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INFECTIVITY OF *SCHISTOSOMA MANSONI* CERCARIAE

LOUIS J. OLIVIER*

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Parasitic Diseases, Bethesda, Maryland 20014

There appears to be no published information on the length of time the cercariae of *Schistosoma mansoni* remain infective after they leave the snail. Numerous observations have been made on the survival of schistosome cercariae as judged by their activity and by their appearance under the microscope.[1-3] In general, it has been found that under relatively favorable conditions in the laboratory some schistosome cercariae can survive for two days or more. Maldonado estimated that in a pond near San Juan, Puerto Rico, the life span of *S. mansoni* cercariae was about 10 hours and that the water temperature affected survival time.[4]

However, the crucial consideration concerning cercarial survival is their capacity to enter a host and develop into adult worms. Surely the cercariae lose their capacity to penetrate and develop to adulthood some time before they die. It is necessary, then, to test them for infectivity as they age. This was done by Miller and Edney with *Schistosomatium douthitti*.[5] Though they gave no details, they reported that infectivity of the cercariae declined as the larvae aged and that the decline was more rapid at higher temperatures. The experiments to be described were designed to test the infectivity of *Schistosoma mansoni* cercariae at intervals after they were shed from snails.

EXPERIMENTAL PROCEDURE AND RESULTS

Cercariae were obtained by placing numerous *Biomphalaria glabrata* (*Australorbis glabratus*) infected with a Puerto Rican strain of the parasite in a beaker in approximately 200 ml of water, known to be favorable to snails and cercariae, and placed under an incandescent lamp so that the cercariae were stimulated to emerge by both light and rising temperature. After stimulation for 25 (exp. I) or 40 minutes (exp. II) and cercariae were emerging in large numbers, the snails were transferred to a new beaker containing water of the same temperature and exposure to light and stimulation was continued. The water temperature during escape of the cercariae did not exceed 28°C. After an additional 45 minutes, during which large numbers of cercariae entered the water, the snails were removed and the cercariae were retained for experimental use.

The mice were exposed individually to cercariae by a standardized procedure. While the cercaria suspension was stirred constantly with a wooden paddle, portions were drawn into a Cornwall automatic syringe (5-ml syringe, adjustable holder and 14 gauge, 4 in. cannula) and one released into each of three plastic petri dishes with bottoms cross-scored for counting. A drop of saturated aqueous nile blue hydrochloride solution was added to each dish and counts were made after a few minutes to allow time for staining. The concentration of cercariae was determined by taking the average of the three counts. The average fell between 75 and 100 cercariae in each experiment. Since the counts were within the desired range no adjustment of the syringe was necessary and the same sized portions (1.4 ml for exp. I and 1.0 ml for exp. II) were rapidly delivered into Kahn tubes (glass; 12 x 75 mm) while the suspension was stirred constantly. Enough water was added to bring the suspension in each tube to a total of 3.5 ml. This filled each tube to about 1 cm from the top. All tubes for the experiment plus several extra tubes were charged with cercariae in a short space of time and all were held in racks and kept covered until used.

Groups of either 15 or 20 mice were then exposed to the cercariae at selected time intervals. The first group of mice in each experiment was exposed when the "average age" of the carcariae was 38 minutes (0.6 hours). Young female white Swiss mice, about 5 weeks old and weighing 20 to 21 grams, were used for all the tests in each experiment. Just before exposure to cercariae the mice were injected with 0.01 ml per gram of 14% solution of sodium pentobarbital in saline intra-

* With the technical assistance of Rodney Duvall and Judith McBride.
Present address: Pan American Health Organization, Washington, D. C.

TABLE 1

Infectivity of S. mansoni *cercariae at intervals after they were shed**

Exp. no.	Age of cercariae (hours)	No. mice used	Temp. in tubes at start of mouse exposure	Temp. in tubes at end of exposure	No. mice at end of experiment	No. worms recovered at autopsy		Percent of cercariae used found as worms in mice
						Range	Mean ± S.E.	
I	0.6	20	23.8	24.5	20	7-22	11.7 ± 1.0	12.3
II	3.6	20	23.7	23.8	20	5-22	12.3 ± 1.0	12.9
III	6.6	20	24.2	24.4	19	1-16	9.5 ± 1.0	10.0
IV	12.6	20	23.2	25.2	19	0-5 (5 neg.)	2.2 ± 0.4	2.3
V	24.6	20	23.5	24.7	19	0-3 (13 neg.)	0.5 ± 0.2	0.5
VI	30.6	20	24.6	24.8	20	0-1 (16 neg.)	0.2 ± 0.1	0.2

* Each mouse was exposed for 30 minutes to 95 cercariae.

peritoneally, a quantity sufficient to anesthetize them for 60 minutes or more. Each mouse was exposed by immersing its tail in one of the Kahn tubes.[6] Exposure time was exactly 30 minutes after which the tail was removed from the tube and allowed to dry. The mice were then returned to plastic cages.

All mice were perfused 42-44 days after exposure using a standardized and very simple method, the sensitivity and reliability of which has been tested.[7] In each experiment all mice were perfused by one highly skilled person in two days, equal numbers of mice from each group being perfused each day. In the second experiment the groups were coded so that the person perfusing and counting the worms did not know to which exposure group they belonged.

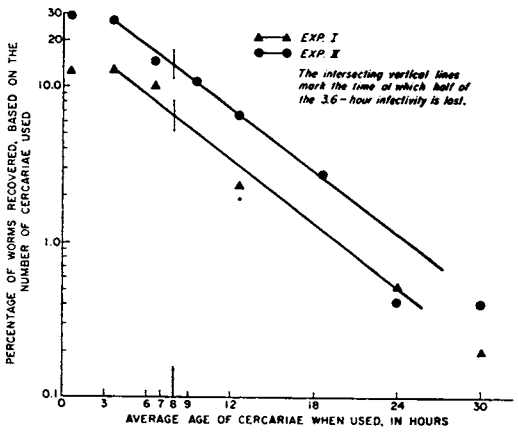

FIGURE 1. Number of worms recovered in relation to the age of cercariae used for infecting mice.

EXPERIMENT I

In the first experiment six groups of 20 mice were exposed using 95 ± 1.5 cercariae per Kahn tube. The mice were perfused 42 or 43 days after the first group was exposed. Data from the experiment are presented in Table 1 and Figure 1.

The decline in infectivity of the cercariae with age is evident. Infectivity of the cercariae fell rapidly after the cercariae were 6.6 hours old, and when the cercariae were 12.6 hours old their infectivity was low. When they were 24.6 or 30.6 hours old, only an extremely small proportion of the cercariae was able to infect mice.

The proportion of fresh cercariae successfully completing penetration and development in the mice of this experiment was relatively low (12.3% for cercariae 0.6 hr old). In our experience the return of worms is usually larger though there is considerable variation from experiment to experiment. However, the return in this experiment is not considered low enough to affect the significance of the results in any way.

EXPERIMENT II

In the second experiment two mouse groups were added and the number of mice per group was reduced to 15. The number of cercariae per mouse was 84 ± 1.5. The procedure was the same as that used in experiment I except that, after removal of the mouse's tail, each Kahn tube was checked for cercariae that had not penetrated. This was done by washing the water from each tube into a plastic petri dish, adding nile blue and counting the cercaria bodies, *i.e.* the anterior portions of the cercariae whether or not the tail was still attached.

The mice were perfused 43 and 44 days after the first group was exposed. Data from the experiment are presented in Table 2 and Figure 1.

TABLE 2

Infectivity of S. mansoni *cercariae at intervals after they were shed**

Exp. no.	1 Age of cercariae (hours)	2 No. mice used	3 Temp. in tubes at start of mouse exposure	4 Temp. in tubes during mouse exposure (range)	5 Cercariae in tubes after exposure (mean + range)	6 Percent of cercariae recovered in tubes	7 No. mice at end of experiment	8 No. of worms recovered at atuopsy		9 Percent of cercariae found as worms in mice
								Range	Mean ± S.E.	
I	0.6	15	25.7	26.2–27.0	21.1 (12–30)	25.1	15	15–38	23.9 ± 1.8	28.5
II	3.6	15	24.5	26.5–27.2	18.6 (14–25)	22.1	14	11–32	22.6 ± 1.7	26.9
III	6.6	15	24.0	24.7–26.0	27.2 (11–57)	32.4	14	8–19	12.1 ± 1.4	14.4
IV	9.6	15	24.0	25.4–27.0	31.9 (17–48)	38.0	15	3–24	9.1 ± 1.2	10.8
V	12.6	15	23.7	25.0–26.4	32.3 (22–50)	38.5	14	2–9	5.5 ± 0.7	6.5
VI	18.6	15	22.7	24.5–25.6	43.1 (30–63)	51.3	15	0–6 (2 neg.)	2.3 ± 0.4	2.7
VII	24.6	15	23.6	24.5–25.6	62.7 (48–84)	74.6	15	0–2 (12 neg.)	0.3 ± 0.2	0.4
VIII	30.6	15	24.0	25.5–26.4	63.1 (51–73)	75.1	15	0–1 (11 neg.)	0.3 ± 0.2	0.4

* Each mouse was exposed for 30 minutes to 84 cercariae.

The decline in infectivity of the cercariae with time is again obvious. The 6.6-hour-old cercariae were much less infective than the fresh cercariae and by 18.6 hours a great proportion of the cercariae could not infect mice. The number of cercariae recovered from the exposure tubes rose steadily as time passed, indicating that failure to enter the mice accounts for at least a large proportion of the decline in numbers of adults recovered from the mice.

DISCUSSION

The data presented provide quantitative evidence as to the decline in infectivity of one strain of *S. mansoni* cercariae as a function of time. It is considered that the cercariae in these experiments were held, before they were used, under conditions that favored their survival. They were not subjected to temperature stress or unusual light stimulation, there was no agitation of the water, except when the cercariae were transferred to the tubes, there were no predators and the water in which they were held had no known harmful ingredient. Moreover, the cercariae were brought into very close proximity to the mouse skin at the time of exposure. Therefore, it is concluded that the indicated infectivity of the cercariae was probably near maximum for the strain and for the temperature at which they were held. It is not believed that loss of infectivity of the cercariae was caused by lack of oxygen in the tubes. Probably the cercariae lost infectivity by using their energy reserves.

The curves for the two experiments, plotted by inspection in Figure 1, are essentially parallel indicating that loss of infectivity followed the same pattern. As can be seen in the figure, the time at which the infectivity of the cercariae fell from the infectivity found at 3.6 hours to one-half of that quantity was less than five hours in both experiments. The estimated time for infectivity to fall to one-tenth of the maximum level was 14 or 15 hours. Thus, under conditions considered highly favorable for survival, loss of infectivity was relatively rapid.

Since these data probably represent near maximal persistence of infectivity, one can expect that in natural habitats the persistence of infectivity of the cercariae will fall below that reported here. Climatic stress is probably greater under field conditions since high temperature and ultra-violet light are injurious to the cercariae. Certain forms of pollution may shorten their lives. In some situations preda-

tors probably reduce their numbers significantly. Fish have been implicated[8,9] in reduction of cercaria populations and Pellegrino *et al.*[10] have striking quantitative evidence as to what *Lebistes* can do to a cercaria population in a natural habitat. Although one can expect great variation in survival time of cercariae under field conditions, it is probably reasonable to estimate that the "infective half-life" of *S. mansoni* cercariae in some field habitats is not more than three or four hours even in the absence of predators. Rowan has shown that the number of cercariae recoverable by filtration falls rapidly after the peak density is reached, indicating a rapid attrition presumably due to multiple causes.[9] In Rowan's data for the Mayaguez Pond (margin, Nov. 20) the recovery of cercariae fell to one-half of the highest number in less than two hours and to one-tenth in less than six hours.

The demonstrated disappearance of cercariae in the field as judged by filtration results and the data presented here make it probable that, in some *S. mansoni* endemic foci at least, the water may contain infective cercariae for only a portion of the day and that during the night and early morning hours the water may have few infective cercariae or may even be free of them. However, Pellegrino and de Maria were able to infect mice in a natural habitat at midnight.[11]

The worm counts we obtained using cercariae with an average age of 0.6 and 3.6 hours are worthy of special note. In neither experiment was there evidence of loss of infectivity of the cercariae during this 3-hour period. Perhaps infectivity of the cercariae does not change appreciably in that period.

SUMMARY

The decline in infectivity of *Schistosoma mansoni* cercariae was studied under controlled laboratory conditions considered favorable for cercarial survival. Cercariae of known age were tested by mouse exposure challenge at intervals up to 30 hours from the time they were shed from snails. Infectivity decreased with time and the decrease was rapid. About eight hours after the cercariae were shed, one-half the infectivity had been lost. Since there was no detected loss of infectivity in the first three hours, the time for infectivity to fall to one-half of the maximum level was less than five hours. Infectivity was about one-tenth the initial level after 14–15 hours.

Since conditions for cercarial survival in the experiments were relatively favorable, it can be expected that under field conditions, which are almost always more rigorous, the cercarial half-life is probably only a few hours even in the absence of predators.

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