THE EFFECTS OF TEMPERATURE, LIGHT AND WATER UPON THE HATCHING OF THE OVA OF *SCHISTOSOMA JAPONICUM*

Xiao-Ping Ye[1,3], Ying-Ling Fu[2], Zhong-Xing, Wu[3], Roy M Anderson[1] and Alison Agnew[4]

[1]The Wellcome Trust Centre for the Epidemiology of Infectious Disease, University of Oxford, Oxford OX1 3PS, UK; [2]Yujiang City Hospital of Schistosomiasis, Yujiang, Hunan, PR China; [3]Jiangsu Provincial Institute of Parasitic Diseases, Wuxi, Jiangsu 214064, PR China; [4]Department of Biology, University of Leeds,Leeds LS2 9JT, UK

Abstract. Eggs of *Schistosoma japonicum* were obtained from infected patients' feces from Yujiang City, China to observe the effects of temperature, light and water on the hatching of eggs. The temperature of water and light played important roles on the hatching of *S. japonicum*, but the type of water did not. A constant temperature of 28°C and electrical light produced the highest rate of hatching, and reproducible results, whereas a temperature of 4°C or 37°C, and the absence of light inhabited the hatching of eggs. The percentage of eggs hatched during the first 8 hours of 24 hours incubation was 94.90%, so that using the hatching rate of the first 8 hours could approximate the total hatching rate of samples.

INTRODUCTION

When schistosomiasis is suspected but the concentration of eggs by the usual sedimentation and floatation technics is unsuccessful, the presence of relatively few viable eggs can be demonstrated by the use of a hatching technic and identification of the liberated miracidia (Faust and Melency, 1924). Research workers have met with unexplained and inconsistent hatching failures of *S. japonicum* eggs, even though the feces were obtained from the same individual animal and treated according to known optimal conditions.[1] Although these inconsistencies are undoubtedly due in part to fluctuations in egg numbers, unrecognized factors probably still exist (Li and Hu, 1989).

A number of factors have been considered, including light, temperature and pH of the water (Faust and Melency, 1924; Li and Hu, 1989). Lutz (1919) concluded that light plays some part in the hatching process; Faust and Hoffman (1934) observed that few ova of *S. mansoni* hatched after they had been 8 hours in fresh water and that the majority appeared after 16 hours but did not, however, appreciate the importance of light. Maldonado and Acosta-Matienzo (1948) showed that in fresh water and with suitable irradiation many *S. mansoni* ova hatched within 1 hour. Standen (1951) also showed that both light and temperature influenced the hatching of laboratory maintained *S. mansoni* eggs and concluded that low (4°C) and high (37°C) temperatures as well as absence of light inhibited or retarded hatching. Thus, although there was a general indication that light, temperature and hypotonicity were controlling factors in egg hatching, optimal conditions, especially for *S. japonicum* have not yet been well defined.

MATERIALS AND METHODS

Hatching assay

Feces containing ova of *S. japonicum* were obtained from infected patients in Yujiang city of China. The feces were macerated with 10-15 times their volume of 0.9 % saline and shaken mechanically for 10 minutes. This suspension was filtered through three layers of different size nylon gauze (60, 150 and 300 mesh nylon gauze, of which 300 mesh nylon gauze that is the smallest mesh is about 45-55 μm pore size) and the residue on last gauze washed with saline to remove any adherent ova. The filtrate was maintained in a state of suspension and divided by volume into the required number of equal parts. These were each made up to 200 ml with 0.9% saline at 20°C and allowed to sediment at this temperature for 15 minutes. All sedimentations were carried out in the dark. The supernatant was then decanted and the deposit resuspended in saline and allowed to sediment. Each sample was washed three times in this way and after the final decantation the sediment was washed into a 100 ml beaker with

boiled water and made up to volume. The temperature of the fresh water was always the same as the temperature maintained during the subsequent incubation period. When required, illumination was provided by ten 40-watt daylight fluorescent tubes in an incubator. Two of the ten fluorescent tubes were fixed on the top wall of incubator, and 2 × four tubes were fixed on left and right wall respectively. The distance of fluorescent tube from samples was about 30-40 cm. To measure hatching, miracidia were removed with a fine-drawn glass pipette, killed and stained with aqueous iodine solution and counted on a slide using a microscope at 1 hour intervals over 24 hours.

Temperature

A test was carried out to study the hatching probability at temperatures of 4°C, 24°C, 26°C, 28°C, 30°C and 37°C. The other testing conditions were the same, *ie* artificial light and tapwater.

Light

Three experiments were carried out to determine the effects of light upon hatching of *S. japonicum* ova. The temperature was maintained at 28°C throughout all experiments and the water was as above. Each of the three groups (12 stool samples) was kept either in natural light (indoor), electrical light, as described, or in the dark, for 8 hours.

Water

The influence of natural water (river water), tapwater and distilled water on the numbers of ova hatched was also tested. The temperature was maintained at 28°C and illumination was provided by ten 40 watt daylight fluorescent tubes. Each experiment consisted of a 8 hours incubation period using different water for each sample from 8 stool samples.

The reproducibility of hatching in optimal conditions

Two to 4 replicate hatching tests from 27 stool samples were observed to determine whether hatching was reproducible or not at the optimal hatching conditions. The hatching conditions were the same in all tests; 28°C, ten 40 watt fluorescent lights and tap water (pH 6.8). The incubation period was 8 hours for each experiment.

Hatching rate of egg of *S. japonicum* at various hourly intervals

12 samples were used to check the hourly hatching rate. The hatching conditions were fixed at temperture 28°C, ten 40W fluorescent light and tap water at pH 6.8. In all cases examinations were made at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours after the final sedimentation.

RESULTS

The effects of temperature on the hatching of *S. japonicum* eggs

Table 1 shows the effect of different temperatures on the numbers of eggs hatched. Of the six temperatures tested, 4°C, 24°C, 26°C, 28°C, 30°C and 37°C, it was shown that a constant temperature between 24°C and 30°C, following the standard sedimentation technic, produced the greatest rate of hatching in the first 4 hours. Hatching continued thereafter but at a decreased rate and after 8 hours' incubation the hatched miracidia totalled 94.90% of those hatched after 24 hours. It was also shown that a temperature of 4°C inhibits hatching, with only 1.29% of the eggs hatching compared to those

Table 1

Effect of temperature on the hatching of *S. japonicum* eggs.

Temperature (°C)	No. of eggs hatched in 4 stools			
	1	2	3	4
4	4	5	6	7
24	275	305	541	531
26	287	321	495	452
28	289	328	532	553
30	281	324	522	547
37	49	53	83	78

at 28°C. The initial rate of emergence at higher temperatures was much the same at 37°C as at 24°C, 26°C, 28°C and 30°C.

The effects of light on the hatching of *S. japonicum* eggs

Results from 3 experiments on the role of light as a stimulus to hatching are shown in Table 2. A very different picture emerged in the natural light and dark groups. Here, very few miracidia hatched under natural light or darkness so that at the end of 8 hours only 29.05% in the natural light group and 10.35% in the dark group had hatched compared to the electrical light group during the same period. Exposure to light, especially intense electrical light, exerted a pronounced effect on the emergence of miracidia.

The effects of water on the hatching of *S. japonicum* eggs

The results showed that there were no significant differences in the hatching rates of *S. japonicum* eggs between the river, tap and distilled water treatments in all 4 tests (Table 3). In the first two hours, the number of eggs hatched in river water was more than in the groups of tap and distilled water, but after 8 or 24 hours, there were no significant differences between them.

The variability in the hatching of *S. japonicum* eggs under optimum conditions

Table 4 shows that, of the 27 stool samples tested, there were no significant differences between or

Table 2

Effect of light on the hatching of *S. japonicum* eggs.

Time (hours)	No. of eggs hatched in electrical light				Natural light				Dark			
	1	2	3	4	1	2	3	4	1	2	3	4
0.5	4	2	3	0	0	0	0	0	0	0	0	0
1	10	11	11	11	0	0	0	0	0	0	0	0
2	7	8	6	4	0	0	0	0	0	0	0	0
3	14	32	49	25	0	0	2	5	0	0	0	0
4	41	22	19	23	3	5	4	7	0	0	0	1
5	46	30	45	41	7	4	6	11	3	2	2	3
6	3	4	10	15	4	10	7	13	3	2	3	4
7	4	6	11	6	16	10	14	15	8	3	5	1
8	7	13	5	6	10	9	11	6	6	8	2	2
24	17	8	14	5	6	12	4	3	0	0	2	2
Total	153	137	173	136	46	50	48	60	20	15	14	13

Table 3

Effect of water on the hatching of *S. japonicum* eggs.

Water	pH value	No. of eggs hatched in different types of water of 7 stools						
		1	2	3	4	5	6	7
River	7	112	358	335	362	390	139	130
Tap	6.7	126	352	326	378	381	114	133
Distilled	6.4	110	325	331	331	352	119	109
p-value		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Table 4

Consistency of the hatching rate of *S. japonicum* eggs in replicate samples from the same stools.

Stool no.	Hatching rate in 2-4 tests				p-value (Chi test)
	1	2	3	4	
1	0.70	0.69	0.65	0.66	> 0.05
2	0.70	0.65	0.76	0.63	> 0.05
3	0.68	0.64	0.72	0.72	> 0.05
4	0.28	0.36	0.26	0.30	> 0.05
5	0.39	0.36	0.45		> 0.05
6	0.32	0.32	0.31		> 0.05
7	0.34	0.26	0.19		> 0.05
8	0.56	0.74	0.60		> 0.05
9	0.25	0.35	0.24		> 0.05
10	0.37	0.51	0.31		> 0.05
11	0.35	0.37	0.33		> 0.05
12	0.44	0.46	0.38		> 0.05
13	0.65	0.87	0.34		< 0.001
14	0.60	0.66			> 0.05
15	0.54	0.59			> 0.05
16	0.60	0.66			> 0.05
17	0.61	0.60			> 0.05
18	0.48	0.53			> 0.05
19	0.44	0.41			> 0.05
20	0.35	0.43			> 0.05
21	0.48	0.44			> 0.05
22	0.51	0.53			> 0.05
23	0.33	0.31			> 0.05
24	0.04	0.47			> 0.05
25	0.47	0.64			> 0.05
26	0.51	0.56			> 0.05
27	0.51	0.53			> 0.05

among parallel tests in 26 of them. This means that hatching is consistent at the hatching conditions of temperature 28°C, tap water (pH = 6.8) and ten 40 watt fluorescent tubes in an electrically illuminated incubator for 8 hours.

The changes in rate of hatching of *S. japonicum* eggs over 24 hours of incubation

Counts were made at 15, 30, 45, 60 minutes and thereafter at hourly intervals for the duration of 24 hours. It was found that of the total eggs hatched at the end of 24 hours 3.85% hatched after 1 hour, 16.21% after 2 hours, 57.91% after 4 hours, and 94.90% after 8 hours (Table 5).

DISCUSSION

The mechanism of hatching of schistosome eggs has been the subject of several studies. Although much attention has been paid to outside factors that inhibit or initiate hatching, relatively little effort has been devoted to studying the intrinsic hatchability of the egg itself. It is clear, however, that not every egg can hatch. There is an important distinction between viability and hatchability of schistosome eggs; Stirewalt (1973) states that eggs 'destined to play a part in the life cycle of the parasite' have 'motile miracidia visible within

Table 5

Number of *S. japonicum* eggs hatched at various hourly intervals in 24 hours incubation.

Stool no.	No. of eggs hatched at different time (hours)										
	0.5	1	2	3	4	5	6	7	8	24	Total
1	1	5	5	7	76	23	37	0	0	7	159
2	3	7	13	25	45	23	26	11	0	6	161
3	2	9	18	32	40	17	18	9	0	4	149
4	1	15	38	36	22	19	12	3	0	7	153
5	4	10	21	41	46	3	4	7	13	12	161
6	2	11	40	22	30	4	6	13	5	15	149
7	3	11	55	19	45	10	11	5	9	7	175
8	0	11	29	23	41	15	6	6	3	12	146
9	0	0	21	74	106	89	18	6	38	15	367
10	0	2	13	30	130	23	25	18	85	19	345
11	0	7	53	78	98	94	25	16	7	10	388
12	0	1	24	36	130	67	74	27	22	9	390
13	0	1	14	16	7	11	41	23	13	12	138
14	2	1	19	4	22	42	37	10	13	3	153
15	2	9	16	16	23	17	10	6	7	19	125
16	5	2	28	26	28	19	13	2	3	11	137

them', but Hairston (1973) points out that not all eggs with a mature, moving miracidium are able to hatch. The actual hatching percentage of egg preparations from stool varies from approximately 20% to 100% (Maldonado and Acosta-Matienzo, 1948; Standen, 1951; Samuelson *et al*, 1984; Xu and Dresden, 1986). It is affected by many factors such as extrinsic factors, the immune response of host and worm age. Extrinsic factors that control whether a potentially hatchable egg will hatch are known to include temperature, pH, salinity, light and redox potential (Standen, 1951; Pitchford, 1971; Upatham, 1972; Bair and Etges, 1973).

From this study it is clear that absence of light has a considerable retarding influence on the hatching of eggs although a small number of miracidia do emerge in the dark. Maldonado and Acosta-Matienzo (1948) reported that 83% and 31% of *S. mansoni* eggs obtained from mice hatched in bright artificial light and room light, respectively, similar to this study of *S. japonicum*. However, Kassim and Gilbertson (1976), and Blair and Etges (1972) reported that there was no difference in the proportion of *S. mansoni* eggs that hatched in light and in darkness, distinct from the results shown by Luts (1919), Standen (1951) and the results presented here for *S. japonicum*. The contradiction may be due to differences in experimental procedure or to species and strain differences in the schistosomes. However, the consensus is that light plays an important part in the process of hatching even when conditions of temperature and osmotic pressure are at their optimum.

Magath and Matheson (1946) showed that eggs of *S. japonicum* are inhibited from hatching at 37°C and 4°C and Standen (1951) obtained similar results with eggs of *S. mansoni*, but Pitchford and Visser (1971), and Bair and Etges (1972) reported different results with eggs of *S. mansoni*. This study showed the same results as Magath and Matheson (1946), *ie* that the temperature of the water also played an important role for the hatching of *S. japonicum* eggs and that temperatures as great as 37°C were definitely harmful. Thus, body temperature is sufficiently high to prevent eggs being hatched either in the tissues or in fecal material within the intestinal tract. In order to hatch, the eggs must be reduced in temperature and the optimum appears to be within the range of tropical fresh water. Faust and Melency (1924) apparently did not appreciate the fact that although body temperature was optimal for development of the embryo, a

lower temperature might be optimal for hatching. This has been proved to be the case in this study and previous studies. Our experiments also indicated that water condition, within the limited range tested, was not an important factor in the hatching of *S. japonicum* eggs. The most important result was that the hatching rate of *S. japonicum* eggs was highly reproducible if the conditions were fixed at a temperature 28°C, tap or river water and good light. In addition, 95.56% of the eggs that hatched in 24 hours hatched within 8 hours' incubation. These data established reproducible conditions for assessing relative hatch rates in fecal eggs from infected humans.

ACKNOWLEDGEMENTS

This work was supported by UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Medicine.

REFERENCES

Baird RD, Etges FJ. *Schistosoma mansoni*: Factors affecting hatching of eggs. *Exp Parasitol* 1972; 33 : 155-67.

Faust EC, Hoffman WA. Studies on schistosomiasis mansoni in Puerto Rico. III. Biological studies. 1. The extra-mammalian phase of the life cycle. *Puerto Rico J Public Health Trop Med* 1934; 10 : 1-47.

Faust EC, Melency HE. Studies on schistosomiasis japonica. *Am J Hyg Monogr Ser* 1924; 3 : 349.

Hairston NG. The dynamics of transmission. Basel: S. Karger 1973.

Kassim O, Gilbertson DE. Hatching of *Schistosoma mansoni* eggs and observations on motility of miracidia. *J Parasitol* 1976; 62 : 715-20.

Li Y, Hu S. Experimental study on hatching of *Schistosoma japonicum*. *Chin J Schisto Cont* 1989; 3 : 33-6.

Lutz A. O *Schistosomum mansoni* e a Schistosomatoes segundo observacoes, feitas no Brasil. *Mem Inst O Cruz* 1919; 11 : 121-55.

Magath TR, Matheson DR. Factors effecting the hatching of *Schistosoma japonicum*. *J Parasitol* 1946; 32 : 64-8.

Maldonado JF, Acosta-Matienzo J. Biological studies on the miracidium of *Schistosoma mansoni*. *Am J Trop Med* 1948; 28 : 645-57.

Pittchford RJ, Visser PS. Some observation on the hatching pattern of *Schistosoma mansoni* eggs. *Ann Trop Med Parasitol* 1971; 66 : 399-407.

Samuelson JC, Quin JJ, Caulfield JP. Hatching, chemokinesis, and transformation of miracidia of *Schistosoma mansoni*. *J Parasitol* 1984; 70 : 321-31.

Standen OD. The effects of temperature, light and salinity upon the hatching of the ova of *Schistosoma mansoni*. *Trans R Soc Trop Med Hyg* 1951; 45 : 224-41.

Stirewalt MA. Important features of the schistosomes. Basel: S Karger 1973.

Upatham ES. Rapidity and duration of hatching of *Schistosoma mansoni* eggs in outdoor habitats. *J Helminthol* 1972; 46 : 271-6.

Xu YZ, Dresden MH. Leucine aminopeptidase and hatching of *Schistosoma mansoni* eggs. *J Parasitol* 1986; 72 : 507-11.