STUDY OF THE MORPHOLOGICAL CHARACTERISTICS OF *SCHISTOSOMA MANSONI* IN MICE, SQUIRRELS, AND HAMSTERS

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Abstract. This study attempted to describe mophological characteristics of *S. mansoni* worms. In the present study, 6 hamsters, 35 squirrels and 141 mice were infected with pooled cercariae of *Schistosoma mansoni* by intraperitoneal and percutaneous routes. The worm recovery rate was 18.2% (257/1,412) in hamsters (*Mesocricetus auratus*); 10.1% (3,310/32,792) in squirrels (*Callosciurus erythraeus roberti*) and 16.2% (4,328/26,720) in mice (Swiss strain). There were no significant differences between the sizes of 332 adults studied from three kinds of experimental animals. However, the worms collected from the hepatic portal system were usually larger than those from the peritoneal cavity because the latter almost always remained in the immature stage. We found male *S. mansoni* with tandem (17-22%), non-tandem (80-83%) and unusual/irregular arrangement (3-5%) of testes. The number of *S. mansoni* testes found were from 3 to 15 in mice, 3 to 11 in hamsters and 4 to 15 in squirrels. Mature worms had a tendency to reduce their size with aging. The number of *S. mansoni* cecal loops were from 1 to 5 in mice and hamsters and 1 to 4 in squirrels. The location of the first cecal loop was usually in the anterior part of body (1/4 in females and 1/3 in males), but there were some exceptions. The number of eggs in the uterus of each female worm, were 0-3 in mice, 0-1 in squirrels and hamsters. The average number was 0.75. The location of the ovary was usually situated in the anterior part of body of the worm in the three kinds of experimental animals. A few mated male and female worms of *S. mansoni* being free in the peritoneal cavity were found to develop to sexual maturity, because eggs were observed in their uteri. Their size was usually found to be considerably smaller than the worms seen in the hepatic portal system, and they had no hematin in their intestinal ceca. Encapsulated eggs were found from the peritoneal cavities of a few mice following intraperitoneal and percutaneous methods of infection.

INTRODUCTION

There are a number of reports regarding the morphological characteristics of *Schistosoma mansoni* which indicate that hermaphroditic males of *S. mansoni* have been found in various experimental animals (Vogel, 1947; Short, 1948; LaGrange and Scheecqmans, 1949; Buttner. 1950; Saoud, 1965, 1966). More recently, Fan and Khaw (1969) found that the presence of more than one cecal loop was observed in some male *S. mansoni* worms.

This study attempts to describe the morphological characteristics of *S. mansoni*. Measurements were made to determine the size of the males, females, their organs, the number and patterns of testes, number and position of cecal loops, and the location of the ovaries and number of intrauterine eggs in female worms. Eggs in the peritoneal cavity were also noted.

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MATERIALS AND METHODS

Source of *S. mansoni*

A total of 7,895 worms were collected from 141 albino mice (Swiss strain), 35 Taiwan squirrels (*Callosciurus erythraeus roberti*) and 6 golden hamsters (*Mesocricetus auratus*) infected with *S. mansoni* cercariae from *Biomphalaria glabrata* of Puerto Rico origin. The infected animals were sacrificed 33-482 days after infection. The worms were carefully picked up from each vessel of the hepatic portal system with an automatic pipetting machine (Baltimore Machine

and Equipment Inc), placed immediately in tap water at the room temperature, then fixed and preserved in 10% formalin.

Examination of *S. mansoni* worms before staining

The individual worms were placed on microscopic slides with a drop of normal saline. The saline was drained slowly with a strip of filter paper, until the worm was straightened out. The number of cecal loops and the location of the first cecal loop in the males and the number of intrauterine eggs and the position of ovaries in the females were determined.

Staining and mounting of worms

After rinsing with tap water then 70% alcohol, the worms were stained with carmine for 3-5 minutes, destained with 3% acid alcohol, adjusted under the microscope, then washed with 70% alcohol to remove the acid alcohol. The worms were dehydrated in 70, 80, 90, and 95% alcohol, 30 minutes each, then in 100% alcohol for 20 minutes, and 100% again for 10 minutes. After clearing slowly in cresol for at least 10 minutes, each worm was transferred to a slide with a fine-pointed tooth pick. One drop of neutral medium was put on the worm and then it was covered with a cover glass.

Examination of *S. mansoni* worms after staining

The number and pattern of united ceca in male worms and the number of eggs in the uteri of female worms were examined and counted under a microscope. Worms of special interest were evaluated with the aid of a lucida camera. Washings from the peritoneal cavities of mice infected with *S. mansoni* were examined under a dissecting microscope for mature worms and eggs.

RESULTS

Worm recovery rate

The figures in Table 1 show the following intraperitoneal and percutaneous infections: 4,328, 3,310 and 257 worms were recovered from 141 mice, 35 squirrels and 6 hamsters, respectively, after exposure to 26,720, 32,792 and 1,412 cercariae of *S. mansoni*, respectively, giving worm recovery rates of 16, 10 and 18%, respectively. The above results indicate that hamsters and mice are favored more as hosts for *S. mansoni* than squirrels.

Number and location of cecal loops in male worms

Table 2 shows that the major location of the first cecal loop in males was in the first 31-50% of the worm length and that of females was before this in the first 21-40% of the worm length. All females had their cecal loop in the anterior part of the body, but there were some exceptions in male worms from the squirrels and hamsters.

Table 1
Recovery of *Schistosoma mansoni* in mice, squirrels and hamsters following intraperitoneal and percutaneous infections

Animal	No. of animals used	Age of worms (days)	Cercariae inoculated	Worms recovered			
						Total	
				Males	Females	No.	%
Intraperitoneal infection							
Mice (Ann Arbor)	59	74-108	11,680	253	966	1,215	10
Squirrels (NIH)	16	50-482	14,117	1,731	1,238	2,969	21
Hamsters (Ann Arbor)	6	39-426	1,412	183	74	257	18
Percutaneous infection							
Mice (NIH)	82	73-146	15,040	833	2,278	3,113	21
Squirrels (NIH)	19	33-398	18,675	194	147	341	2
Both types of infections							
Mice (NIH/Ann Arbor)	141	73-146	26,720	1,086	33,244	4,328	16
Squirrels (NIH)	35	33-482	32,792	1,925	1,385	3,310	10
Hamsters (Ann Arbor)	6	39-426	1,412	183	74	257	18

Table 2
Location of cecal loop in *Schistosoma mansoni* for different species of experimental animals.

Animals		Percentage of location of united ceca[b]							
		Mice (Ann Arbor)[a]		Squirrels (NIH)		Hamster (Ann Arbor)		Squirrels (NIH)	
No. of worms		Male (100)	Female (100)	Male (100)	Female (72)	Male (73)	Female (27)	Male (104)	Female (96)
Percentage of location of united ceca[b]	16-20	-	4	-	-	-	-	-	-
	21-30	8	76	11	55	10	6	7	37
	31-40	59	19	42	17	32	19	59	56
	41-50	33	1	25	-	29	2	36	3
	51-85	-	-	22	-	2	-	2	-

No. of worms examined in parenthesis; [a]Strain of worm.
[b]The distance between the anterior end of worm body and the junction of the first intestinal ceca to full body length.

Table 3
Number of cecal loops in male *Schistosoma mansoni* for different species of experimental animals.

Animal	Strain of worm	No. of worms examined	No. of cecal loops							
			1	2	3	4	5	6	7	9
Mice	Ann Arbor	138	112 (81)[a]	18 (13)	5 (4)	2 (1)	1 (<1)			
Squirrels	NIH	100	75 (75)	16 (16)	6 (6)	3 (3)				
Hamsters	Ann Arbor	71	52 (73)	9 (13)	5 (7)	3 (4)	2 (3)			
Squirrels	NIH	104	39 (38)	27 (26)	15 (14)	8 (8)	6 (6)	7 (7)	1 (<1)	1 (<1)

[a]Percentage in parenthesis.

Table 4
Number of eggs found in the uterus of mature female *Schistosoma mansoni* for different species of experimental animals.

Animal	Strain of worm	No. of worms examined	No. of eggs in the uterus				
			0	1	2	3	4
Mice	Ann Arbor	485	137 (28)[a]	343 (71)	4 (<1)	1 (<1)	-
Mice Squirrels	NIH	70	8 (11)	62 (89)	-	-	-
Hamsters	Ann Arbor	32	7 (22)	25 (78)	-	-	-
Squirrels	NIH	96	3 (3)	93 (97)	-	-	-

[a]Percentage in parenthesis.

The number of cecal loops in male *S. mansoni* for different species of experimental animals

Table 3 shows that the number of cecal loops varying from 1 to 5. The worms had more than one cecal loop in 19-27% of the three different animals.

The number of eggs in the uteri of mature female *S. mansoni* in different species of experimental animals

None of the 587 female worms had 4 eggs in its uterus. In 152 female worms, no eggs were found. In most (430) of the worms only one egg was seen, and in a few worms there were 2 or 3 eggs. The average number was 0.75 eggs per worm (Table 4).

The number and patterns of testes in male *S. mansoni* for different species of experimental animals

Two testes arrangement patterns were found, one was tandem in a single column, the other was non-tandem, including the unusual/irregular arrangements of the testes. The number of testes found were from 3 to 15 in a non-tandem arrangement. The most frequent number of testes for both tandem and non-tandem arrangements in the three kinds of animals were from 6-9 (Table 5).

Unusual/irregular arrangements of testes in male *S. mansoni* for different species of experimental animals

The results in Table 6 show that the number of testes varied from 4 to 15. All had an unusual/irregular arrangement. A large group of testes was usually located in the normal position, and a smaller number were located posterior to the normal testes. The frequency of unusual/irregular arrangements of testes for the worms collected in mice was 2.5% (17/685);

Table 5
Number and pattern of testes in male *Schistosoma mansoni* for different species of experimental animals.

Pattern of testes	No. of testes	Mice (Ann Arbor) (685)[a]		Squirrels (NIH) (184)		Hamsters (Ann Arbor) (168)		Squirrels (NIH) (104)	
		No.	%	No.	%	No.	%	No.	%
Tandem	3	4	4	-	-	-	-	-	-
	4	6	5	3	8	1	3	2	6
	5	12	11	1	3	1	3	10	29
	6	23	20	9	23	10	29	8	24
	7	38	33	16	40	14	41	13	38
	8	22	19	6	15	7	21	1	3
	9	7	6	4	10	1	3	-	-
	10	2	2	1	3	-	-	-	-
	Total	114	17	40	22	34	20	34	33
Non-tandem	3	3	<1	-	-	1	<1	-	-
	4	8	1	1	<1	-	-	1	1
	5	36	6	1	<1	-	-	5	7
	6	75	13	7	5	15	11	16	23
	7	146	26	36	25	35	26	23	33
	8	138	24	52	36	47	35	14	20
	9	77	14	22	15	21	16	9	13
	10	46	8	11	8	10	8	1	1
	11	20	4	8	6	3	2	1	1
	12	10	2	4	3	2	2	-	-
	13	7	1	1	<1	-	-	-	-
	14	4	<1	-	-	-	-	-	-
	15	1	<1	1	<1	-	-	-	-
	Total	571	83	144	78	134	80	70	67

[a]No. of worms examined in parenthesis.

Table 6
Unusual/irregular arrangement of testes in male *Schistosoma mansoni* for different species of experimental animals.

No. of testes	Pattern of testes	Mice (Ann Arbor)	Squirrels (NIH)	Hamsters (Ann Arbor)	Squirrels (NIH)
4	1-3	-	-	1	0
5	4-1	3	-	-	0
	3-2	1	-	-	0
6	5-1	2	-	-	0
	2-4	-	-	1	0
7	6-1	1	2	1	0
	3-4	1	-	-	0
	5-2	-	-	1	0
8	7-1	-	1	1	0
	6-2	3	-	-	0
	5-3	-	-	-	0
9	8-1	1	1	-	0
	1-8	1	-	-	0
10	9-1	1	-	-	0
	8-2	-	-	1	0
11	9-2	-	2	-	0
	10-1	1	2	-	0
14	11-3	1	-	-	0
15	12-3	-	1	-	0
Total		17	9	6	0

Table 7
Location of ovary in female *Schistosoma mansoni* for different species of experimental animals.

Location percentage of ovary[b]	Mice (Ann Arbor) (100)[a]		Squirrels (NIH) (72)		Hamsters (Ann Arbor) (27)		Squirrels (NIH) (96)	
	No.	%	No.	%	No.	%	No.	%
12-20	5	5	-	-	1	3	-	-
21-30	87	87	29	40	10	37	37	39
31-40	7	7	43	60	16	61	56	58
41-46	1	1	-	-	-	-	3	3

[a]No. of female worms examined in parenthesis.
[b]The distance between the anterior end of the worm body and the overy as a percent of the total length of the worm.

squirrels, 4.9% (9/184); and hamsters, 3.5% (6/168).

Location of ovaries of female *S. mansoni* in different species of experimental animals

The measurement of ovary was made from the middle of the ovary to the anterior end of worm body. The figures in Table 7 indicate that the ovaries in all the female worms were located in the anterior part of worm body. The average location was from 21 to 40% of the total worm length in case of the three species of animals.

Finding of mature worms and eggs in the peritoneal cavity of mice

On examination of washings from the peri-

Table 8
Mature worms and eggs of *Schistosoma mansoni* found in the peritoneal cavities of mice.

Type of infection	No. of mice used	Cercariae inoculated		Age of worms (days)	Findings in peritoneal cavity			
		Male	Female		Copula	Male	Female	Egg
Intraperitoneal	82	1,640	13,400	73-146	2 (2)[a]	26 (11)	12 (7)	+ (19)
Percutaneous	59	1,180	10,500	74-107	0	0	0	89 (15)

[a]No. of mice in parenthesis.

toneal cavities of 141 mice under a dissecting microscope, there were 2 copulated worms, 26 single males and 12 single females which were sexually matured. Encapsulated eggs were also found in the peritoneal cavity in 19 and 15 mice, respectively, infected by intraperitoneal and percutaneous routes. In the latter, there were 89 encapsulated eggs found. Most of the encapsulated eggs were observed to be embryonated and a few miracidia were seen to escape through a linear break in the encapsulated eggs and swam rapidly away. These eggs were no doubt fertilized.

DISCUSSION

It is generally recognized that within the mammalian host, the developmental stages of *S. mansoni* are: (1) metacercariae during their migration to liver, (2) immature forms in the liver, and (3) maturing forms migrating to the mesenteric or portal vessels (Belding, 1965). The figures in the present experiment show that many worms recovered from the vessels of hepatic portal system still remained in the immature stage even 60 days after exposure to cercariae. This seems to indicate that the developmental stages of *Schistosoma* worms may be found in the same condition in the vessels of large and small intestines as well as the portal vein.

Male maturing worms increased continuously in size until about eight weeks, but females required one additional week more. After reaching maximum, the number and size of the worms usually decreased. This point was proved in the present study. There were no significant differences in the sizes of the mature worms recovered from the various vessels of the portal system in the three kinds of experimental animals.

The number of worms with more than one cecal loop were so great that those worms should not be considered abnormal.

The worms found without eggs in their uterus may be explained by their being expelled during relaxing in the tap water.

The testes located posteriorly to the normal group were named by Najim (1951) and Saoud (1965, 1966) as “supernumerary testes”. The term “testes in an abnormal location” termed by Hsu and his co-worker (1957) is probably better than the term “supernumerary testes”, because the total number of these unusual/irregularly arranged testes is no greater than the number of normal testes. To our knowledge, this was the first instance involving the presence of more than 13 and less than 4 *S. mansoni* testes in worms in mice, squirrels and hamsters.

Previously, all worms had been described as having their first cecal loop located in the anterior part of body, but in the present study, the first cecal loop was found in the posterior part of the worm body in 22 worms from mice and squirrels, and in 2 worms from hamsters.

In our experiment 14 female worms in the peritoneal cavity of 9 mice were observed to each have a typical egg in their uterus. Two of them were mated each with 1 male and the remaining 12 were unmated. Encapsulated eggs were also observed free in the peritoneal cavity of 19 mice following intraperitoneal infection, and in the cavity of 15 mice following percutaneous infection. The number of eggs in the latter group in 7 mice were 1, 1, 6, 11, 12, 21 and 37, re-

spectively. Most of the encapsulated eggs had viable miracidia, some moving actively. To determine the source of these eggs, the whole length of the intestines of 4 mice with eggs in their peritoneal cavities were carefully examined from the stomach to the lower end of the rectum in a 1,000 ml cylinder filled with 0.5% sodium chloride solution. Eight pin-point-like whitish spots about, 0.2 mm in length, were found in the small intestine of two mice. Under a dissecting microscope, 6 out of the 8 spot samples were each found to have typical eggs; one egg encapsulated in dark-brownish fibrous material was found in two samples; two eggs, in three samples; and three eggs, in one sample. These findings clearly demonstrate that the pin-point-like whitish spots contained encapsulated eggs, which became detached from the serosa and dropped into the peritoneal cavity. The findings confirmed previous reports by Fan and Fao (1970) and Fan (1971). The normal eggs produced by the fertilized females in the peritoneal cavities of infected mice induced a local reaction and inflammation in the same way as foreign bodies. The pathological changes of the peritoneal membrane may increase secretions and/or exudation, which then gradually surrounds the eggs, and finally encapsulates them.

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