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THE SHEDDING CYCLE OF CERCARIAE OF *PARAGONIMUS WESTERMANI* FROM ITS SNAIL HOST *SEMISULCOSPIRA LIBERTINA*[1]

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ABSTRACT

The present study was made to determine the time of most prolific emergence of *Paragonimus* cercariae from the snail host, *Semisulcospira libertina*, and to study the influence of temperature and light on this phenomenon. The results are as follows: The shedding of cercariae of *Paragonimus westermani* from the snail host, *Semisulcospira libertina*, occurred mainly at night from 6 p. m. to 6 a. m., with 96.72% of a total 1,253 cercariae during a period of 24 hours. There was probably a nocturnal periodicity. The number of cercariae shed in 24 hours varied with the individual snail from 116 to 326 at the temperature range of 19–20 C. The average number was 179 per snail. This might be increased by raising the temperature within the limited range. No cercariae were seen to emerge at temperatures at or below 12 C. The almost inhibition of cercariae emergence in the daytime may be reversed by cutting off light by placing the infected snails in a closed cabinet at 24 C.

Concerning the shedding of cercariae from their snail hosts, different species of trematodes have their own specific pattern in time. As to the genus *Paragonimus* there was still no other work than that of Ameel regarding the emergence of *P. killicotti* in the late afternoon or at night (1); and that of Yokogawa claiming that *P. westermani* did not ordinarily escape from the snail host (2). Therefore the problem of the shedding cycle of cercariae of *P. westermani* was taken up again in the present study from November 1963 to January 1964. The factors of light and temperature were also considered.

MATERIALS AND METHODS

Seven naturally infected mature snails of *Semisulcospira libertina* were selected from a lot of living snails collected from a mountain brook in the heavy paragonimiasis endemic village of Alilao, in Northern Taiwan. Each infected snail was kept in a 30 ml beaker, filled with filtered river water and covered with a wire gauze. The snails were maintained at room-temperature of 19–20 C for 24 hours from noon of one day to that of the next. At the end of every two hours each snail was routinely transfered from the old beaker to a new one. The cercariae from the old beaker were carefully counted under a dissecting

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microscope. With the same procedure, observations were made on four infected snails (Nos. II, III, IV, and VII) kept at a low temperature of 6.5–12.0 C for 24 hours; and on another two snails (Nos. IV and V) in a dark cabinet at 24 C in the day-time for 2 hours.

RESULTS

The data in TABLE I indicate that the total number of cercariae shed by 7 infected snails in 24 hours was 1,253 with an average of 179 per snail. The majority (96.72%) escaped from the snail host at night from 6 PM to 6 AM when the average temperature was 19.0 C; and very few (3.28%) in the daytime with a temperature difference of only 0.7 C. The maximum number of cercariae shed was 326 in Snail no. II and the minimum 116 in Snail no. VII.

TABLE I

Number of cercariae of ***Paragonimus westermani*** *shed from the snail hosts,* ***Semisulcospira libertina,*** *in 24 hours at room temperature (19–20 C)*

Date: Jan. 14, 12.00 AM – Jan. 15, 12.00 AM 1964

Snail no.	2 pm	4 pm	6 pm	8 pm	10 pm	12 pm	2 am	4 am	6 am	8 am	10 am	12 am	Total
	20.0 C	19.5 C	19.0 C	19.0 C	19.0 C	19.0 C	19.0 C	19.0 C	19.0 C	19.5 C	19.5 C	20.0 C	
I	0	0	0	36	48	31	9	0	0	2	1	0	127
II	0	0	0	90	142	57	28	5	1	2	0	1	326
III	0	0	7	49	46	25	13	1	0	0	0	0	141
IV	0	1	0	35	39	87	31	11	1	0	3	2	210
V	3	0	3	2	22	40	58	53	12	11	1	0	205
VI	0	0	0	31	45	26	18	3	5	0	0	0	128
VII	0	0	0	0	29	45	28	14	0	0	0	0	116
Total	3	1	10	243	371	311	185	87	19	15	5	3	1,253
Average no. per snail per 2 hour period	0.43	0.14	1.43	34.71	53.00	44.43	26.43	12.43	2.71	2.14	0.71	0.43	179

The influence of low temperature on the shedding of cercariae is indicated in TABLE II. At a temperature range of 6.5–12.0 C, the cercariae did not emerge at all from the 4 heavily infected snails (Snail nos. II, III, IV, and VII) either in daytime or at night.

TABLE II

The influence of low temperatures (6.5–12.0 C) on the shedding of ***Paragonimus cercariae*** *in 24 hours from the snail hosts,* ***Semisulcospira libertina,***

Date: Jan. 25, 12.00 AM – Jan. 26, 12.00 AM 1964

Snail no.	2 pm	4 pm	6 pm	8 pm	10 pm	12 pm	2 am	4 am	6 am	8 am	10 am	12 am
	12.0 C	11.5 C	10.5 C	9.5 C	8.5 C	8.0 C	8.0 C	6.5 C	7.0 C	8.0 C	9.5 C	11.0 C
II, III, IV, VII.	No shedding of cercaria											

Absence of light likewise exerted a great influence on the shadding pattern as shown in TABLE III. Two moderately infected snails, Nos. IV and V, were kept in the dark in a cabinet at 24 C. Within 2 hours, 1-3 PM. 205 and 108 cercariae were collected respectively, whereas only 3 were shed by snail No. V at a lower temperature of 20 C at the same time of the day but in the presence of light (TABLE I).

TABLE III

Number of cercariae of ***Paragonimus westermani*** *shed in two hours from the snail hosts,* ***Semisulcospira libertina,*** *in the dark cabinet in the day-time, 1-3 PM*
Date: Jan. 18, 1964, Temperatue 24 C

Snail no.	No. of cercariae shed
IV	205
V	108

DISCUSSION

The present finding shows that the shedding of cercariae of *P. westermani* from *S. libertina* snail was nocturnal, occuring between 6 PM to 6 AM during the night (TABLE I and *Fig. 1*). This result agrees with the observation of Ameel which reported that the largest number of cercariae of *P. kellicotti* was naturally shed from *Pomatiopsis lapidaria* in the late afternoon or at night (1). However, Yokogawa found a different result on the escape of cercariae of *P. westermani* from infected snails, *S. libertina* (2). In one series of his studies, 16 infected snails were placed each separately, in water in test tubes, and he observed that 20 cercariae escaped from 1 snail when the temperature was accidentally raised from 25 C to 37 C, while only 13 were obtaind from the other 15 snails at 25 C. Therefore he concluded that under natural condition the cercariae of *P. westermani* did not ordinarily escape from the snail host.

The time of daily cercaria emergence

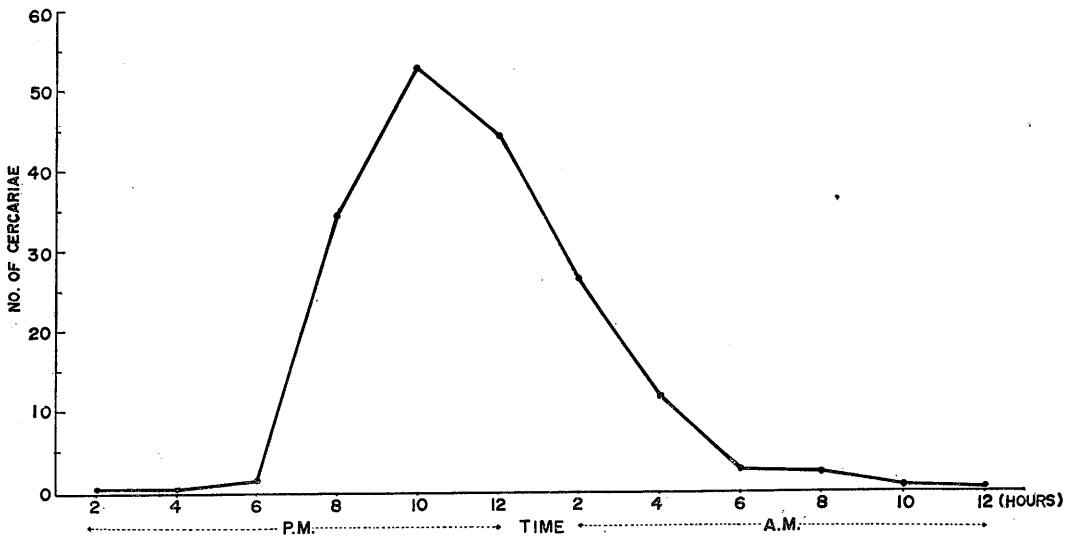

Fig. 1. Normal shedding periodicity of cercariae of *Paragonimus westermani* from *Semisulcospira libertina* in laboratory (19-20 C)

varies with the species of trematodes. For example, Giovannola recorded that the normal cycle of three species: *Diplostomum flexicandum, Cercaria brevifurca* and *Cercaria reniferinae*, had a regular periodicity in their emission, with the peak from 11:30 AM to 5:30 PM, 5:30 AM to 11:30 AM, and 5:30 AM to 11:30 AM respectively (3). Olivier found that under normal laboratory condition and presumably under field condition too, the cercariae of *Schistosomatium douthitti* emerged daily during the early evening hours (4). Kuntz stated that *Oncomelania* snails normally shed cercariae of *Schistosoma japonicum* once daily between 10 AM and 2 PM (6). Faust and Russell wrote that "The cercaria is a free-living aquatic form, which in species of blood flukes typically emerges from the mollusc in swarms during the sunlight hours in the morning" (5). Therefore the references at hand obviously indicate the existence of the shedding cycle of cercariae from the host in 5 species. The present definite finding of the nightly emergence pattern of cercariae of *P. westermani* is in accord with the general tendency. The shedding of *P. westermani* in the absence of light corresponds possibly with the nocturnal activity of the crab host in nature, thus affording a good opportunity for crabs to be infected.

The number of *P. westermani* cercariae shed was directly influenced by temperature (TABLES I and III). No cercariae were observed to escape from snails at and under 12 C. (TABLE II). This phenomenon may correspond with the period of crab hibernation and also explains why the crab host do not acquire the infection of *Paragonimus* in the cold months under natural condition. Kuntz pointed out that the shedding of cercariae of *S. japonicum* is affected by both temperature and light, but sudden changes in temperature exert a greater effect than abrupt changes in light (6). The writers also obtained the same results under reverse conditions in this study.

The present results that 96.72% of the cercariae escaped at night and that the escape of the larvae was also induced when the infected snails were placed in a dark cabinet (TABLE III) in the daytime indicate the influence of light on the emergence of *P. westermani*. The authors are aware of the danger of drawing conclusion from one experiment only, yet the emergence in the absence of light is so striking as to make the conclusion inescapable. Some investigators reported comparable experiments with other species. As mentioned earlier, Giovannola claimed that he could reverse the cycle of emergence of cercariae of three species, *D. flexicandum, Cercariae brevifurca* and *Cercaria reniferinae* by reversal of light and darkness (3). In the first two cases the reversal did not occur immediately but took place after a period of a day or more when shedding was erratic and less definitely cyclic. The third species apparently shed according to the reversed cycle immediately. Rees noted that shedding cycle of *Cercaria purpurae* was reversed immediately and completely when the light cycle was reversed (7). For getting cercariae of *S. douthitti* from their snail host, under both normal and reveresed light conditions, Olivier tested five infected snails, two *Lymnaea stagnalis* and three *Lymnaea palustris*, (4). First he kept them in the dark from 6 PM to 6 AM and in the light from 6 AM to 6 PM and found that the snails shed almost all of their cercariae in the 6-hour period between 4 PM and 10 PM, next in the reversed condition he found that the reversal of the shedding cycle was also complete and almost immediate following the reversal of the light cycle and that almost all of the cercariae emerged between 4 AM to 10 AM. Based on this result, Olivier suggested that there was a sort of trigger mechanism to induce the shedding of cercariae.

In general the ability of the cercaria to exist in free-living state is limited at most to 3 days, usually 24 hours or less. The shedding time varies with the species of trematodes, the snail hosts, light and temperature. Thus the cercariae may establish their specific emergence pattern according to the ecological background of the hosts.

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