increased dramatically from 4 to 7 weeks PI, but did not increase significantly after that. The results were the same in this study. Warren and Moore (1966) suggested that the discharged schistosome eggs and dying eggs in the liver might balance out after 7 weeks. However, the number of eggs in the intestinal wall increased continuously and was more than in the liver after 6 weeks PI. This result probably means that the portal vein flow was affected by the eggs trapped in the intrahepatic portal vein and most of the eggs produced were deposited in the wall of the intestine. Ito (1953) reported that at 5 weeks PI about half of the worms remained in the portal vein of the liver, but after 7 weeks PI all of the worms moved into the mesenteric vein. Our results corroborate this.

In man, the colon is severely affected by the *S. japonicum* infection and granuloma resembling carcinoma (bilharzioma) is seen in the colon. Moore and Sandground (1956) reported that in hamsters with *S. japonicum* infection an average of 50% of the eggs were found in the large intestine and only 10% of the eggs produced were deposited in the small intestine. Our results about the distribution of eggs in the intestine were different from theirs. In ddY mice only 1.4% of schistosome eggs were found in the large intestine, and 61.8% of eggs were found in the proximal small intestine at 15 weeks PI. Hsü and Hsü (1960) studied distributions of eggs of the four geographic strains of *S. japonicum* in the viscera of infected mice. In their results, the percentage distribution of eggs in the viscera of albino mice infected with the Japanese strain of *S. japonicum* was 81% in the small intestine, 11% in the liver and 7% in the large intestine. The pattern of distribution of eggs was almost the same as our results at 9 weeks PI. Moore and Sandground (1956) also speculated that the localization of the worms in certain areas of the alimentary canal might vary with the species of host. Hsü and Hsü (1960) indicated that the distributions of *S. japonicum* eggs in the visceral organs of its host were influenced by the strain of the parasite, the species of the host, and the intensity of infection in the host.

In this study, schistosome eggs were first investigated in the feces of mice between 36 and 42 days PI. Tanaka *et al.* (1951) reported that the schistosome eggs in the feces of goat began to be found on 35 days PI. The number of eggs in the feces on increased until 8 weeks PI, but decreased after that. This result may be concerned with the volume of food eaten, because after 8 weeks PI mice lost their appetites. In our study, about 28.2% of schistosome eggs produced passed in the feces. In Moore and Sandground's study (1956), only 16% of the *S. japonicum* eggs produced passed in the feces. This difference is probably due to the difference of hosts; *i.e.* mice vs. hamsters. Cheever *et al.* (1980) reported that in rabbits a mean of 289 ±41 eggs per worm pair per day were found in the feces. In our study, a mean of 653 ±110 eggs per female worm per day were found in the feces of mice. One reason for this difference, also, might be concerned with the difference of hosts, especially the thickness of the intestinal wall. In mice, the deposited eggs in the intestinal wall could pass easily through into the lumen of the intestine, because their intestinal walls were thin.

If it is speculated that the adult female worm begins to produce eggs after 4 weeks PI and continues to produce at the same rate until 15 weeks PI, an adult female worm produces about 2,100 eggs per day. Moore and Sandground (1956) speculated that in the hamster each female *S. japonicum* produced an average

of 3,500 eggs per day. There is a relatively large difference in number between their results and ours. One reason for this difference is the estimation of the first day of egg production. They regarded the day on which eggs first appeared in the feces as the basis for the calculation of daily egg output per female worm. They pointed out that, on the average, the *S. japonicum* eggs appeared first in the feces on the 42nd day. In our study, the schistosome eggs were recognized in the liver, but not in the feces at 4 weeks PI. The schistosome eggs in the feces were first found between 36 and 42 days PI. From this observation, we suspected that female worms began to produce schistosome eggs on 28 days PI and the eggs needed about 10 days to pass through into the feces. Of course the above may be also affected by the strain and the number of adult worms in the host.

In this study, we disregarded the destruction of eggs in tissues. Warren and Moore (1966) suggested that the discharged schistosome eggs and dying eggs in the liver might balance out after 7 weeks PI. The destruction of eggs could be explained by greater host reactivity or by preferential obstruction of eggs laid more recently (Cheever and Anderson, 1971). However, it was impossible to speculate on the number of eggs destroyed in the tissues. Nevertheless, when we calculated the mean number of schistosome eggs in the tissues and the mean cumulative number of eggs in the feces at 6, 9 and 12 weeks PI, the mean number of eggs produced by a female worm per day was almost the same as the counts of eggs at 15 weeks PI. From this outcome, we can suggest that the change in the count of eggs in the liver is mainly affected by the change in egg deposition; the eggs produced were mainly deposited in the small intestine in the later course of infection. Actually, the number of schistosome eggs produced per female worm per day will change in the course of infection. Thus, in mice we estimate that in the Japanese strain of *S. japonicum* a female worm produces at least 2,100 eggs per day and under good conditions it will produce more.

In mice infected with *S. mansoni* eggs accumulated in the tissues in near linear fashion at a rate of 250 to 300 eggs per day per pair of worms for the first 4 to 6 months. About 70 to 90 eggs per pair of worms passed in the feces throughout this period. From these results, Cheever (1969) estimated an average egg production of 330 to 380 per worm pair per day. Therefore, the most important pathological difference between schistosomiasis mansoni and japonica will be affected by the difference in the number of eggs produced per female.

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