REACTIONS OF *CERCARIA HAMATA* TO LIGHT AND TO MECHANICAL STIMULI

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The swimming behavior of a holostome larva, *Cercaria hamata* Miller, 1923, which McCoy (1928) found to penetrate sunfishes and develop in them to the meta-cercarial stage, has been studied, as well as the effect of light upon its activity and its reactions to shadow and to mechanical stimuli. Great variety in the swimming behavior and reactions of larval trematodes, particularly to shadowing, has been found among more than fifty species studied in more or less detail by one of us, (Miller, 1927–1930). In view of the meager knowledge of the life histories of these trematodes any statement concerning the adaptive value of the behavior of any species would be mere speculation.

In the development of the work certain facts of general physiological interest were found, and the results of preliminary studies are reported in this paper. The most outstanding is the fact that a dual mechanism of stimulation is involved in the response to mechanical stimulation and to a change of light intensity (shadow).

Material and Methods

The snail host of *C. hamata* is *Planorbis trivolvis* Say. Snails from Ramona Lake, St. Louis County, were isolated in water in shell vials; cercariæ emerged, usually in large numbers, into the water from those snails in which the infestation was mature. The snails were easily kept alive in the laboratory and were isolated from time to time to secure cercariæ. It is assumed that these cercariæ, which are fully formed and emerge spontaneously, constitute a uniform material for experimental purposes. They live approximately 48 hours after emergence, during which period they do not feed or reproduce; hence these factors do not enter into their behavior.

Observations and experiments were made in the laboratory before a window, using diffuse daylight or sunlight, and were also carried out in a darkroom. The emerged cercariæ were placed in glass spectroscope absorption cells (40 × 40 × 20 mm.) or in small cells of various sizes made from high grade glass slides and DeKhotinsky cement. All observations were made with a binocular microscope held in a horizontal position.

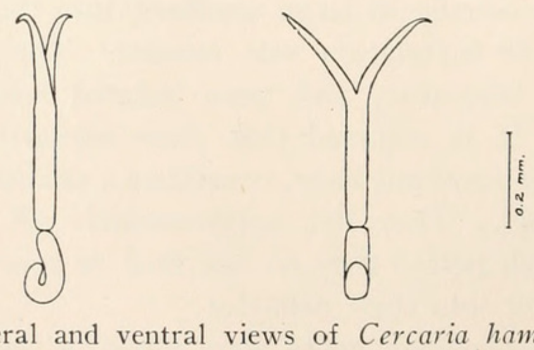
Intervals of five minutes or longer were measured with a "Reminder" clock and one minute intervals by the aid of an Eastman timer, the hand dipping into mercury and making contact every minute with a buzzer circuit. Short intervals were measured with a split-second timer registering tenths of seconds.

The light source in the darkroom was a 115 volt-200 watt, concentrated filament Mazda lamp in a 200 cm. beaver board tunnel, painted flat black. A camera shutter with a 1 inch opening was installed in the end of the tunnel. The time-setting lever was connected to an electromagnet and was operated by tapping a key. Observation in the "dark" was made by means of a weak red darkroom light behind the cell containing the cercaria. A large water cell was placed in front of the red light to absorb any heat given off.

In experiments where temperature of the water was controlled, the cell containing cercariæ was placed in a water bath and a mercury thermostat was used, connected with a heating coil and a 6-volt storage battery.

Swimming Behavior

Cercaria hamata is an intermittent swimmer. Ordinarily a short rapid upward swim alternates with a long period of sinking. The path of a swim may be changed suddenly and erratically, and occasionally an individual will make an uninterrupted swim lasting as long as 6 seconds, with many changes in direction. The tail is directed forward in locomotion, and lashes vigorously. At the end of a swim the anterior third of the body bends ventrally and posteriorly, so that the body is in the form of a hook (Text-figure), and as a cercaria sinks it



Diagrams of lateral and ventral views of *Cercaria hamata* sinking through water.

slowly comes to a position with the body down. The body retains this hook-shape until swimming is suddenly resumed. At the beginning of a sinking period the forks of the tail are spread at an angle of slightly less than 180°, but this angle gradually decreases until it is about 90°.

A great deal of variation was found among individual cercariæ in the length of a swim and of the succeeding sinking period. Ten alternating periods of sinking and swimming were measured for 75 cercariæ; sometimes a long swim followed a long period of sinking, other times a short swim followed, and thus there seems to be no correlation between them. Tables I and II show the ten observations on one individual and the averages for 10 others.

TABLE I

Variation in periods of passive sinking and of alternate spontaneous swims of one cercaria.

	Period of sinking	Duration of following swim
	seconds	*seconds*
	25.2	2.2
	48.0	3.2
	34.0	2.2
	41.0	3.0
	45.0	1.8
	107.0	2.4
	20.2	1.2
	55.6	1.0
	50.4	1.2
	39.4	3.8
Average	46.6	2.2
Range	20.2–107.0	1.0–3.8

TABLE II

Averages of periods of passive sinking and of alternate spontaneous swims of ten cercariæ.

	Average period of sinking	Average duration of swim
	seconds	*seconds*
	31.0	2.4
	45.0	1.4
	42.8	0.8
	42.7	1.2
	48.6	1.3
	80.1	1.2
	39.5	1.2
	57.1	1.2
	79.0	0.9
	26.9	1.2
Range	26.9–80.1	0.8–2.4

Effects of Temperature on Activity

The temperature of the water was noted in every case; it was found that the average period of swim did not change significantly in warming the water from 18° to 35° C., but the length of the period of sinking decreased, so that swims were more frequent at the higher temperatures.

Below 15° C. swimming behavior varied from the preceding account. The body was usually held extended when the cercaria came to rest, instead of bending. In place of swimming the cercaria exhibited jerking and lashing movements; the body sometimes bent and unbent, and occasionally, held at full length, lashed back and forth. Convulsive twitching of body and tail occurred, and the forks of the tail jerked separately. When the cercaria swam, it was only for a short distance. One individual kept up the twitching movements continuously for over 21 minutes, after which it sank to the bottom. Below 7° C. the cercariæ were either motionless on the bottom or jerked about only occasionally. Above 38° C. the body of the cercaria was held extended, but twitching did not occur.

Effect of Light on Swimming

It was observed that *C. hamata* swam more frequently in strong than in weak light; this was later tested in a darkroom. Thirty groups of ten cercariæ each were used; they were placed in a small cell and dark-adapted for an initial period of 20 or 30 minutes. During the entire experiment a weak red observation light was burning. After the cercariæ were dark-adapted, the number of swims during a one minute period was counted, and then light admitted by means of the shutter and the number of swims during a one minute period in the light was counted. These procedures were repeated, with five minute periods of dark-adaptation followed by the admission of light of diferent intensities. In each case the number of swims in the "dark" and in the light was counted. The following approximate light intensities, obtained by placing a 115 volt-200 watt lamp at different distances from the cell, were used: 2000, 1550, 1100, 650, 200, 100, 50 meter candles. Each group of ten cercariæ was subjected only once to each light intensity; the different intensities were used in alternation of low and high intensities so that the factor of progression would not enter.

The summary of observations on the 30 groups of cercariæ is shown in Table III. The average number of swims at each intensity was compared with the average number during the preceding one minute period of dark, and the percentage of increased activity calculated.

Reaction to Shadowing

When *C. hamata* is in a resting state, it is usually stimulated to swim, instantaneously, either by the shadow of an opaque object or by other means of decreasing the light intensity. It was thought that when failure to swim occurred it might be due to the fact that the stimulus was applied just at, or too soon after, the cessation of a spontaneous swim. Experiments were carried out on about forty individuals to test this hypothesis. A shadow was thrown upon a cercaria at the instant that it ceased swimming, and at varying intervals (1 to 10 seconds) after the cessation. Each cercaria was allowed to swim spontaneously twice between trials. A great deal of variability was found. Some individuals almost invariably swam when shadowed as soon as they came to rest; but in general the longer the interval be-

Table III

Effect of Light on Frequency of Swim of Cercaria hamata

Thirty groups of 10 cercariæ used. Total number of swims during one minute of dark and during a succeeding one minute period in light of a given intensity counted, and percentage of increased activity in the light calculated. Five minute intervals between trials. High and low intensities used, alternately; each group of cercariæ exposed only once to a given intensity.

Light Intensity in Meter Candles..	2000	1550	1100	650	200	100	50
Average Per cent of Increased Activity......................	626	585	408	228	94	12	8

tween cessation of swimming and stimulation, the more certain the response.

When a shadow is thrown upon a cell containing a large number of cercariæ, concerted, or almost concerted, swimming results. *C. hamata* is stimulated to swim by touch and by currents of water, and these stimuli, occasioned by individuals which responded to the shadow, are undoubtedly factors in the general response. When the cercariæ are isolated these factors of mechanical stimulation are eliminated.

Cercariæ of this species are inhibited by repeated shadows, after responding to the first or second of the series, when an interval of one or two seconds intervenes between shadows. After a lapse of a minute or more almost all of the cercariæ swim again when shadowed, and again may be inhibited by repeated stimuli. This may be shown by the following experiment. The number of swims made by five cercariæ during 90 seconds was counted, and then a shadow cast on the cercariæ every second and the number of swims during 90 seconds again counted, and the procedures repeated. The first shadow always brought about a response on the part of many individuals; this initial response was ignored and the number of swims in the ensuing 90 seconds counted. The data are shown in Table IV.

The effect of temperatures through a range from below 10° C. to 35° C. was studied in reference to response to shadowing; isolated cercariæ and groups of five individuals were used. Below 10° C. only an occasional cercaria swam when shadowed; from 10° C. to 35° C. the curve is practically a straight line, with 10 per cent of the cercariæ responding at 15° C. and 70 per cent at 35° C.

Table IV

Showing the inhibiting effects of repeated stimulation (shadowing) on the activity of C. hamata.

	Number of swims of 5 cercariae, counted in alternate 90 second periods							
Not stimulated	24		26		27		23	
Cercariae shadowed every second; swims in response to the first stimulus not counted		2		7		5		6

Response to Mechanical Stimulation

C. hamata when sinking quietly may be stimulated to swim instantly by mechanical stimuli, such as touch on any part of the body or tail, or jarring the container, or a fine stream of water. Whereas repeated stimulation by shadowing at short intervals resulted in failure to swim, it was found that this cercaria may be kept in almost continuous locomotion by repeated stimulation by touch. Forty cercariæ were used. Each was isolated in a small cell and touched twenty-five times with a fine wire on the body, tail-stem, or a fork of the tail.

In the case of twenty cercariæ the stimulation was applied as soon as possible after the individual had stopped swimming. Only seventeen failures to respond occurred in the total of 500 trials; twelve of the cercariæ swam every time. The other twenty cercariæ were permitted to assume a vertical sinking position before being touched; this allowed them an average recovery period of about 15 seconds. Thirty-five failures to swim occurred during 500 trials; seven of the cercariæ had a perfect response record for the twenty-five trials.

Several other individuals were repeatedly stimulated until fatigued.

One of these, allowed to resume the vertical sinking position between stimuli, responded 120 times and then failed to swim when stimulated ten times at short intervals. Another was touched " immediately " upon cessation of each swim. It responded to 79 successive stimuli, but after the fiftieth trial the swims became shorter and near the end of the trials the reaction consisted only of a few vibrations.

From these data and those on response to shadow stimuli, it seems clear that two different mechanisms are involved; a relatively long interval is necessary to secure anything like a regular response to the shadow stimulus, when repeated, whereas a very brief interval is sufficient in the case of stimulation by touch.

Effect of Temperature.—Between 20° C. and 38° C., the cercariæ usually swam when touched by a needle, but at lower temperatures a high percentage of failure occurred. Below 10° C. the response consisted of the jerkings which are the only movements found at these temperatures.

TABLE V

Comparison of duration of spontaneous swim with that following stimulation by shadow, touch, and stream of water. Duration of swimming in seconds.

	Cercaria *a*	Cercaria *b*
Unstimulated	1.16 ± 0.17	0.69 ± 0.07
Stimulation by:		
Shadow	2.15 ± 0.16	1.84 ± 0.17
Touch	3.24 ± 0.29	1.95 ± 0.16
Stream of Water	2.66 ± 0.24	2.01 ± 0.16

COMPARISON OF SPONTANEOUS WITH STIMULATED SWIMMING

It was observed that when *C. hamata* swam spontaneously, the average duration of swim was shorter than when it was stimulated by any of the methods used. This was tested experimentally on a number of individual cercariæ, in the following manner. The duration of a spontaneous swim was measured, and then fifteen seconds after the cercaria had stopped a shadow was cast upon it and the duration of the resulting swim measured. This was followed by touch with a fine wire and stimulation by a fine stream of water from a pipette, and the series was repeated twenty-five times for each individual. The cercaria was always allowed to swim twice between stimuli and the stimulus was then applied 15 seconds after the cessation of the second swim; occasionally the duration of the period of sinking was shorter than 15 seconds, in which event three spontaneous swims intervened between stimuli.

Data for one hundred observations on each of two cercariæ are given in Table V. It was calculated that the difference between the mean for the spontaneous swims and those for the three types of stimuli is 4.2 (shadow), 6.2 (touch), and 5.1 (stream of water) times the probable errors in the case of cercaria *a,* and 6.2 (shadow), 7.2 (touch), and 7.4 (stream of water) in the case of cercaria *b.* For nine other cercariæ the probable errors were not calculated, but it is evident that the differences are statistically significant.

Discussion

Reactions of the sort described in this paper have been found in some species of most of the large groups of animals, but among the larval trematodes they are quite common, particularly the response to shadowing. The extent to which these varied responses aid cercariæ to reach an intermediate or final host has been studied in only one instance. Miller and McCoy (1929; and in press) have shown that the reaction of *Cercaria floridensis* to shadows, cast by fish intermediate hosts, carried the cercaria to the upper levels of the water and hence possibly was a factor in the infestation of the fishes. On the other hand, Cort and Brooks (1928), who studied the characteristic behavior but not the reactions to stimuli, of a number of fish-penetrating holostome larvæ, believe that the activity of the fish intermediate hosts is such that they would come in contact with the cercariæ, so that there is no necessity for activity on the part of the latter. Until experiments have been carried out on each species, the adaptive value of these reactions to shadow and to mechanical stimuli cannot be determined. The reactions to shadow described for *C. hamata* could be brought about in nature by animals swimming above the cercariæ, and the swimming upward would carry the cercaria toward a possible host. Judging from Cort's studies (1922) on the escape of cercariæ from their snail hosts it is likely that *Cercaria hamata* would emerge in but small numbers at temperatures below 17° C., and, on the basis of the present data on effect of low temperatures, would only occasionally react to a shadow cast by a possible host.

From the results of the present preliminary study it seems evident that a dual mechanism is involved in the response to shadow and to touch, because the cercaria may be kept in almost continuous locomotion by touch stimuli, whereas a relatively long interval must intervene between shadow stimuli to secure anything like a regular response. Experiments are now under way which are so planned as to elucidate further the nature of this dual mechanism. Effects of curare, nicotine, and other substances which specifically affect myoneural junctions are being studied, as well as the effects of polarizing currents, ultra-sonic radiation, and other forms of stimulation.

Summary

1. *Cercaria hamata* swims intermittently. A short, rapid swim alternates with a relatively long period of quiet sinking.

2. The duration of the period of sinking decreases with rise in temperature. Below 15° C. the cercariæ exhibit jerking and lashing movements in place of swimming.

3. Shadowing a dense group of cercariæ results instantly in the almost simultaneous swimming of most of them. The response is partly due to the shadow stimulus and partly to the mechanical stimulus occasioned by the colliding of active individuals with quiet ones.

4. Repeated shadowing at short intervals results in inhibition of swimming.

5. Mechanical stimulation by touch (or a stream of water) initiates swimming instantaneously. Only a very short interval between stimuli is necessary to keep a cercaria in almost continuous locomotion. This indicates that a dual mechanism is involved in response to shadowing and to touch.

6. The duration of swim in response to shadow, touch, and stimulation by a stream of water is significantly greater than that of a spontaneous swim.

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