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Host-Parasite Relationship of *Bulinus truncatus* and *Schistosoma haematobium* in Iran

2. Effect of Exposure Dosage of Miracidia on the Biology of the Snail Host and the Development of the Parasites

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Laboratory experiments were carried out to study the development of Bulinus truncatus *and the larval stages of* Schistosoma haematobium *after the snails had been exposed to various numbers of miracidia. The results showed: (1) that in the cercarial-incubation period the growth and survival rate of snails was not influenced by the development of the larval stages of* S. haematobium, *but that in the cercaria-shedding period the life-span of the infected snails was shorter than that of the non-infected controls; (2) that reduction of oviposition was proportional to the exposure of miracidia; (3) that the length of the cercarial-incubation period in snails was inversely proportional to the exposure number of miracidia; (4) that all the snails exposed to 20 miracidia (the maximum exposure number used) shed cercariae; (5) that snails exposed to one miracidium each shed fewer cercariae than those exposed to two or more miracidia each; and (6) that the peak of cercaria-shedding occurred 40 to 90 days after the shedding had started, varying in different groups.*

The effect of the exposure dosage of miracidia on the biology of the snail host and on the subsequent development of the larval stages of the parasites is an interesting problem involving the host-parasite relationship of *Bulinus truncatus* and *Schistosoma haematobium*. Except for the study made by Najarian (1961) on the egg-laying capacity of *B. truncatus* after its exposure to infection with *S. haematobium*, no work has previously been done on this problem. The present paper describes some research on this topic carried out by us in Iran.

MATERIALS AND METHODS

Newly laid egg-masses of *B. truncatus* were removed from an aquarium and put into crystallizing dishes, which were paved with a mud substratum supporting a flourishing growth of algae, and the newly hatched snails were maintained in these dishes. When the snails were three to four weeks old, 300 of them were measured with a precision of 0.2 mm and divided into six groups containing 50 each. The length of the snails ranged from 2.8 to 4.6 mm, the average being 3.48 mm. Snails in Group I were used as controls. Each individual snail in Groups II, III, IV, V and VI was exposed to 1, 2, 5, 10 and 20 miracidia respectively. The miracidia were obtained from the pooled urine of 15 patients who were in the active stages of bilharziasis caused by *S. haematobium*. The miracidia were counted under a stereoscopic microscope and transferred with a fine pipette to the bottom of a 10-ml test-tube. One snail was added to each tube. The amount of water in the tube was just sufficient to cover the exposed snail. The room temperature during exposure was about 25°C, and the exposure time was six hours. After the exposure, snails in each group were maintained in separate crystallizing dishes. Laboratory-stored river-water was used for a daily change. Fresh lettuce was used as food. The water temperature during the first 40 days ranged from 22°C to 24°C and in the following period from 21°C to 24°C. The experiment was started in November and continued until April. Every time the water was changed in the crystallizing dishes, the numbers of egg-masses and dead snails were recorded.

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TABLE 1

LONGEVITY OF NON-INFECTED AND CERCARIA-SHEDDING *B. TRUNCATUS*

Time since exposure (days)	Surviving snails, Group I (non-infected)		Surviving snails, Group II (1 miracidium)		Surviving snails, Group III (2 miracidia)		Surviving snails, Group IV (5 miracidia)		Surviving snails, Group V (10 miracidia)		Surviving snails, Group VI (20 miracidia)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
50	30 [a]	100.0	9 [a]	100.0	20 [a]	100.0	21 [a]	100.0	30 [a]	100.0	30 [a]	100.0
60	30	100.0	9	100.0	19	95.0	21	100.0	27	90.0	28	93.3
70	29	96.7	8	88.9	19	95.0	20	95.2	26	86.7	27	90.0
80	27	90.0	5	55.6	16	80.0	10	47.6	26	86.7	22	73.3
90	24	80.0	3	33.3	13	65.0	9	42.9	21	70.0	20	66.7
100	24	80.0	0	0	10	50.0	5	23.8	17	56.7	1	3.3
110	24	80.0			6	30.0	4	19.1	15	50.0	1	3.3
120	23	76.7			6	30.0	4	19.1	12	40.0	1	3.3
130	19	63.3			5	25.0	2	9.5	7	23.3	1	3.3
140	18	60.0			2	10.0	0	0	4	13.3	1	3.3
150	17	56.7			2	10.0			2	6.7	0	0
160	16	53.3			1	5.0			0	0		
170	15	50.0			0	0						

[a] Number of snails used when experiment was started.

Beginning on the 35th day after exposure, snails in the five exposed groups were tested for cercaria-shedding. On the 38th day after exposure, a few snails were noted to be shedding cercariae, and the size of each snail in the six groups was measured at that time. The number of days from the date of exposure to the date of the first shedding of cercariae was regarded as the cercarial-incubation period for each respective snail. On the 66th day after exposure, all the snails that had not shed carcariae were crushed and the presence of cercariae in these crushed snails was recorded. Snails that died through crawling out of the water during this period were also examined for the presence of cercariae. The infection rate of the snails was calculated from the number of cercaria-positive snails present among the surviving snails at the time of the first shedding of cercariae. Dead snails found to be cercaria-positive after crushing were regarded as positive snails in the calculation of the infection rate.

After the snails had started shedding cercariae, their mortality rates, oviposition capacity, and daily output of cercariae were observed. Thirty snails were randomly selected from the surviving snails in Group I, 9 from Group II, 20 from Group III, 21 from Group IV, 30 from Group V, and 30 from Group VI. All the snails from Groups II-VI were shedding cercariae. These snails were maintained in crystallizing dishes with a constant water volume of 1 litre and with 10 snails or less per dish. The number of dead snails and the number of newly laid egg-masses were recorded daily. The daily output of cercariae from the infected snails was measured in the following way. Every day the snail room was artificially lighted from 12 noon until 3 p.m. At 3 p.m. the water was changed. All the water from each dish was poured into a separate 1-litre cylinder and formol added in a sufficient quantity to give a concentration of 5%. After standing overnight, the upper portion of the solution in each cylinder was decanted and the lower portion of the water from all cylinders belonging to the same group was pooled in a 1-litre cylinder. Tap-water was added to bring the final volume up to 1 litre. Each cylinder thus contained the total number of cercariae from one of the five groups of infected snails. After the water had been shaken manually to ensure an even distribution of cercariae, five 1-ml samples were taken from each cylinder and the number of cercarie counted. Thus, the total number of cercariae in the five samples, multiplied by 200 and divided by the number of snails in each group,

TABLE 2
SECOND EXPERIMENT ON THE LONGEVITY OF CERCARIA-SHEDDING *B. TRUNCATUS*

Time since exposure (days)	Snails surviving of those exposed to 1 miracidium		Snails surviving of those exposed to 5 miracidia		Snails surviving of those exposed to 20 miracidia	
	No.	%	No.	%	No.	%
30	28	100.0	30	100.0	44	100.0
40	23	82.1	28	93.3	26	59.1
50	22	78.6	19	63.3	17	38.6
60	22	78.6	14	46.7	11	25.0
70	21	75.0	8	26.7	8	18.2
80	20	71.4	7	23.3	2	4.6
90	12	42.9	7	23.3	0	0
100	5	17.9	5	16.7		
110	4	14.3	4	13.3		
120	3	10.7	2	6.7		
130	2	7.1	2	6.7		
140	1	3.6	1	3.3		
150	1	3.6	1	3.3		
160	1	3.6	0	0		
170	0	0				

gave the average number of cercariae shed per snail per day.

The statistical analyses used in this work were mainly significance tests for the means and the calculation of correlation coefficients. As the method for the significance tests for the means is a complicated one, a short description of the method seems necessary. Our method followed that of Hsü & Hsü (1960). For the comparison of the means of samples from two groups, the homogeneity of the variances was investigated by the *F*-test. If the variances were homogeneous, Student's *t*-test was then performed. If the variances were heterogeneous, the modified procedure developed by Welch & Aspin was used. For the comparison of the means of samples from more than two groups, the homogeneity of variances was tested by Bartlett's method. If the variances were homogeneous, analysis of the variance was made by the *F*-test. Pairwise comparisons of the means by Student's *t*-test were made if the general significance test pointed to real differences between the means. If the variances were heterogeneous, the Kruskal-Wallis one-way analysis of variance by rank was performed. When real differences in variance among the groups were found, the means of any pair of the groups were again tested for the significance of difference by the modified procedure of Welch & Aspin. A difference at the 5% level was regarded as significant.

RESULTS

Biology of B. truncatus

Growth of the exposed snails. At the time of miracidial exposure, snails in all groups were of a similar size. On the 38th day after the exposure, namely, the date of the first shedding of cercariae, the means of the size of the snails in Groups I-VI

TABLE 3
EGG-LAYING CAPACITY OF *B. TRUNCATUS* DURING THE MINIMUM CERCARIAL-INCUBATION PERIOD (0-38 DAYS AFTER EXPOSURE)

Group	No. of snails	No. of egg-masses	No. of eggs	Eggs/mass (mean)	Egg-masses/ snail (mean)	Eggs/snail (mean)
I (controls)	42	44	250	5.7	1.1	6.0
II (1 miracidium)	41	55	243	5.5	1.3	6.4
III (2 miracidia)	45	45	208	4.6	1.0	4.6
IV (5 miracidia)	44	35	179	5.1	0.8	4.1
V (10 miracidia)	42	18	94	5.2	0.4	2.2
VI (20 miracidia)	42	18	95	5.3	0.4	2.3

TAB

EGG-LAYING CAPACITY OF INFECTED AND NON-INFECTED *B. TRUNCATUS* DURING TI

Time since exposure (days)	Group I (non-infected)			Group II (1 miracidium)			Group III (2 miracidia)		
	No. of surviving snails	No. of eggs	Eggs/snail (mean)	No. of surviving snails	No. of eggs	Eggs/snail (mean)	No. of surviving snails	No. of eggs	Eggs/snai (mean)
53-62	30	334	11.1	9	12	1.3	20	15	0.8
63-72	29	392	13.5	8	40	5.0	19	70	3.7
73-82	27	545	20.2	7	35	5.0	18	64	3.6
83-92	26	535	20.6	4	25	6.3	15	98	6.5
93-102	24	537	22.4	3	23	7.7	12	95	7.9
103-112	24	839	35.0	0	0	0	8	92	11.5
113-122	24	837	34.9				6	108	18.0
123-132	22	646	29.4				6	81	13.5
133-142	19	154	8.1				5	0	0
143-152	18	883	49.1				2	10	5.0
153-162	16	568	35.5				2	10	5.0

were 6.8, 6.8, 6.9, 7.1, 6.8 and 7.0 mm respectively, and their standard deviations were 0.57, 0.59, 0.53, 0.92, 0.85 and 0.81. The differences between these mean sizes were not significant. The results showed that, in the cercarial-incubation period, the growth of the control snails in Group I did not differ from that of the exposed snails in Groups II-VI and that the growth of exposed snails was not influenced by the intensity of infection. No snails were measured thereafter.

Survival of the exposed snails. At the time of exposure to miracidia there were 50 snails in each of the six groups. At the end of the minimum cercarial-incubation period (38 days after exposure), the number of the surviving snails in the control group was 42, and the number of surviving snails in Groups II-VI were 41, 45, 44, 42 and 42. The differences between these numbers was not significant. Thus in the cercarial-incubation period the survival rate of the control snails was not essentially different from that of the exposed snails; nor did the survival rate of snails exposed to light doses of miracidia differ significantly from that of snails exposed to heavy doses.

The life-span of the cercaria-shedding snails was, however, shorter than that of the non-infected snails in the control group. This is best demonstrated by the fact that on the 170th day after exposure, all the 110 cercariae-shedding snails in the exposed groups were dead, whereas in the control group 15 out of the 30 snails were still alive (Table 1).

The mean survival periods of the cercaria-shedding snails were 87.8, 110.0, 95,7, 105.3 and 94.0 days from the time of exposure for Groups II-VI respectively, and the maximum survival periods were 100, 170, 140, 160 and 150 days after exposure respectively. The data showed that the mean life-spans of the cercaria-shedding snails of Groups II, IV and VI were shorter than those of Groups III and V. This variation was clearly inconsistent with the number of miracidia used to infect each snail in the different groups and the results were therefore difficult to interpret. Accordingly, a second experiment was conducted.

One hundred snails were exposed to one miracidium each, 50 to five miracidia each, and 50 to 20 miracidia each. Because the age of the snails and the temperature in the laboratory were not the same as in the first experiment, some snails started to shed cercariae on the 33rd day after exposure. The number of cercaria-shedding snails was 28 for the one-miracidium group, 30 for the five-miracidia group, and 44 for the 20-miracidia group (Table 2). Results of further calculation of the data in the table showed that the mean post-exposure survival for the cercaria-shedding snails in the one-miracidium

:ERCARIA-SHEDDING PERIOD (ALL INFECTED SNAILS BEING CERCARIA-SHEDDING)

Group IV (5 miracidia)			Group V (10 miracidia)			Group VI (20 miracidia)		
No. of surviving snails	No. of eggs	Eggs/snail (mean)	No. of surviving snails	No. of eggs	Eggs/snail (mean)	No. of surviving snails	No. of eggs	Eggs/snail (mean)
21	36	1.7	30	12	0.4	30	5	0.2
21	151	7.2	26	90	3.5	27	31	1.1
19	92	6.1	26	22	0.8	26	0	0
15	17	1.9	25	46	1.8	22	0	0
9	21	2.3	20	53	2.7	13	0	0
4	51	10.2	17	43	2.5	1	0	0
4	46	11.5	14	76	5.4	1	24	24.0
2	5	2.5	11	46	4.2	1	0	0
0	0	0	7	0	0	1	0	0
			3	56	18.7	0	0	0
			2	26	13.0			

group was 89.3 days; in the five-miracidia group 71.3 days and in the 20-miracidia group 54.5 days. The standard deviations for these three groups were 31.7, 31.8 and 16.2 respectively. Statistical analysis showed that the differences between the three means were significant. The mean life-span of the one-miracidium group was longer than that of the five-miracidia group, which was in turn longer than that of the 20-miracidia group. We cannot definitely state that the results of the first experiment were due to experimental error, but those obtained in the second experiment seem more reasonable.

TABLE 5

MEAN NUMBER OF EGGS PER MASS

Time since exposure (days)	Non-infected snails			Cercaria-shedding snails		
	No. of egg-masses	No. of eggs	Eggs/mass (mean)	No. of egg-masses	No. of eggs	Eggs/mass (mean)
53-62	62	334	5.4	20	80	4.0
63-72	64	392	6.1	95	383	4.2
73-82	78	545	7.0	51	212	4.2
83-92	72	535	7.4	41	186	4.5
93-102	71	537	7.6	41	192	4.7
103-112	112	839	7.5	43	186	4.3
113-122	103	837	8.1	56	254	4.5
123-132	79	646	8.2	21	132	6.3
133-142	20	154	7.7	0	0	0.0
143-152	106	883	8.3	9	66	7.3
153-162	67	568	8.5	5	36	7.2

TABLE 6

EFFECT OF DOSAGE OF MIRACIDIA ON THE CERCARIAL-INCUBATION PERIOD OF *S. HAEMATOBIUM* IN *B. TRUNCATUS*

Group	Number of miracidia used to infect each snail	Total number of cercaria-shedding snails	Number of snails shedding cercariae for the first time on the following days after exposure:																				Mean length of cercarial-incubation period in days (and standard deviation)
			38	39	40	41	42	43	44	45	46	47	48	49	50–54	55	60–61	62	63	64	65	66	
II	1	10	0	0	0	2	2	2	0	2	0	0	0	1	0	0	0	0	0	0	0	1	45.7 (7.5)
III	2	26	2	4	2	4	2	5	0	0	2	0	1	1	0	1	0	1	0	1	0	0	44.0 (6.8)
IV	5	24	1	3	1	3	5	3	0	3	1	0	1	3	0	0	0	0	0	0	0	0	43.0 (3.3)
V	10	35	2	7	5	6	4	3	0	2	1	0	3	2	0	0	0	0	0	0	0	0	42.0 (3.3)
VI	20	42	10	18	2	5	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39.6 (1.4)

Egg-laying capacity. Studies were carried out on the egg-laying capacity of the exposed snails. These studies were divided into two periods: (1) the minimum cercarial-incubation period, i.e., from the time of miracidial exposure to the time of the first shedding of cercariae, covering 38 days, and (2) the cercaria-shedding period itself, starting from the 53rd day after exposure until the death of all the cercaria-shedding snails, covering 110 days.

In the minimum cercarial-incubation period the average number of eggs laid per snail was 6.0 for the control group and 6.4, 4.6, 4.1, 2.2 and 2.3 for Groups II-VI respectively (Table 3). While no reduction of egg-laying capacity was shown in snails exposed to one miracidium each (Group II), a significant reduction of this capacity was shown in snails exposed to two or five miracidia (Groups III and IV) and a particularly significant reduction in the snails exposed to 10 or 20 miracidia each (Groups V and VI).

In the cercaria-shedding period, a total of 110 egg-counting days was recorded. Table 4 shows that the mean number of eggs per snail in successive 10-day periods was lower in the infected groups than in the non-infected group. The average mean numbers of eggs laid per snail per 10-day period were 25.4, 5.1, 6.9, 5.4, 4.8 and 2.8 for Groups I-VI respectively. The egg-laying capacities of infected snails (compared with that of control snails) were thus reduced in the ratios 5.0, 3.7, 4.7, 5.3 and 9.1 for Groups II-VI respectively.

In the minimum cercarial-incubation period, the mean numbers of eggs per mass for the snails in Groups I-VI were 5.7, 5.5, 4.6, 5.1, 5.2 and 5.3 respectively (Table 3). Thus, there was apparently a slight decrease in the number of eggs per mass for snails in the exposed groups compared with the number for snails in the control group. In the cercaria-shedding period, the average numbers of eggs per mass for the cercaria-shedding snails were 4.2, 5.0, 3.6, 5.2 and 4.6 in Groups II-VI respectively. There was no significant difference between the mean numbers of eggs per mass in these five groups, which indicates that the number of eggs per mass produced by the infected snails was not affected by the number of miracidia originally received. However, if the pooled results of egg-depositions by all cercaria-shedding snails recorded at 10-day intervals are compared with the results obtained from non-infected snails under similar conditions (Table 5), two interesting facts emerge—the number of eggs per mass increases with time in both the non-infected

TABLE 7

EFFECT OF EXPOSURE DOSAGE ON INFECTION RATE

Group	Number of miracidia used to infect each snail	Number of snails exposed to infection	Number of snails surviving at the time of first shedding of cercariae	Number of surviving snails cercaria-positive	Percentage of surviving snails cercaria-positive
II	1	50	41	10 [a]	24.4
III	2	50	45	26	57.8
IV	5	50	44	27 [b]	61.4
V	10	50	42	35	83.4
VI	20	50	42	42	100.0

[a] Including one snail found to be cercaria-positive after crushing on day 66.
[b] Including three cercaria-positive snails that died through crawling out of the water before the first shedding of cercariae.

TABLE 8

EFFECT OF EXPOSURE DOSAGE ON PRODUCTION OF CERCARIAE DURING SUCCESSIVE TEN-DAY INTERVALS

Number of days after first shedding of cercariae [a]	Group II (1 miracidium)		Group III (2 miracidia)		Group IV (5 miracidia)		Group V (10 miracidia)		Group VI (20 miracidia)	
	No. of cercaria-shedding snails (mean)	No. of cercariae per snail per day (mean)	No. of cercaria-shedding snails (mean)	No. of cercariae per snail per day (mean)	No. of cercaria-shedding snails (mean)	No. of cercariae per snail per day (mean)	No. of cercaria-shedding snails (mean)	No. of cercariae per snail per day (mean)	No. of cercaria-shedding snails (mean)	No. of cercariae per snail per day (mean)
0-10	4	18.4	14	31.9	12	28.2	21	30.9	29	70.0
11-20	9	89.8	19	63.6	21	106.9	28	133.9	29	139.2
21-30	8	62.7	19	65.3	20	53.3	27	53.4	28	44.0
31-40	7	123.1	17	173.8	17	199.3	26	189.3	24	163.8
41-50	4	62.0	15	227.1	10	275.6	24	156.7	21	178.5
51-60	1	53.3	12	428.6	7	393.1	19	224.3	10	242.5
61-70	0	0	8	307.4	4	344.5	15	163.1	1	180.0
71-80			6	510.0	4	790.0	13	279.7	1	740.0
81-90			5	523.0	3	445.0	9	173.6	1	1180.0
91-100			4	451.4	2	15.0	5	113.0	1	456.4
101-110			2	410.0	0	0	3	138.3	1	20.0
111-120			2	441.9			2	14.6	0	0
121-130			1	369.1			0	0		
131-140			0	0						

[a] 0 days after first shedding of cercariae = 38 days after exposure.

and cercaria-shedding groups; and the mean number of eggs per mass for the cercaria-shedding snails is significantly less than that for the non-infected snails in all the 10-day intervals.

Development of the parasites

Length of the cercarial-incubation period. The average length of the cercarial-incubation period for the snails in Groups II-VI was 45.7, 44.0, 43.0, 42.0 and 39.6 respectively (Table 6). Thus, the length of the cercarial-incubation period was shorter for the snails that had been exposed to more miracidia.

Infection rate. The infection rates of snails in Groups II-VI were 24.4%, 57.8%, 61.4%, 83.4% and 100% respectively (Table 7), indicating that the infection rate increased with the exposure dosage of miracidia.

Number of cercariae shed by an infected snail. The mean total numbers of cercariae shed by a snail during the first 50 days after the first cercarial shedding were 3560, 5617, 6633, 5642 and 5955 for Groups II-VI respectively. Thus the snails in Group II (exposed to one miracidium each) appear to shed fewer cercariae than those in other groups. It is interesting to note that one surviving snail in Group VI shed about 25 764 cercariae during the last 50 days of its life (Table 8).

Cercaria-shedding curve. Beginning from the day of the first shedding of cercariae, an average number of cercariae shed per snail per day in a period of ten days was calculated throughout the whole cercaria-shedding period of the snails (Table 8). In Group II, the peak of cercaria-shedding was reached about 30-40 days after the first shedding; in Group III, about 80-90 days; in Groups IV and V, 70-80 days; and in Group VI, 80-90 days.

DISCUSSION

The results of the present studies show that, when *B. truncatus* is exposed to the miracidia of *S. haematobium*, there is a relationship between the dosage of miracidia, the biology of the snail, and the development of the succeeding larval stages of the parasite. The effects on the biology of the exposed snails were manifested by changes in their growth rate, survival rate and egg-laying capacity. The effects on the development of the parasites were shown in the length of the cercarial-incubation period, the infection rate, the total number of cercariae shed by an infected snail, and the cercaria-shedding curve.

As mentioned previously, Najarian (1961) has studied the egg-laying capacity of *B. truncatus* after exposure to infection with *S. haematobium* in Iraq. He reported that the reduction in the number of eggs per mass was more marked than the decrease in the number of egg-masses, and that the total number of eggs laid by non-infected snails was about eight times the number of eggs laid by infected ones. Our own studies showed that the reduction in the number of eggs per mass was *less* marked than the decrease in the number of egg-masses, and that the rate of reduction in egg-laying was proportional to the dosage of miracidia given to the snails. The egg-laying capacity was more intensely reduced in the cercaria-shedding period than in the cercarial-incubation period; however, complete suppression of reproductive function did not occur even in the snails that had been exposed to 20 miracidia each.

While the host-parasite relationship differs with different species of host and parasite, comparison may be made to a certain extent with closely related species. Of course, such comparison should be made and interpreted with caution.

Pan (1963) found that *Australorbis glabratus* snails, after exposure to the miracidia of *Schistosoma mansoni*, grew faster than the non-infected controls in the first six weeks, but that the size of the snails in these two groups became equal in the seventh week. While such initial growth acceleration was not observed in the present study of the *truncatus-haematobium* relationship, there was, at least, no retardation of the growth of the exposed snails in the cercarial-incubation period.

The reduction of egg-laying capacity of infected snails does not occur only in *B. truncatus* infected with *S. haematobium.* Several authors have observed sporocysts of *S. mansoni* in the ovotestis of *A. glabratus* (Barbosa & Olivier, 1958). Brumpt (1941) reported that infected *A. glabratus* laid fewer eggs than non-infected ones. Coelho (1954) showed that, although a complete suppression of reproduction did not occur in infected *A. glabratus*, the infection was responsible for a significant reduction in the number of egg-masses and eggs. Pan (1963) observed that egg production in infected *A. glabratus* was completely suppressed by the end of the sixth week and was resumed thereafter during a period of eight months. In *B. truncatus* infected with *S. bovis*, Lengy (1962) found that the hepatopancreas and ovotestis of the snail were extensively invaded by numerous saccular and convoluted daughter sporocysts. Pesigan et al. (1958) reported that *Oncomelania quadrasi* snails, infected with *Schistosoma japonicum*, laid fewer eggs than the non-infected ones. We may conclude that the invasion of larval stages into the ovotestis of their snail intermediate hosts is a common phenomenon for bilharziasis parasites, and the egg-laying capacity of the infected snails is accordingly reduced.

Pesigan et al. (1958) studied the cercarial-incubation period of *Schistosoma japonicum* in *Oncomelania quadrasi* and found that there was no difference in the length of cercarial-incubation period between the snails exposed to one miracidium and those exposed to two to five miracidia. The results of our studies on the cercarial incubation period in *B. truncatus* exposed to various numbers of miracidia of *S. haematobium* were at variance in this respect with those obtained by Pesigan et al. We found that the length of the cercarial-incubation period was inversely proportional to the exposure dosage. It would be of great interest if groups of *O. quadrasi* were exposed individually to 1, 2, 5, 10 and 20 miracidia of *S. japonicum*, as performed in the present experiment, and the resulting cercarial-incubation periods from these groups compared.

Investigating the infection rate of snails to miracidia, Schreiber & Schubert (1949a) exposed individual specimens of *A. glabratus* to five to seven miracidia of *S. mansoni* and found that 25% of the snails were refractory to infection. In another paper (1949b), they found an increasing percentage of snails shedding cercariae as a result of exposure to increasing numbers of miracidia. Etges (1963) indicated that *A. glabratus* should be 100% susceptible to *S. mansoni* infection if snails were exposed to a sufficiently large number of miracidia. He was of the opinion that failure to achieve 100% infection of the snails is attributable to deficient infectivity of the miracidia rather than to innate resistance of the snails. We are in favour of Etges's point of view in this respect, because we have achieved a 100% infection rate in *B. truncatus* that have been exposed to 20 miracidia of *S. haematobium*.

With regard to the effect of the exposure dosage of miracidia and the number of cercariae shed by the infected snails, Pesigan et al. (1958) reported that *O. quadrasi* exposed to one miracidium of *S. japonicum* shed twice as many cercariae per shedding day as the snails exposed to two to five miracidia. The results of our studies indicated that *B. truncatus* exposed to two or more miracidia of *S. haematobium* shed more cercariae than do snails exposed to one miracidium. It is our opinion that snails exposed to two or more miracidia may eventually develop more sporocysts and more cercariae than the snails exposed to one miracidium. We may consider that the difference in the natural sizes of *B. truncatus* and *O. quadrasi* might play an important role in their optimum exposure dosage of miracidia. Since *O. quadrasi* is much smaller than *B. truncatus*, the optimum exposure dosage for the former could be one miracidium instead of two or more.

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RÉSUMÉ

Dans ce deuxième article sur les relations hôte/parasite entre *Bulinus truncatus* et *Schistosoma haematobium*, les auteurs étudient l'influence du nombre de miracidiums infectants sur la biologie du vecteur et le développement larvaire du parasite. Ils ont commencé leur expérimentation en novembre et l'ont poursuivie jusqu'en juin. Cinq groupes de 50 mollusques chacun ont été exposés respectivement à 1, 2, 5, 10 et 20 miracidiums provenant des urines mélangées de 15 malades; un groupe de 50 mollusques non infectés servait de témoin. Chaque groupe a été conservé dans un cristallisoir distinct. Les cristallisoirs contenaient de l'eau de rivière, conservée au laboratoire et changée chaque jour; à cette occasion, le nombre de pontes et celui des mollusques morts étaient relevés. Les

auteurs ont étudié l'influence du nombre de miracidiums sur la croissance, le taux de survie et la capacité de ponte des mollusques, sur la durée de l'incubation cercarienne, le taux d'infection, le nombre total des cercaires émises par un mollusque et la courbe de l'émission cercarienne. Les taux de survie suivant l'importance de l'infection initiale n'ayant pas montré de différences significatives, les auteurs ont exposé 100 autres mollusques à un miracidium chacun, 50 à cinq et 50 à vingt miracidiums. Les résultats de ces expériences ont montré que: 1) au cours de la période d'incubation, le développement larvaire de *S. haematobium* ne modifiait ni la croissance ni le taux de survie des mollusques mais, après le début de l'émission des cercaires, les mollusques infectés se développaient moins vite et vivaient moins longtemps que ceux du groupe témoin; 2) leur capacité de ponte était diminuée proportionnellement au nombre de miracidiums auxquels ils avaient été exposés; la ponte n'a jamais été entièrement supprimée; 3) la durée de l'incubation cercarienne était inversement proportionnelle au nombre des miracidiums infectants; 4) tous les mollusques exposés à 20 miracidiums (nombre maximal utilisé) émettaient des cercaires; 5) les mollusques exposés à 2 miracidiums émettaient le plus grand nombre de cercaires; 6) la courbe de l'émission cercarienne présentait un pic entre le 40^{e} et le 90^{e} jour après le début de l'émission, suivant les groupes.

Discutant leurs résultats, les auteurs ont fait une revue des expériences semblables réalisées antérieurement avec d'autres hôtes et d'autres espèces de schistosomes. Ils estiment que la limitation de la taille et de l'alimentation du mollusque fait que des mollusques infectés par 5-20 miracidiums fourniront de plus petits sporocystes et un nombre moindre de cercaires que les mollusques exposés à deux miracidiums. La différence de la taille normale de divers mollusques peut être un facteur important déterminant le nombre optimal de miracidiums au cours de l'exposition, qui variera ainsi suivant l'hôte.

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