3-1-1994

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Am. J. Trop. Med. Hyg., 50(3), 1994, pp. 281-295

KINETICS OF EGG PRODUCTION AND EGG EXCRETION BY *SCHISTOSOMA MANSONI* AND *S. JAPONICUM* IN MICE INFECTED WITH A SINGLE PAIR OF WORMS

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Abstract. Individual male and female schistosomes approximately three weeks of age were implanted into the portal venous system of C57Bl/6 mice to produce infections with a single pair of *Schistosoma mansoni* or *S. japonicum*. Mice were killed between seven and 54 weeks after infection. Worm fecundity was measured by counting eggs accumulating in the tissues and eggs passed in the feces. *Schistosoma mansoni* worm pairs laid approximately 350 eggs per day with no change in the apparent rate of egg laying between eight and 52 weeks after infection and approximately one-third of the eggs were passed in the feces. *Schistosoma japonicum* worm pairs laid approximately 2,200 eggs per day initially and this decreased to 1,000 eggs per day by the end of the experiment, with one-third to one-half of the eggs being passed in the feces. There was marked variability in the fecundity of individual worm pairs, but the number of eggs passed in the feces of individual mice correlated well with the number of eggs in the intestines at all time points in *S. mansoni*–infected mice and at the seventh and tenth week of *S. japonicum* infection.

Mice infected with even a single pair of schistosomes are very heavily infected compared with humans with schistosomiasis.[1] Therefore, we consider infections with a single pair to be more desirable than infections with multiple worm pairs for examining the parasitologic and pathologic results of infection in mice. Infection with a single pair should facilitate quantitative analyses because the numbers of tissue eggs per worm pair, the degree of hepatic fibrosis, and the size of granulomas in the liver may vary with infection intensity.[2] Furthermore, some worms may die or be lost from the portal system and the eggs found at the time of necropsy may include eggs laid by worms not recovered by perfusion. Therefore, we surgically implanted single worm pairs into the portal system of mice and examined the accumulation of eggs in the tissues, passage of eggs in the feces, and pathologic parameters from the third week after egg laying began until approximately one year after infection.

MATERIALS AND METHODS

Worms used for transplantation were recovered from donor mice exposed by subcutaneous injection of 100 *S. japonicum* (Lowell Philippine strain)[3] or 300 *S. mansoni* (NMRI strain)[4] cercariae. Donor mice were killed approximately three weeks after infection by intraperitoneal injection of 10 mg of pentobarbital solution containing 20 units of heparin. The liver was perfused with sterile, cold RPMI 1640 culture medium and the worms were separated from blood cells by sedimentation. Pentobarbital, 0.5 mg/ml, was added to the medium to anesthetize the worms and prevent them from adhering to the needle and tubing used to transfer the worms. One male and one female were aspirated into a butterfly infusion set (Abbott Hospitals Inc., North Chicago, IL) bearing a 21-, 23-, or 25- gauge needle.

Recipient C57Bl/6 (B6) mice were anesthetized with intraperitoneal pentobarbital or with methoxyflurane (Pitman-Moore, Mundelein, IL) by inhalation. Worms were injected into a mesenteric venule in approximately 0.3 ml of RPMI 1640. After removal of the needle, pressure was applied using a small piece of gelfoam (Upjohn Co., Kalamazoo, MI) to control bleeding. Approximately 80% of the mice survived surgery and bisexual infections were present in 80% of the *S. mansoni* transfers and 65% of the *S. japonicum* transfers. Two additional groups of mice were either unexposed or given a sham operation and then evaluated.

The duration of infection is calculated from the time of exposure of donor mice. Mice given *S. mansoni* were killed 8, 12, 20, or 52 weeks after infection and those given *S. japonicum* were killed 7, 15, 26, or 54 weeks after infection. Stools were collected from individual mice for one or two periods of 24 hr^3 immediately before the mice were killed by intraperitoneal injection of a solution containing 10 mg of pentobarbital and 50 units of heparin. The number of eggs found on the first and second days did not differ significantly. Worms were recovered by perfusion of the hepatic portal system via the aorta.[5] Different portions of the liver showed regeneration or atrophy in chronically infected mice. Therefore, we selected at all time periods at least two representative areas, one from the large left lobe of the liver and one from the small suprarenal lobe, and pooled these for determination of collagen content. Liver samples for histology and for counting of eggs were similarly divided. Two small pieces of intestine were fixed for histology and the remainder were frozen for egg counts.

Tissues for histologic examination were fixed in Bouin-Hollande solution. The diameter of granulomas around single eggs containing a mature miracidium was measured with an ocular micrometer. Granulomas surrounding 3–5 eggs were also measured in *S. japonicum*–infected mice. Granuloma volumes were calculated assuming a spherical shape. Eggs in the liver, intestines, and lungs were counted after digestion for 14–18 hr at 37°C in 4% KOH.[6] Feces were collected for 24 hr just prior to killing the mice, which were maintained in one-liter beakers on wire mesh and provided with mouse chow and water ad libitum. Feces were fixed in 20 volumes of 10% neutral-buffered formalin, dispersed in a food processor for 1 min at half speed, and the resulting suspension was passed through nytex monofilament cloth with an aperture of 110 microns. *Schistosoma mansoni* eggs were then collected on 44-micron nytex and *S. japonicum* eggs were collected on 28-micron nytex cloth. Eggs were counted in 1-ml chambers (Sedgwick Rafter; Thomas Scientific, Swedesboro, NJ) with an equivalent of 2–5 mg of feces/ml. If no eggs were found in 2 ml, additional aliquots were counted until the calculated count, assuming one egg had been found, was less than half the mean count in the positive mice. The number of eggs in negative fecal samples was calculated assuming that 0.5 eggs had been present in the volume examined, i.e., if 10 ml was examined, 0.5 eggs/10 ml was used for the calculation. The mean count for negative fecal specimens averaged 15% of that of positive specimens.

Liver collagen was measured as hydroxyproline in 200 mg liver digested in 5 ml of 6N HCl for 18 hr at 110°C. After removal of humin pigments and neutralization, technique B of Bergman and Loxley[7] was used to measure hydroxyproline in duplicate aliquots. The hydroxyproline content of normal livers was measured in sham-operated mice and in unexposed mice. Normal livers contained a mean of 1.6 μmoles of hydroxyproline per liver at 7–15 weeks and 2.3 μmoles at 20–54 weeks.

Infections were considered to be active if eggs were found in the feces or if eggs in tissue sections or in small portions of crushed, fresh liver contained miracidia that were viable in appearance. The viability of these eggs was frequently confirmed by demonstration of active flame cells in miracidia in fragments of liver to which dechlorinated tap water was added, and in no case did we fail to demonstrate flame cells in at least one miracidium in lives judged to contain viable miracidia on morphologic grounds. All three of these indices were positive in nearly all *S. mansoni*–infected mice with active infection but in several *S. japonicum* infections, no viable eggs were found in the liver and eggs in the feces were the only indicator of active infection.

Calculations and statistical analysis

The relationship between eggs in gut tissue and eggs in feces was examined using log-log regressions. Fecundity was calculated as [(Te1 − Te2)/d + (Fe1 + Fe2)/2], where Te1 and Te2 are the tissue eggs at times 1 and 2, d is the number of elapsed days between times 1 and 2, and Fe1 and Fe2 are the fecal eggs per day passed at times 1 and 2. We did not think it was appropriate to analyze differences in fecundity statistically but we have presented standard errors of the difference for (Te1 − Te2)/d and have analyzed the changes in fecal egg passage by one-way analysis of variance (ANOVA).

We also have estimated the possible effects destruction of eggs in the tissues might have had on our calculations of fecundity. We let E(t) be the number of eggs in the tissue at any time t,

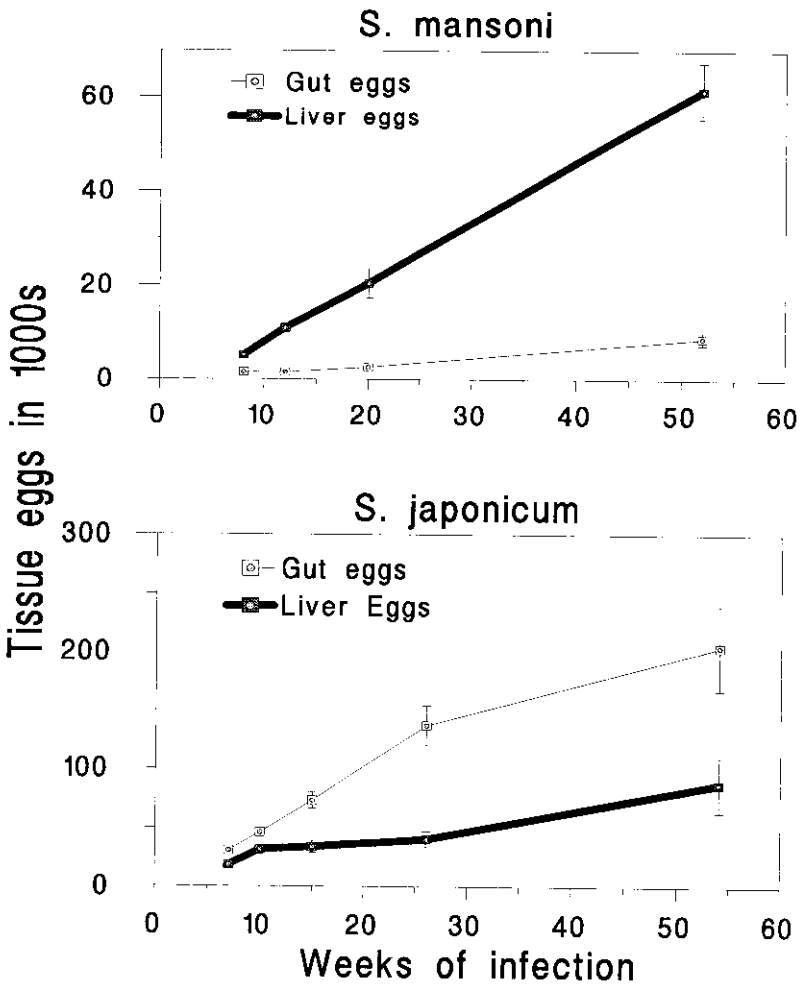

FIGURE 1. Number of eggs in the liver and the gut during the course of the experiment. Bars show the mean ± SEM.

T(t) the total number of eggs laid in the tissue until time t, K(t) the number of eggs laid per day, and the rate of egg destruction is given by a time constant, τ (units of day $^{-1}$). Before egg destruction begins, the number of eggs laid per day is equal to the number of eggs accumulated in the tissue per day: $K(t) = dE(t)/dt$. After egg destruction begins, the number of eggs laid per day is equal to the number accumulated in the tissue plus the number destroyed:

$$K(t) = \frac{dE(t)}{dt} + \frac{E(t)}{\tau}$$

The quantity E(t) is known only at those times when data were taken. Assuming that E(t) varies linearly between these measured values, we can calculate values of E(t) or K(t) at specified times. Since K(t) is the rate of eggs being deposited in the tissue, the total number of eggs that have been laid in the tissues is given by the integral of K(t):

$$T(t) = \int_0^t K(t')\, dt'$$

RESULTS

Parasitologic findings for S. mansoni

Inactive infections were found in 2 of 16 mice at eight weeks, 0 of 23 mice at 12 weeks, 2 of

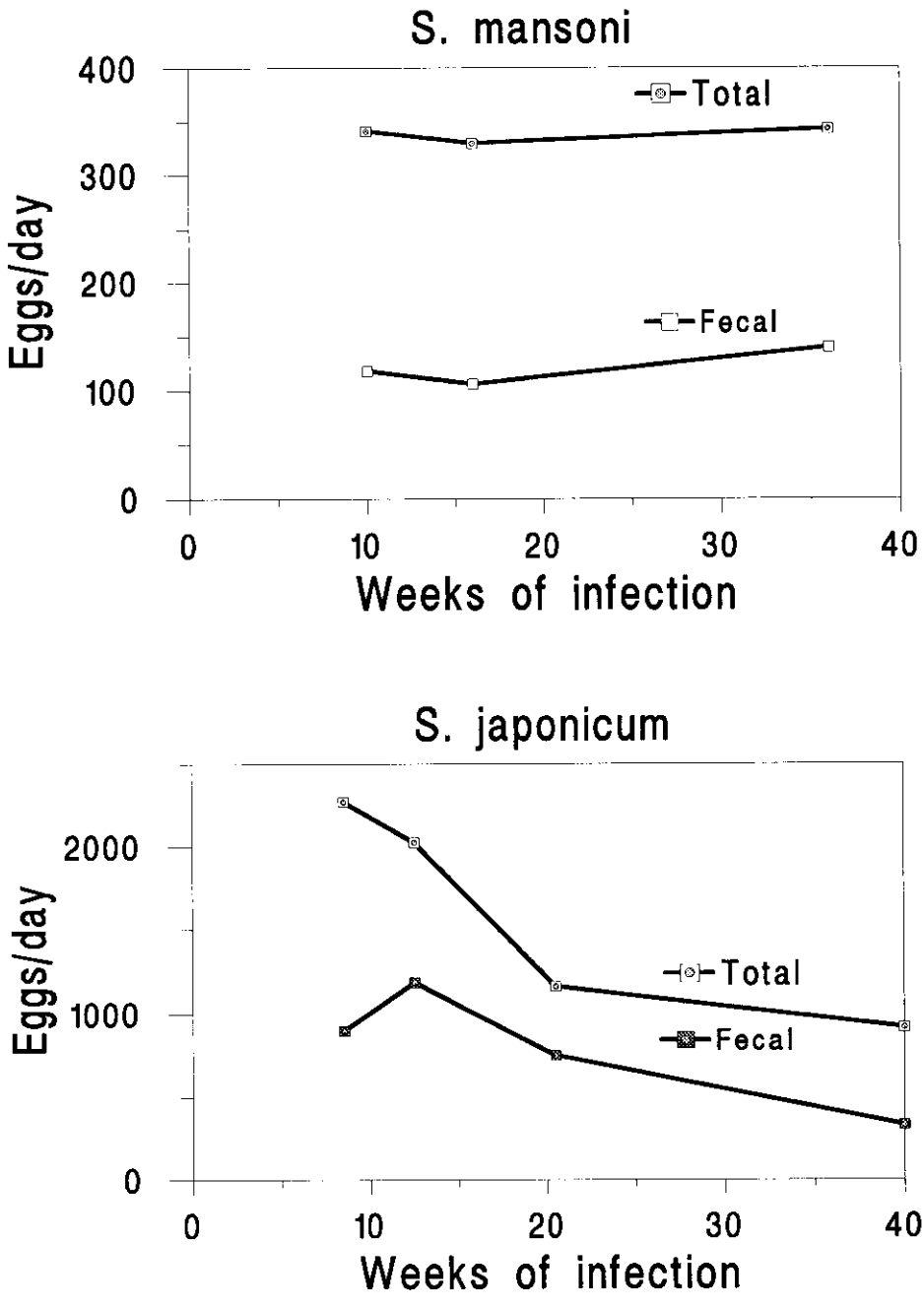

FIGURE 2. Calculated fecundity (tissues plus feces) and the rate of egg passage in the feces. Only active *Schistosoma mansoni* infections are plotted, but the results are similar if based on all *S. mansoni*–infected mice.

20 mice at 20 weeks, and 7 of 31 mice at 52 weeks after infection. Worm pairs were recovered from eight of these 11 mice with inactive infections, but no live eggs were found in crushed fragments of liver or in histologic sections and no eggs were found in the feces. One egg was found in the uterus of the female of half of the worm pairs recovered from mice with in-

TABLE 1

Correlation between fecal eggs and eggs in the gut or liver of mice infected with a single pair of Schistosoma mansoni *or* S. japonicum *(log-log regressions of fecal eggs on gut eggs)**

	Weeks	Gut		Liver	
		Slope	*P*	Slope	*P*
S. mansoni	8	+0.50	<0.01	+1.45	<0.05
	12	+0.34	<0.001	+1.29	<0.001
	20	+0.71	<0.001	+0.88	<0.07
	52	+0.26	<0.01	+0.58	<0.001
S. japonicum	7	+1.32	<0.05	+0.63	NS
	10	+0.94	<0.01	−0.46	NS
	15	+0.57	NS	−0.54	NS
	26	+0.27	NS	+0.27	NS
	54	−0.15	NS	−0.39	NS

* NS = not significant.

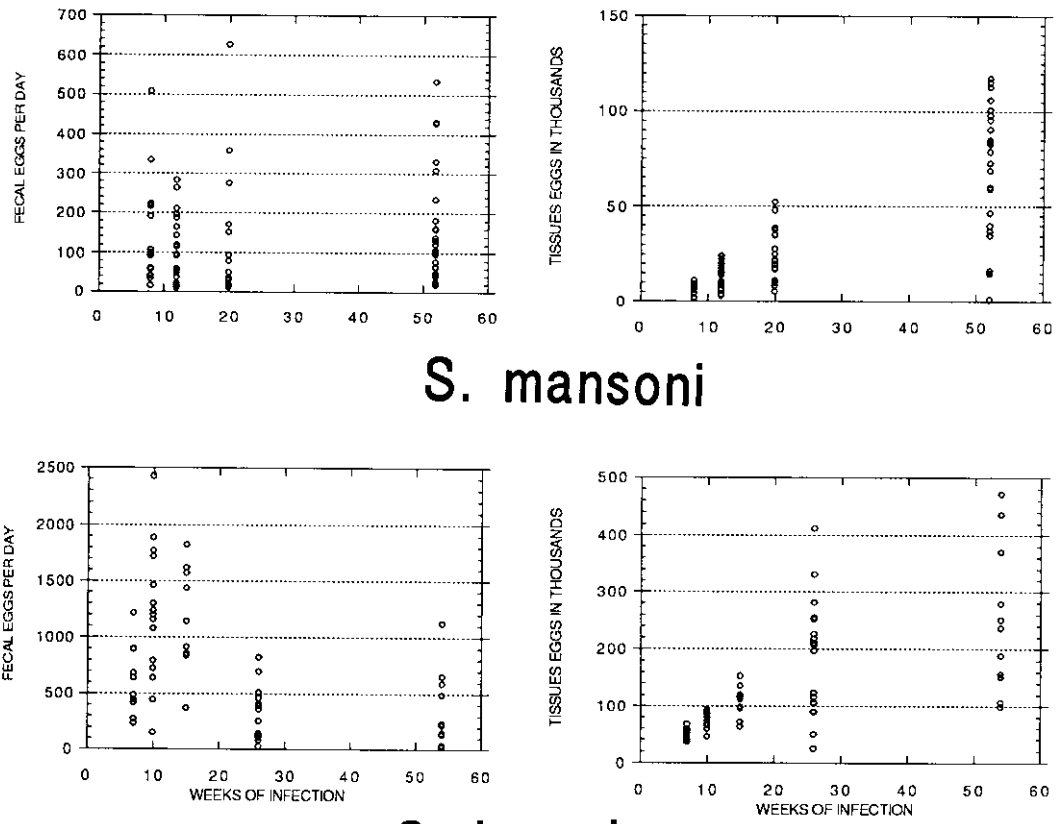

FIGURE 3. Number of fecal and tissue eggs. Passage of eggs in the feces of *Schistosoma mansoni*–infected mice (active infections only) is shown in the upper left quadrant and that of *S. japonicum*–infected mice in the lower left quadrant. Tissue eggs are plotted in the right-hand panels.

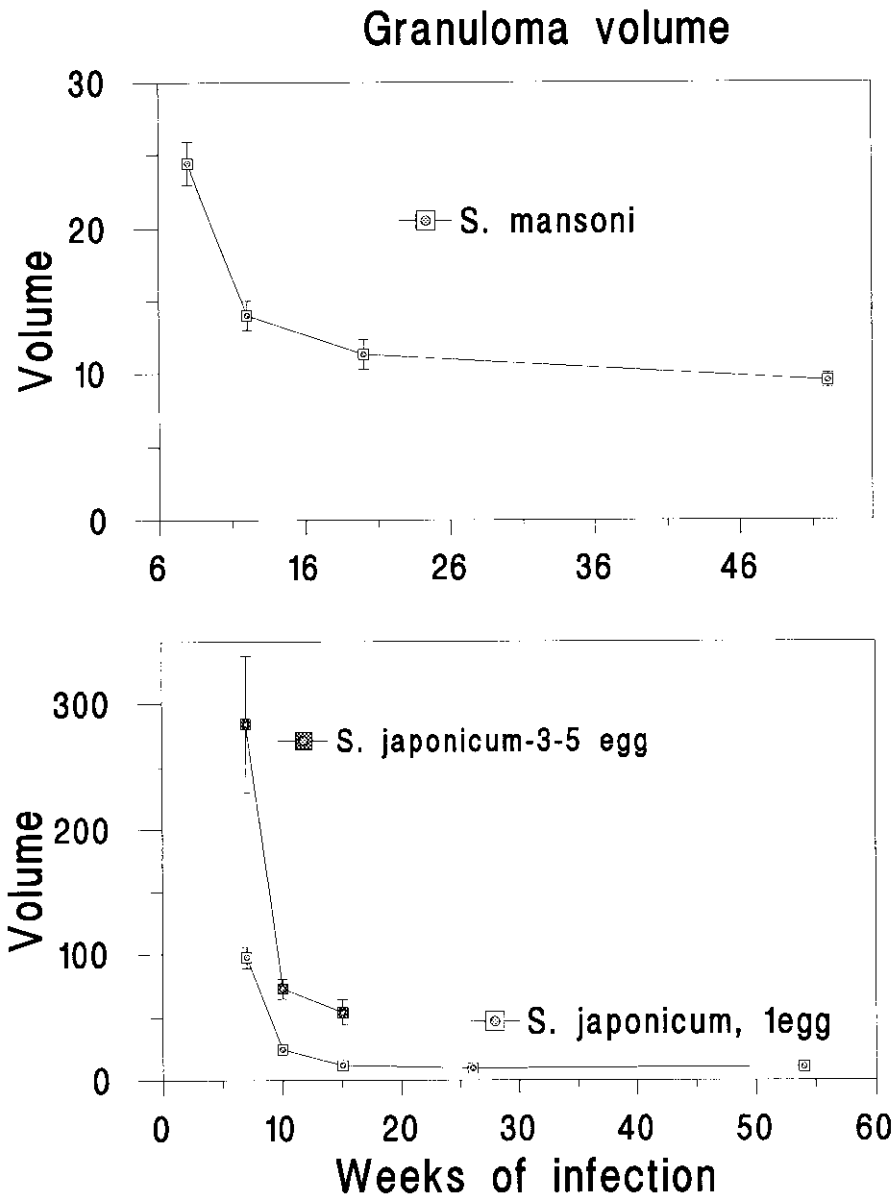

FIGURE 4. Granuloma volume ($mm^3 \times 10^{-3}$) as a function of time. Granulomas around both *Schistosoma mansoni* and *S. japonicum* eggs became smaller as the infection progressed, but did not change significantly after the 12th week for *S. mansoni* or after the 10th week for *S. japonicum*. Insufficient granulomas containing 3–5 *S. japonicum* eggs were available for measurement at 26 and 54 weeks. Bars show the mean ± SEM.

active infections and in the same proportion of worms from mice with active infections. Mice with inactive infections were not generally included in the analysis of egg production and excretion. The number of eggs in the tissues of mice with inactive infections averaged 13% of that of mice with active infections at eight weeks, 5% at 20 weeks, and 19% at 52 weeks.

The livers of *S. mansoni*–infected mice contained a mean of 79–90% of the eggs in the tissues and the intestines contained 10–21% of the eggs (Figure 1). The lungs contained no

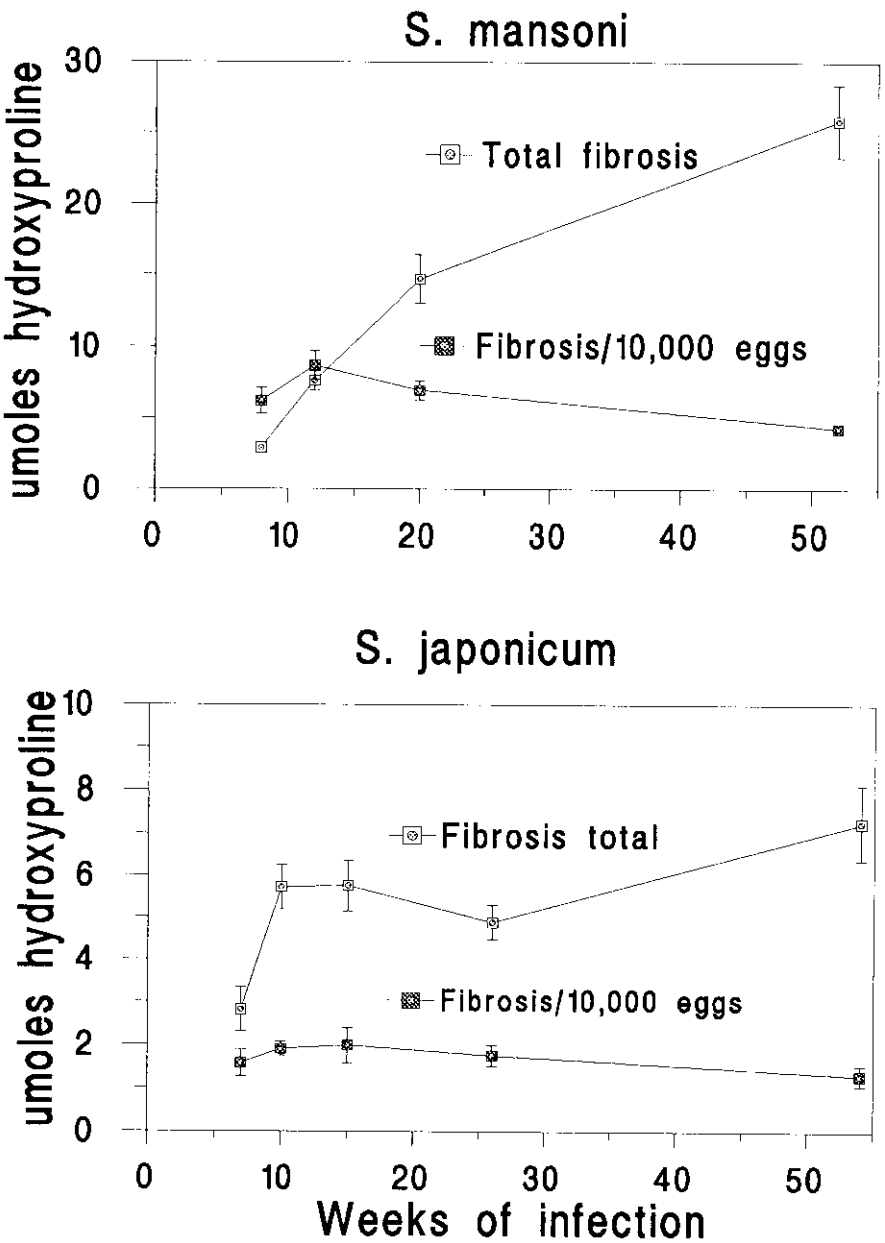

FIGURE 5. Total hepatic fibrosis and fibrosis per 10,000 eggs in mice infected with either schistosome species. The hydroxyproline content of normal livers has been subtracted. Hepatic fibrosis per egg and per liver was much more marked in *Schistosoma mansoni*–infected mice. Bars show the mean ± SEM.

eggs at eight weeks and 1% of the eggs by 52 weeks.

The number of eggs passed in the feces remained constant at approximately 120 eggs/day and the rate of accumulation of eggs in the tissues did not vary significantly with time, averaging approximately 220 eggs/day. Thus, we estimate the overall rate of egg production by single *S. mansoni* worm pairs in mice with active infections to be approximately 340 eggs/day

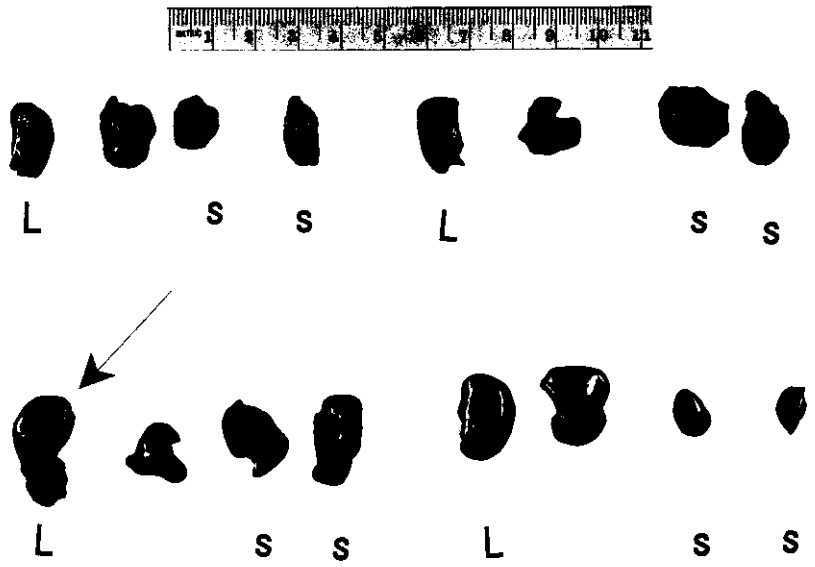

FIGURE 6. Hepatic regeneration and atrophy in *Schistosoma mansoni*–infected mice one year after infection. A normal liver is shown in the lower right quadrant. The smallest lobes of the liver **(S)** are markedly enlarged in all three infected livers. The large left lobe of the liver **(L)** is reduced in size in the infected animals. This lobe shows a large hypertrophic area in one infected mouse **(arrow)**. Similar changes were seen in the livers of mice with chronic *S. japonicum* infection.

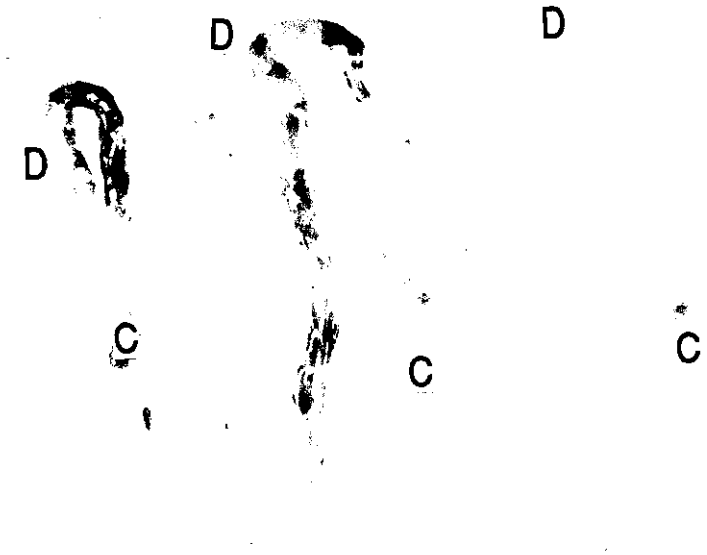

FIGURE 7. Small intestines of two *Schistosoma japonicum*–infected mice 26 weeks after infection, showing marked thickening and dilatation. A normal intestine is shown on the right. The duodenum **(D)** and cecum **(C)** are labeled. No dilatation of the gut is noted proximal to the lesions, indicating that intestinal obstruction is not present.

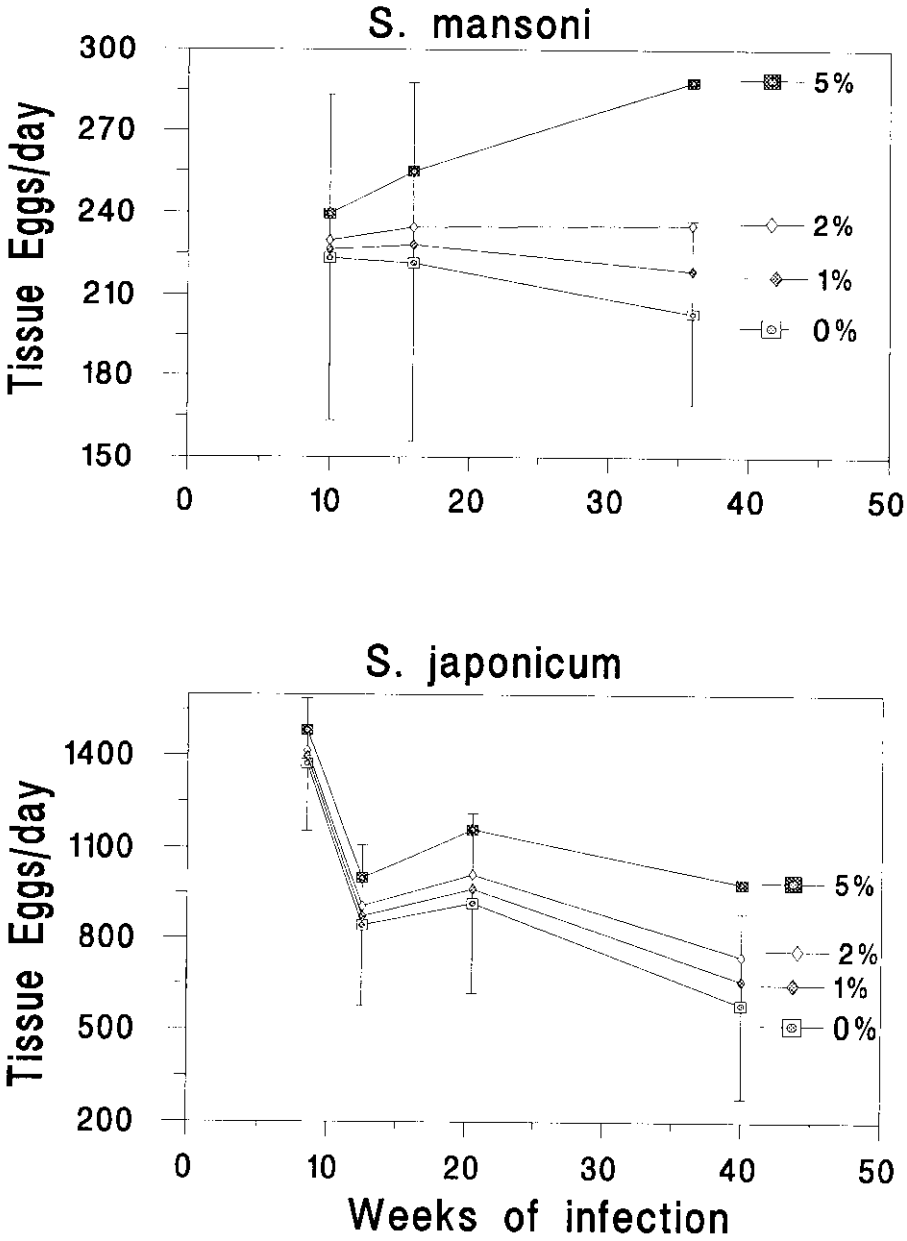

FIGURE 8. Effects of egg destruction (at rates of 1%, 2%, or 5% of tissue eggs/month) on the calculated daily rate of egg accumulation in the tissues for *Schistosoma mansoni*– and *S. japonicum*–infected mice. The rate of egg destruction is uncertain but is probably not greater than 2% for *S. mansoni*[9] and less than 1% for *S. japonicum*[8] infection in mice. Bars show ± one standard error of the difference of the means for calculations assuming 0% egg destruction.

(Figure 2), assuming that no eggs were destroyed in the tissues. Mean fecundity did not differ greatly when mice with inactive infections were included in the calculations.

The number of eggs in the feces of individual mice at each time period correlated well with the number of eggs in the intestines or livers of the same mouse (Table 1).

TABLE 2

Estimates of fecundity of Schistosoma mansoni *in other studies**

Host	Worm strain	Mean no. of worm pairs	Interval, weeks (w) or days (d)	Tissue eggs/day ± SE†	Fecal eggs/day	Total eggs/day	Reference
C57Bl/6 mouse	NMRI-PR	6	8–12 w	275 ± 31	161	436	13
C57Bl/6 mouse	NMRI-PR	6	12–20 w	243 ± 30	115	358	13
BALB mouse	NMRI-PR	6	8–12 w	204 ± 23	111	315	13
BALB mouse	NMRI-PR	5	12–20 w	120 ± 27	65	185	13
Swiss mouse	NA	3	40–54 d	202–274	NA	NA	14
NIH mouse	PR	1–4	80–100 d	102	NA	NA	15
CBA mouse	PR	1–7	80–100 d	117	NA	NA	15
Albino mouse	Egypt	3–19	~8 w	264	98	362	16
Albino mouse	Egypt	4–20	~12 w	224	143	367	17
Swiss mice	PR-1	18	7–15 w	310 ± 89‡	38	356	18
Swiss mice	PR-1	8	15–26 w	204 ± 124‡	40	235	18
Swiss mice	PR-2	12	7–15 w	146 ± 43‡	47	196	18
Swiss mice	PR-2	7	15–26 w	133 ± 51‡	53	186	18
Swiss mice	LC-1	6	7–15 w	295 ± 177‡	47	276	18
Swiss mice	LC-1	5	15–26 w	14 ± 65‡	37	92	18
Swiss mice	NIH-PR	3	7–11 w	139 ± 31	110	249	19
	experiment 1		11–19 w	284 ± 40	80	364	
			19–27 w	148 ± 54	43	191	
Swiss mice	NIH-PR	3	7–11 w	239 ± 45	NA	NA	19
	experiment 2		11–19 w	221 ± 36	NA	NA	
			19–27 w	0 ± 37	NA	NA	
Swiss mice	W-PR	3	7–11 w	150 ± 18	NA	NA	19
			11–19 w	180 ± 16	NA	NA	
			19–27 w	79 ± 35	NA	NA	
Swiss mice	Bahia, Brazil	3	7–11 w	164 ± 29	59	223	19
			11–19 w	213 ± 36	67	270	
			19–27 w	86 ± 44	37	105	
Swiss mice	St. Lucia	3	7–11 w	204 ± 29	91	295	19
			11–19 w	225 ± 25	79	304	
			19–27 w	89 ± 34	51	140	
BH Swiss mice	Belo Horizonte, Brazil	3	7–11 w	100 ± 23	110	210	19
			11–19 w	168 ± 25	80	248	
			19–27 w	34 ± 33	43	77	
BH Swiss mice	Mwanza	3	7–11 w	196 ± 41	82	278	19
			11–19 w	155 ± 30	60	215	
			19–27 w	91 ± 91	NA	NA	
BALB mouse/ *Mastomys*	South African	3–4	~10–12 w	95/77	3/0.1	98/77	20
BALB mouse/ *Mastomys*	PR	4–6	~10–12 w	234/232	22/4	256/236	20
Hamster	PR	29	42–66 d	227	64	291	21
African green monkey	NIH-PR	126	13 w	475	182	657 ± 21§	22
African green monkey	NIH-PR	109	26 w	469	142	611 ± 30§	22
African green monkey	NIH-PR	134	134 w	431	97	528 ± 54§	22
Baboon	Kenya	247	20 w	829	278	1,107§	23
						730 ± 80§	
Rhesus monkey	NIH-PR	200	13 w	561	169	460 ± 85§	24
Rhesus monkey	NIH-PR	36	35 w	362	98	534 ± 46§	24
Rhesus monkey	NIH-PR	18	12 w	416	118	659 ± 67§	25
Rhesus monkey	NIH-PR	16	27 w	379	280	742 ± 44§	25
Rhesus monkey	W-PR	114	27 w	509	233	702 ± 45§	26
Rhesus monkey	St. Lucia	137	27 w	506	196	1,123 ±	26
Rhesus monkey	Belo Horizonte, Brazil	78	27 w	699	424	227§	26
Rhesus monkey	Mwanza	219	27 w	530	138	668 ± 32§	26

Parasitologic findings for S. japonicum

Active infections were present in all 11 mice examined at seven weeks, in all 15 examined at 10 weeks, and in all 10 examined at 15 weeks. Three apparently inactive infections were found among 21 mice examined at 26 weeks, but since two of these had normal numbers of eggs in the tissues, we considered all these mice in our analysis. In five of 12 mice examined at 54 weeks of infection, an occasional egg in the feces was the only sign of activity. The number of eggs in the tissues of these mice did not differ significantly from the number in the remaining mice.

The livers of *S. japonicum*–infected mice contained an average of 23–42% of the eggs in the tissues (Figure 1), the lungs 0.04–0.4%, and the intestines the remainder.

The number of eggs in the feces of these mice increased between seven and 10 weeks after infection and averaged 1,200 eggs/day at 10 and 15 weeks, and then decreased to 300–500 eggs/day 26–54 weeks after infection. Changes in fecal egg excretion with time were highly significant ($P < 0.001$ by ANOVA). The rate of accumulation of eggs in the tissues followed a similar trend (Figure 2). Few eggs are destroyed in the tissues of *S. japonicum*–infected mice after treatment,[8] and we estimate that egg production by the worms varies from a mean of approximately 2,300/day 10–15 weeks after infection to approximately 1,000/day between 26 and 54 weeks (Figure 2). The number of eggs passed in the feces correlated significantly with the number of eggs in the intestines at seven and 10 weeks but not thereafter (Table 1). No uterine eggs were found in female worms in two of 12 mice with active infections at 54 weeks. The number of eggs present in the tissues or passed in the feces did not correlate with the number of uterine eggs.

The variation in egg numbers in the tissues and in the feces was enormous in both *S. mansoni* and *S. japonicum* infections (Figure 3).

Pathologic findings for S. mansoni

Five mice died after the fifth week of infection. These mice were not examined.

The volume of circumoval granulomas around mature eggs decreased two-fold between eight and 20 weeks and did not change significantly thereafter (Figure 4). Hepatic fibrosis increased steadily throughout the year of infection but the collagen content, normalized for egg number, decreased after the eighth week (Figure 5). At each time period, hepatic fibrosis per 10,000 eggs was inversely related to the number of eggs in the liver (log-log regressions).

Slight-to-moderate regeneration or atrophy of different portions of the liver was evident 20 weeks after infection and by one year nearly all mice with active infections showed marked focal regeneration and atrophy (Figure 6). Atrophic areas showed marked fibrosis and numerous eggs microscopically while regenerating areas contained fewer eggs and less fibrosis.

The small intestine showed only slight thickening in most *S. mansoni*–infected mice and the colon was normal in appearance.

Pathologic findings for S. japonicum

Eighteen mice died after egg laying began. Of these, eight were examined and found to have approximately 1.5 times more eggs in the tissues than expected from calculations based on the mice killed.

Granulomas around mature eggs decreased 10-fold in volume between seven and 15 weeks after infection and did not change in size thereafter (Figure 4). The level of hepatic collagen was approximately three times that of normal livers of unexposed and sham-operated mice at seven weeks and six times normal by 10 weeks but did not change significantly thereafter (Figure 5). The collagen content corrected for egg number did not change significantly after the seventh week of infection.

Marked focal regeneration and moderate focal

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* PR = Puerto Rican; NA = not available.

† Standard error of the difference of the means (the square root of the sum of the squares of the standard errors of the means of the tissue eggs at the two time points used to make the calculation).

‡ Values are calculated from data or graphs in the reference publication.

§ Eggs are assumed to have a half-life of eight days in the tissues of baboons and African green monkeys, the same as that determined for rhesus monkeys.[27] The rate of destruction was assumed to be the same for all strains of *S. mansoni*. Egg destruction has been used to calculate fecundity levels only for these nonhuman primates.

TABLE 3

Estimates of fecundity of S. japonicum *in other studies**

Host	Worm strain	Mean no. of worm pairs	Interval, weeks (w) or days (d)	Tissue eggs/day ± SED†	Fecal eggs/day	Total eggs/day	Reference
Swiss mouse	Jap	1	8–18 w	1,057 ± 263	NA	NA	11
Swiss mouse	Jap	1	18–26 w	214 ± 416	NA	NA	11
Swiss mouse	Phil	1	8–18 w	643 ± 172	NA	NA	11
Swiss mouse	Form	1	8–18 w	1,071 ± 479	NA	NA	11
Swiss mouse	Form	1	18–26 w	875 ± 1,011	NA	NA	11
ddY mouse	Jap	4–7	4–15 w	NA	NA	2,100	28
CBA mouse	Chin	3–5	6–14 w	1,107‡	NA	NA	29
CBA mouse	Chin	3–4	14–28 w	1,561‡	NA	NA	29
Hamster	Form	9	21–42 d	3,112‡	388‡	3,500	21
Rabbit	Jap	67	10–17 w	435 ± 194	403	838	30
Rabbit	Jap	63	17–32 w	550 ± 225	462	1,012	30
Rabbit	Jap	55	32–53 w	490 ± 242	280	770	30
Rabbit	Phil	55	8–18 w	405 ± 129	95	500	30
Rabbit	Phil	38	31–53 w	1,530 ± 294§	71	1,601§	30

* Jap = Japanese; NA = not available; Phil = Philippine; Form = Formosan; Chin = Chinese.

† SED = standard error of the difference of the means (the square root of the sum of the squares of the standard errors of the means of the tissue eggs at the two time points used to make the calculation).

‡ Values are calculated from data or graphs in the reference publication.

§ It is likely that the high number of in tissue eggs per worm pair reflects death of substantial numbers of worm pairs whose eggs contributed to the total eggs in the tissues.

atrophy were noted in the liver 26 and 54 weeks after infection. Hepatic fibrosis was less marked than in *S. mansoni*–infected mice (Figure 5).

The small intestine showed increasing focal distension and mural thickening during the course of infection (Figure 7). Mice that died had marked focal dilatation of the gut without other evident cause of death. Intestinal obstruction, i.e., dilatation of the gut proximal to gross lesions, was present in only one of eight dead mice examined and was not seen in mice that we killed.

DISCUSSION

The fecundity of *S. mansoni* remained constant in mice infected with a single worm pair and the passage of eggs in the feces reflected worm fecundity with time and in individual mice. The estimates of fecundity are minimal because some eggs were probably missed in examining the feces and some eggs were doubtless destroyed in the tissues. Correction for possible destruction of eggs in the tissues, which we estimate to be no more than 2% per month in *S. mansoni* infections[9] and no more than 1% per month in *S. japonicum* infections,[8] has no marked effect on our estimates of fecundity (Figure 8). Although the rate of egg laying by fecund worms did not change with time, substantial numbers of *S. mansoni* worm pairs died or became infertile.

It is evident from Table 2 that the number of eggs found in the feces is not always a good indication of fecundity. Kloetzel found a sharp decrease in passage of *S. mansoni* eggs in heavily infected Swiss mice approximately 60 days after infection, nearly two months before he found a decrease in the rate of egg accumulation in the tissues.[11] In our *S. japonicum*–infected mice, the number of fecal eggs was a poor predictor of the number of tissue eggs, particularly in the liver, in individual animals (Table 1). There was a better correlation between fecal and tissue egg counts in *S. mansoni*–infected mice. Counting of fecal eggs in infected humans continues to be the primary method for estimating intensity of infection and, in addition, is the basis on which one checks the validity of other tests reflecting infection intensity, such as serum or urine antigen concentration. It will be of interest to see whether these methods will give a more valid estimate of egg deposition in situations in which our fecal egg counts were unreliable, e.g., in mice with chronic *S. japonicum* infection.

The fecundity of *S. japonicum* worm pairs decreased markedly between the 15th and 26th weeks of infection and continued to decrease thereafter. Fecal egg passage reflected fecundity

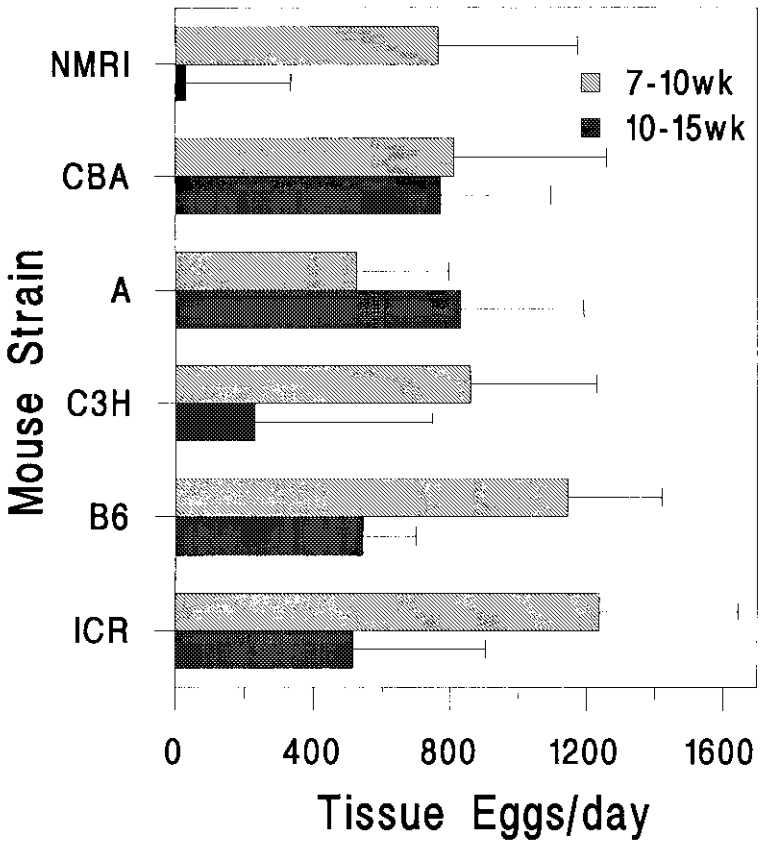

FIGURE 9. Incremental change in tissue eggs between the seventh and 10th weeks of infection compared with that between the 10th and 15th weeks of infection in several strains of mice infected with *Schistosoma japonicum* (data from[10]). Horizontal lines show one standard error of the difference of the means. Apparent fecundity is lower in chronically infected mice of all strains except A. This is probably due to the variability arising from subtracting numerous means rather than a characteristic of the A strain.

only at seven and 10 weeks after infection. This might be related to the marked thickening of the bowel wall (Figure 7) and also to the tendency of *S. japonicum* to nest, i.e., to lay eggs at a few selected sites in the bowel.[12]

We had noted previously that worms containing less than 20 uterine eggs did not lay significant numbers of eggs in mice infected with a single worm pair.[12] This was clearly not the case in the present study. The variability in the number of eggs in the tissues or feces of individual mice was astounding (Figure 3) and increased with time. Delay in mating might contribute to differences in egg number at the first time point, but this seems unlikely to be important later. Some *S. mansoni* worm pairs become infertile and it would seem that this, were it a gradual process, should contribute to variability. However, fecundity remained constant with time and infertile worm pairs were more frequent in mice with chronic *S. mansoni* infection, indicating that there was no prolonged period of markedly decreased fecundity. Genetic differences in the worms seem the most likely cause of varied fecundity but environmental effects on the mice, such as inapparent intercurrent infections, might also affect the worms. Injury to the worms during transplantation seems an unlikely cause of variable fecundity in chronically infected mice.

Our estimates of fecundity in mice infected with a single worm pair are generally similar to those in mice infected with more than one pair of worms (Tables 2 and 3 and Figure 9). A particular concern in the latter is that death of worm

pairs would lead to an apparent increase in tissue eggs per worm pair,[13] while infertile worm pairs or the death of hosts bearing more fecund worms would cause an apparent decrease in eggs per worm pair. Finally, mice infected with a single *S. mansoni* pair have very intense infections compared with most infected primates, including humans,[1] and infections of more than one worm pair in mice may be less relevant to reasonable levels of infection. Our own results in B6 mice infected with multiple pairs of worms differ slightly from those reported here, and the number of eggs per worm pair passed in the feces decreased with time (Table 2). The number of eggs in the feces and tissues of *S. mansoni*–infected BALB/c and B6 mice also differed significantly in that study.[13] Fecundity of *S. mansoni* is apparently higher in nonhuman primates than in mice; however, the calculation of fecundity in primates is critically dependent upon the rate of egg destruction in the tissues (the half-life of eggs in the tissues is only eight days), and this calculation is based on results from a small number of rhesus monkeys.[27]

Our results are clearly not relevant to all host-parasite combinations (Tables 2 and 3). Rhesus monkeys infected with *S. mansoni* show decreasing numbers of eggs per worm pair in the tissues and feces in chronic infections and fecundity in this host is clearly related to intensity[25] and duration of infection (Table 2). More intense infections may stimulate an earlier immune response in this host, which is only temporarily permissive. The strain of *S. japonicum* used to infect rabbits markedly affects the passage of eggs in the feces (Table 3). Although *S. japonicum* eggs are destroyed rapidly in many nonhuman primates,[31] the rate of egg destruction is unknown and consequently the fecundity of *S. japonicum* in primates cannot be estimated.

Hepatic fibrosis overall and fibrosis per egg were much higher in *S. mansoni*–infected mice than in *S. japonicum*–infected ones (Figure 5). The relatively high level of collagen in the liver of *S. mansoni*–infected mice is partly related to the higher proportion of eggs in the liver compared with those in *S. japonicum*–infected animals, but the total number of eggs per liver was higher at all times in *S. japonicum*–infected mice (Figure 1).

Infections with a single worm pair did not lead to the uniform tissue egg numbers and uniform pathologic changes that we had hoped for. However, single worm-pair infections offer a unique opportunity to examine worm fecundity and the great variability among schistosome worm pairs. The fecundity of worms is of basic interest and, in addition, the effects of vaccines on the fecundity of schistosomes may be relevant.[32]

Acknowledgments: The *S. japonicum*–infected snails were provided through an NIAID supply contract (AI-052590) and the *S. mansoni* cercariae were provided by Dr. Fred Lewis (Biomedical Research Institute, Rockville, MD).

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