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RHYTHMIC EMERGENCE OF *SCHISTOSOMA MANSONI* CERCARIAE FROM *BIOMPHALARIA GLABRATA*: INFLUENCE OF THE TEMPERATURE

Célio M. VALLE, J. PELLEGRINO and N. ALVARENGA

SUMMARY

Confirming previous observations it was shown that infected *B. glabrata* kept at constant temperature, but subjected to diurnal light variation, shed cercariae in a characteristic rhythm where about 98% of the cercariae are eliminated between 6 A.M. to 6 P.M.. This rhythm disappears when: a) infected snails are kept at constant temperature and in darkness; b) the temperature is constant and the illumination continuous. Only variation in temperature (snails kept in darkness) can induce a circadian rhythm although not as characteristic as when light and temperature act in the process of cercarial shedding. Infected snails kept at constant temperature and in the dark continue to shed cercariae. Cercarial counts performed within 24-hour periods demonstrated that the numbers of cercariae eliminated under this condition do not differ significantly from those found when infected snails are kept under normal laboratory conditions.

INTRODUCTION

It is well known that the emergence of cercariae of *Schistosoma mansoni* from snail vectors follows a rhythmic pattern, the great percentage of cercariae being emitted during the day (FAUST & HOFFMAN [5], GIOVANNOLA [6], MALDONADO [10], BARBOSA & COELHO [3], PELLEGRINO & DE MARIA [12], WEBBE & JORDAN [16]). There are evidences that the peak of emergence varies according to geographic conditions. Field observations revealed that in Brazil the peak ranges from 11 A.M. to 5 P.M. (BARBOSA & COELHO [3]) in Recife and from 3:30 P.M. to 5:30 P.M. in Belo Horizonte (PELLEGRINO & DE MARIA [12]). In Africa, the exposure of animals to infested waters showed that the greatest infectivity occurs between 10 A.M. to 2 P.M.. Cercarial countings in the field by filtration of large volumes of water demonstrated that the greatest number of cercariae, in Puerto Rico, is found within 11 A.M. to 3 P.M. (ROWAN [13]).

The influence of light and temperature in the process of cercarial shedding has been studied by KUNTZ [7], SCHREIBER & SCHUBERT [14] and, more recently, by VALLE, PELLEGRINO & ALVARENGA [15] and ASCH [1].

In this paper a series of experiments have been designed: a) to study the rhythm under constant temperature; b) to follow rhythmic changes when infected snails are kept under complete darkness or continuous light and constant temperature; c) to investigate the rhythm pattern when infected snails are kept in the dark but are influenced by variation of the temperature during the day-night period; d) to make cercarial countings in 24-hour periods when snails are maintained under complete darkness and at constant temperature.

Contribution n.º 28 from the Schistosomiasis Research Unit

(1) Schistosomiasis Research Unit, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil
Address for reprints: Caixa Postal 1404. 30000 Belo Horizonte, Brasil

MATERIAL AND METHODS

All experiments have been carried out with pigmented *Biomphalaria glabrata* snails (Barreiro's strain, reared in the laboratory) and a strain of *S. mansoni* (L.E.) isolated from an infected patient in Belo Horizonte, Brazil, and maintained through successive passages in hamsters. The livers from 2 infected hamsters were ground and repeatedly sedimented in saline. The saline was then replaced by dechlorinated tap water and exposed to light at 28°C. Miracidia were then concentrated according to the technique of CHAIA [4]. The water with miracidia was poured into a 5-liter glass aquarium containing about 100 snails with a shell diameter of 8-12 mm. Snails after being exposed *en masse* were kept at 28°C in a circulating-water aquarium. Fresh lettuce was used as the only source of food. Infected snails were used within the period of 2 months after the beginning of cercarial shedding.

Experiments were designed as to have controlled environmental conditions of light and temperature. Artificial illumination was supplied by 5 daylight fluorescent tubes (Phillips 15-watt frosted tubes TL|54|L4) within a diffusing globe maintained at 40 cm above the surface of the water. When alternating 12-hour periods of light and darkness was required, infected snails were transferred to a light-tight room in which the temperature could be controlled as desired. Temperature inside the light-tight room was recorded throughout the experiments with a recorder thermograph. A multiple-channel thermistor was used for checking the temperature at the points of special interest. An electric timing device was set so that the light within the room was on from 6 A.M. to 6 P.M. and off from 6 P.M. to 6 A.M.. This cycle approximated the light conditions under which the infected snails had been maintained previously.

Unless otherwise specified, an estimate of the number of cercariae was made every 12 hours, at 6 A.M. and at 6 P.M.. For this purpose each snail was transferred at the end of each 12-hour period to a clean beaker containing dechlorinated tap water kept at the same temperature and thereafter returned to the original environmental conditions dictated by the correspondent experimental design.

The method described by ROWAN [13] was used for cercarial countings. After removing the snails from the containers and gently stirring the water, a sample corresponding to 20 percent of the liquid was filtered through a Büchner funnel fitted with an S & S 404 filter paper. The damp filter paper was transferred to a shallow pie pan containing 5 ml of ninhydrin reagent and finally dried over a hot plate (about 70°C). After staining, each paper was placed between two glass plates in which parallel lines had been drawn with a diamond glass marker. Countings were made under a dissecting microscope using reflected light and a 20 X magnification. Cercariae of *S. mansoni* are easily identified and appear dark blue against a white background. The data provided by serial countings from a standardized cercarial suspension showed that the discrepancy between countings ranged from 10 to 20%.

When the number of cercariae from snails was expected to be low (for instance those emerging from 6 P.M. to 6 A.M.) or when a previous examination of the containers showed only a few organisms, all cercariae were counted. For this purpose, after the snails had been removed, sufficient 5% formalin was added to fix the cercariae. The supernatant was carefully decanted and the cercariae were then counted under the low power of a dissecting microscope. As otherwise stated, the results of the countings were expressed as the percentage of cercariae emerged during the "subjective day" (6 A.M. to 6 P.M.) and "subjective night" (6 P.M. to 6 A.M.) for each 24-hour cycle. Actually, the data concerning the total numbers of cercariae emerged appear to be meaningless to give a clear picture of the rhythm of cercarial shedding. Nevertheless, some data in connection with the mean number of cercariae which emerge per day per snail will be presented.

In all experiments infected snails were kept individually in beakers containing about 100 ml of dechlorinated tap water. Before starting any experiment the snails were maintained for at least 8 days under labora-

tory conditions (natural variation of light, and temperature ranging from 19° to 29°C).

A series of experiments were designed to confirm that: a) there is a circadian rhythm of emergence of cercariae when temperature is maintained constant and snails are influenced by light variation; b) that the rhythm disappears when infected snails are kept under complete darkness or continuous light and constant temperature; c) the emergence continues to be rhythmic when snails are kept under complete darkness but with variation in the temperature; d) cercariae are shed in comparable numbers when snails, kept under normal laboratory conditions, are transferred to complete darkness and constant temperature.

RESULTS

Influence of normal variation of light intensity during the day on the emergence of cercariae when snails are kept at constant temperature

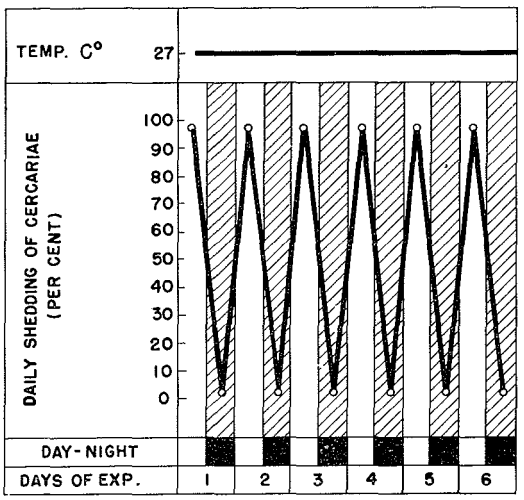

Fig. 1 — Rhythmic emergence of cercariae of *Schistosoma mansoni*: infected *Biomphalaria glabrata* kept at constant temperature but influenced by light variation of the day-night cycle.

In this experiment 5 snails were put individually in small beakers immersed into a large water-bath (100 x 50 x 20 cm) maintained at constant temperature (27 ± 0.5°C). The water bath was placed near a glass window, in order to receive illumination from the light following its normal circadian variation.

The results obtained are shown in Fig. 1. About 98 percent of the cercariae emerged during the day and only 2 percent at night. Cercarial countings showed a mean of 1,977 cercariae/snail/24 hours (range from 784 to 3,277).

Lack of rhythm when infected snails are kept at constant temperature and complete darkness or continuous illumination

The experiment was carried out in 17 days. In the beginning (4 days) the infected snails (15) were kept at room conditions with normal variation of light and temperature. Under these conditions the circadian rhythm was clearly established, 98% of the cercariae being shed during the day (6 A.M. to 6 P.M.). The mean number of cercariae per snail, shed within 24-hour periods, was 1,900 (range 530-5,970). From day 5 on, the snails were kept under complete darkness and at constant temperature. As shown in Fig. 2, the rhythm disappeared although cercariae continued to be shed. During the period of complete darkness and constant temperature the mean number of cercariae shed in 24 hours per snail was 2,980 (range from 340 to 10,080). On day 16 and 17 the snails were again submitted to normal variation of light and temperature and the rhythm of emergence returned promptly.

In the experiment depicted on Fig. 3, during 4 days 15 infected snails were kept under laboratory conditions with variations of light and temperature. Under these conditions, the classic pattern of cercarial emergence is clearly seen. From day 5 on the snails were kept under constant temperature (27°-28°C) and continuous illumination. As shown, the rhythm was still apparent during the first two days but disappeared thereafter. On day 14 the snails were again submitted to the influence of light-dark of the day-night period but the temperature was kept constant. The rhythm returned gradually to the normal pattern.

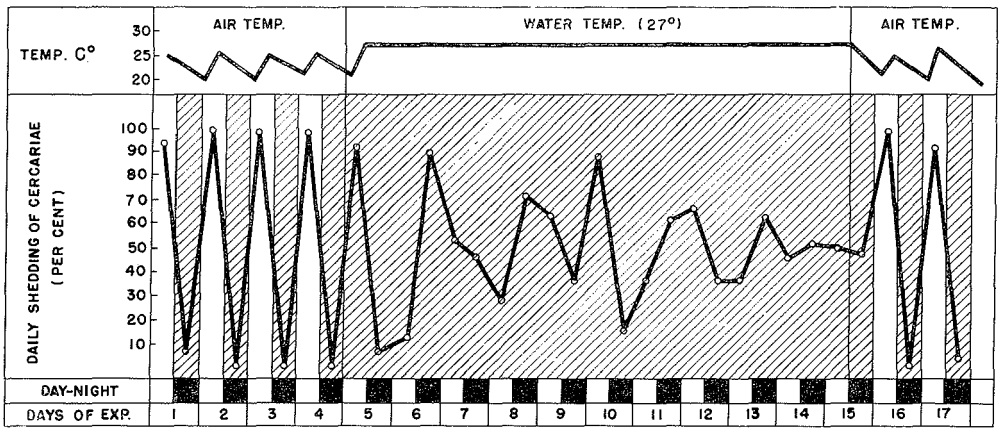

Fig. 2 — Loss of the rhythmic emergence of cercariae: within day 5 and 15, infected snails were kept under complete darkness and at constant temperature. Note that the cercarial shedding followed a regular rhythm at the beginning and at the end of the experiment (normal variation of light and temperature)

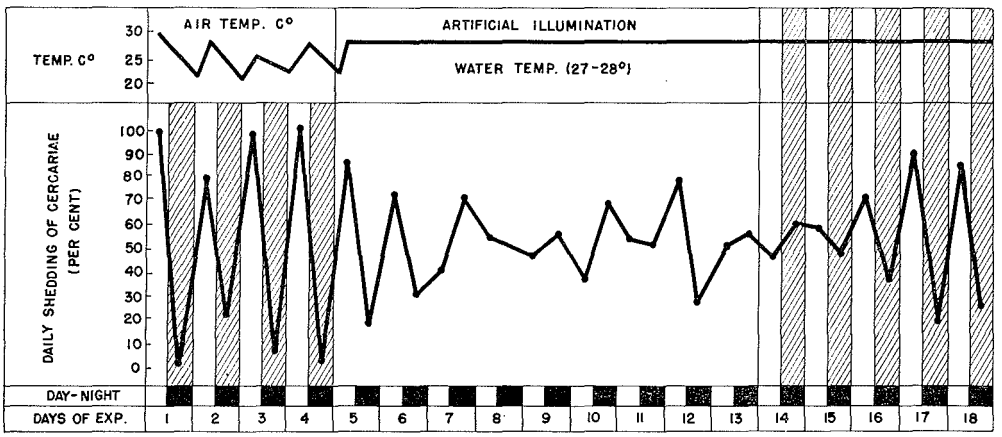

Fig. 3 — Loss of the rhythmic emergence of cercariae: within day 5 and 13, infected snails were kept under continuous illumination at constant temperature. Note that at the beginning and end of experiment the rhythm of cercarial shedding followed a normal pattern

Maintenance of the rhythm of emergence in snails kept under complete darkness but influenced by temperature variation

Five snails were maintained individually in small beakers (100 ml of water). The first day they were kept under laboratory conditions (variation of light and temperature). On the next day, the snails were put in the light-tight room in order to be in complete darkness but still continuining to be influenced by the variation in temperature during the day-night period (26°-28°; 19°-23°C). As can be seen from Fig. 4, the temperature alone was able to maintain a rhythm with more cercariae emerging during the "subjective day". Nevertheless, the difference in the percentages of cercariae emerged during the day and at night was not as clear when both light and temperature acted on the shedding process (days 10, 11, and 12).

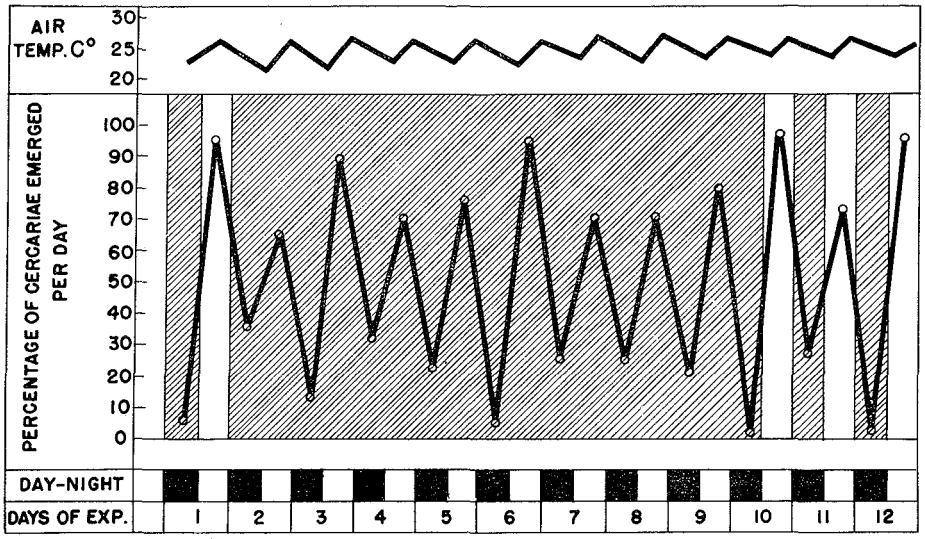

Fig. 4 — Persistence of rhythmic emergence of cercariae when infected snails were kept under complete darkness (day 2 to 10) but influenced by the variation of temperature during the day-night cycle

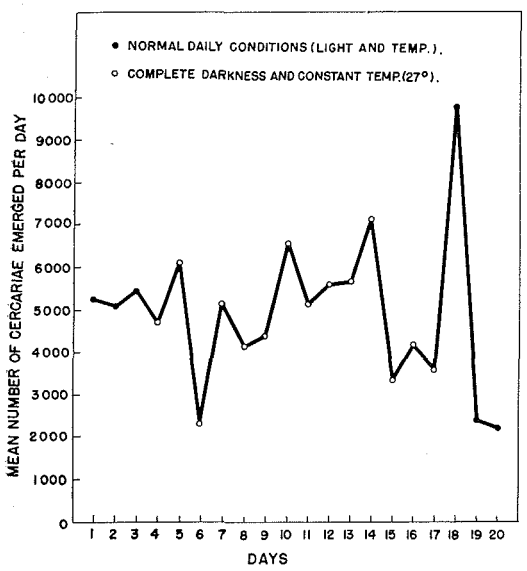

Fig. 5 — Mean number of cercariae emerged per day when infected snails were kept under normal laboratory conditions (●) and under complete darkness and constant temperature (○)

Influence of the suppression of light and of temperature variation on the number of cercariae emerged in 24-hour periods

Ten infected snails were used for this experiment (Fig. 5). In the 3 first days the snails, placed individually in small beakers of 100 ml, were kept under laboratory conditions, near a glass window. Cercariae were counted separately for each snail after a 24-hour period. From day 4 to day 17 the same snails were kept in complete darkness and at constant temperature (27° ± 0.5°C). On days 18, 19 and 20 the snails returned to the initial conditions. For the first period, the mean number of cercariae/snail per 24 hours was 5,257. At complete darkness and constant temperature the mean number was 4,870 and, for the last period (days 10, 11, and 12), 4,605.

DISCUSSION

The data provided by the literature show that the pattern of emergence of cercariae varies according to the species of trematode. In two species zoologically related to *S. mansoni*, LENGY [8] demonstrated that cercariae of *S. bovis* comes out both in dark and light, and for *Schistosomatium douthitti* OLIVIER [11] showed that cercariae are shed mainly in the dark and that the emergence pattern can be readily reversed by keeping snails in the dark during normally daylight hours. This

reversion (emergence of cercariae during normally night hours) was also achieved in experiments performed by LUTTERMOSER [9], VALLE, PELLEGRINO & ALVARENGA [15] and ASCH [1] with *S. mansoni* cercariae.

It was demonstrated by ASCH [1] that exposure of *B. glabrata* to different portions of the light spectrum, within the range of visible light, does not affect the shedding phenomenon. A threshold intensity of approximately 100-200 ergs/cm²/sec. was required to induce emergence.

From the data here presented and taking into consideration the observations of previous workers it seems clear that light represents the most important factor governing cercarial emergence and the circadian rhythm of cercarial shedding in *B. glabrata*. However, it was shown that when infected snails are kept in the dark but are affected by day-night variations of temperature, the rhythm is maintained, a great percentage of cercariae being shed during normally daylight hours. Although ASCH [1] considers that periodic application of light fits most of the criteria for control over cercarial emergence, and that illumination is a "Zeitgeber" according to the definition of ASCHOFF [2], it is clear from our experiments that diurnal variation of temperature alone can keep the cycle when snails are maintained under complete darkness. However, there is no doubt that light is more important than temperature in governing the cercarial rhythm.

It is important to note that the rhythm disappears when snails are kept under complete darkness or continuous illumination and constant temperature. However, cercariae continue to escape from snails. It was also demonstrated that cercarial shedding occurs regularly when snails are kept under complete darkness and constant temperature. Actually, the total number of cercariae shed per day under these conditions does not differ significantly when the same snails were maintained under normal conditions of the laboratory, i.e., with variation in light and temperature.

Although the data existing in the literature seem to favour the hypothesis that the circadian rhythm of cercarial emergence is environmental-dependent (exogenous rhythm) it is also probable that cercariae are responding to a rhythm of the snail which is controlled at least by illumination and temperature.

RESUMO

Ritmo de emergência de cercárias do Schistosoma mansoni *eliminadas por* Biomphalaria glabrata: *influência da temperatura*

Comprovando observações prévias de outros Autores foi mostrado que quando exemplares de *B. glabrata* são submetidos à temperatura constante mas são influenciados por variações de luz no período dia-noite, estabelece-se um ritmo regular no qual 98% das cercárias são eliminadas entre 6 e 18 horas. Esse ritmo desaparece quando: a) caramujos infestados são submetidos à temperatura constante e mantidos no escuro; b) quando a temperatura é constante e a iluminação contínua.

A variação de temperatura pode, isoladamente, manter um ritmo circadiano em caramujos mantidos no escuro, embora não seja tão nítido quando temperatura e luz combinados agem no mesmo processo.

Caramujos mantidos no escuro e à temperatura constante continuam eliminando cercárias, sendo que contagens feitas em períodos de 24 horas mostraram que esse número não difere significativamente quando os caramujos são mantidos em condições normais de laboratório.

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