Journal of Helminthology (2004) **78,** 185–187 DOI: 1079/JOH2003208

Research Note

Effects of glucose on survival, infectivity and linear movement of the cercariae of *Echinostoma caproni*

E.L. Ponder and B. Fried*

Department of Biology, Lafayette College, Easton, PA 18042, USA

Abstract

The effects of glucose in artificial spring water (ASW) on the survival, infectivity, and linear movement of *Echinostoma caproni* cercariae were studied. Cercariae maintained at 23°C in 1% glucose in ASW (ASWG) or ASW alone, reached 50% survival at 26 and 23 h, respectively. All cercariae in ASWG and ASW were dead by 50 and 32 h, respectively. Infectivity to juvenile *Helisoma trivolvis* (Colorado strain) snails was significantly less for cercariae aged 16 h in ASWG compared to cercariae aged 16 h in ASW. Linear movement, i.e. the ability of cercariae to traverse a 1-cm radius, ceased at 16 and 20 h for cercariae maintained in ASWG and ASW, respectively. Glucose added to ASW extended the survival time of *E. caproni* cercariae but decreased their ability to infect snails or move in a linear direction.

The cercariae of trematodes depend on their nutritive reserves to maintain activity since they are a non-feeding larval stage (Ginetsinskaya, 1988). In previous studies, the addition of glucose to artificial spring water (ASWG) enhanced the survival time of *Echinostoma trivolvis* and *Schistosoma mansoni* cercariae when compared to cercariae maintained only in artificial spring water (ASW) (Fried *et al.*, 1998, 2002). Fried *et al.* (2002) also found that *S. mansoni* cercariae maintained in 1% ASWG did not yield a significant increase in adult worm recovery in experimentally infected laboratory mice. No information is available on the effects of exogenous glucose on the survival, infectivity or linear movement of cercariae of *E. caproni*.

Activity of trematode cercariae is dependent, in part, on glycogen stored in the tail and body; additionally, accelerated consumption of glycogen through movement shortens the life span of cercariae (Ginetsinskaya, 1988). Actively swimming cercariae in the genera *Echinostoma* and *Opisthioglyphe* have large glycogen reserves, while the immobile cercariae of *Bilharziella polonica* have only a small amount of glycogen that is consumed slowly (Ginetsinskaya, 1988). Histochemical tests on *E. trivolvis* and *S. mansoni* using the periodic acid-Schiff (PAS) reaction indicated that glycogen stores decreased over time in cercariae maintained in either ASW or ASWG and resynthesis of glycogen did not occur in ASWG (Fried *et al.*, 1998, 2002). In addition to histochemical observations on the presence or depletion of glycogen, the changes in the activity of cercariae can be evaluated directly by measuring linear movement. Such movement can be attained by limiting the volume of medium to restrict vertical movement as described in Meyrowitsch *et al.* (1991). They used linear movement of cercariae in addition to survival and infectivity to evaluate the effects of temperature and host density on the snail-finding capacity of cercariae of *E. caproni*. Following the procedure of Meyrowitsch *et al.* (1991), our study examined differences in the linear movement of cercariae maintained in ASW versus ASWG in a restricted water depth. The aim of this study was to examine the effects of exogenous glucose on the survival and infectivity of *E. caproni*. Additionally, linear movement of cercariae in ASW versus 1% ASWG was also observed.

For studies on cercarial survival, cercariae collected within 1 h postemergence from experimentally infected *Biomphalaria glabrata* snails were placed ten per well in

*Author for correspondence
Fax: (610) 330-5705
E-mail: friedb@lafayette.edu

0.5 ml of ASW or 1% ASWG in a multiwell chamber. The glucose was purchased from Sigma Co. (St Louis, Missouri), and the ASW was formulated as described by Ulmer (1970). All replicates were maintained at 23°C for up to 44 h. Percent survival was determined at 8, 12, 16, 26, 36 and 44 h. Cercariae were considered dead when they no longer responded to a mechanical stimulation.

For studies on cercarial infectivity, cercariae collected within 1 h post-emergence from infected *B. glabrata* snails were placed ten per well in 0.5 ml of ASW or 1% ASWG in a multiwell chamber. At 4, 8, 12, 16 and 20 h each group of ten cercariae was transferred to 0.5 ml of fresh ASW in additional wells of the multiwell chamber and one juvenile *Helisoma trivolvis* (Colorado strain; shell diameter 1 to 3 mm) snail was added. This snail is a good experimental second intermediate host for *E. caproni* cercariae (Fried & LaTerra, 2002). Each snail remained in the wells with the cercariae for 8 to 12 h to ensure maximum infectivity (Fried & LaTerra, 2002). Each snail was removed from a well, crushed between a glass slide and coverslip, and examined under a compound microscope at 100 × to count the number of cysts formed per snail as described by Fried & LaTerra (2002).

Histochemical observations of whole cercariae were made on cercariae collected within 1 h postemergence as well as cercariae maintained in ASW or 1% ASWG for 24 h. Cercariae fixed in 10% neutral buffer formalin (NBF) were treated by the periodic acid-Shiff (PAS) reagent with or without 1% malt diastase digestion as described by Haas & Fried (1974). At least ten cercariae were used for each treatment.

For studies on linear movement, cercariae collected within 1 h postemergence from at least three infected *B. glabrata* snails were placed 50 per dish in Stender dishes containing ASW or 1% ASWG. After ageing for 0, 4, 8, 12, 16 or 20 h at 23°C, ten cercariae from each medium were observed under the dissecting microscope. Each cercaria was placed in the centre of a 2 cm diameter circle drawn on the underside of a 100 × 15 mm square Petri dish containing ASW with a maximum water height of 2 mm to restrict cercarial vertical movement. Time from release of a cercaria until it crossed a 1 cm radius was measured in seconds using a stopwatch. Cercariae that did not cross the radius within 5 min were considered to have lost the ability to move in a linear direction.

The effects of exogenous glucose on survival are summarized in fig. 1. Cercariae maintained in ASW alone reached 50% survival at 23 h, while cercariae maintained in 1% ASWG reached 50% survival at 26 h. The mean cercarial survival in ASWG was not significantly greater (one way ANOVA, $P > 0.05$) than that in ASW at 26 h. All cercariae in ASW were unresponsive by 32 h; all cercariae in ASWG were unresponsive by 50 h.

The effects of exogenous glucose on infectivity are summarized in fig. 2. Using Student's t-test ($P < 0.05$), the percent cyst recovery was significantly greater for cercariae maintained in ASW for 16 h compared to those maintained for the same time in 1% ASWG. There were no significant differences between the two treatments at the other cercarial ages.

The histochemical treatment of fresh cercariae revealed intense PAS positive staining material in the body and tail. Diastase treatment removed the PAS staining material from mainly the tail and some of the body. The cercariae maintained in ASW and 1% ASWG for 24 h showed no visible difference in staining reaction following treatment with PAS; cercariae maintained in ASWG showed no signs of glycogen resynthesis. Cercariae maintained in either ASW or ASWG for 24 h stained similarly to fresh cercariae that had been treated with diastase.

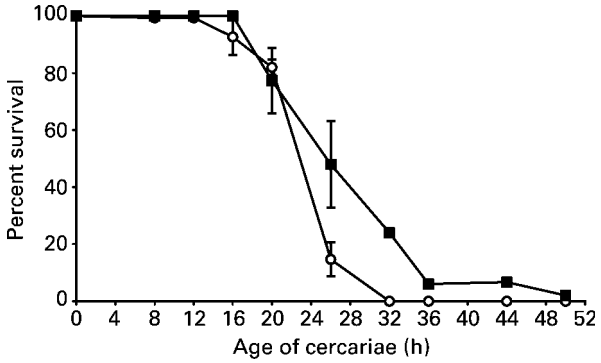

Fig. 1. Effects of exogenous glucose on the survival of *Echinostoma caproni* cercariae in artificial spring water (ASW); ASWG = 1% glucose in artificial spring water. Bar with open circle (○) represents mean ± SE in ASW; bar with closed square (■) represents mean ± SE in ASWG.

Linear movement for fresh cercariae (0-h old) and cercariae maintained in ASW or 1% ASWG for 4 to 20 h are summarized in table 1. Beyond 8 h, some cercariae in both media lost their ability to swim linearly. By 16 h, 40% of the cercariae in ASW could still swim linearly compared to 0% of those maintained in 1% ASWG for this time. All cercariae maintained in ASW lost their ability to swim linearly by 20 h.

Although exogenous glucose enhanced the maximal survival time of *E. caproni* cercariae, there was no histochemical evidence that glycogen was resynthesized in 24 h. Fried *et al.* (1998, 2002) also found that exogenous glucose enhanced the survival of the cercariae of *E. trivolvis* and *S. mansoni*, respectively, without evidence of glycogen resynthesis. Although maximal survival time

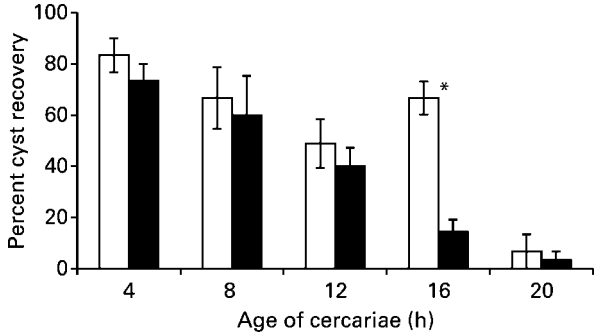

Fig. 2. Effects of exogenous glucose on the infectivity of *Echinostoma caproni* cercariae in artificial spring water (ASW); ASWG = 1% glucose in artificial spring water. The asterisk indicates significant difference (Student's t-test, $P < 0.05$). Bar in open column (□) represents mean ± SE in ASW; bar in closed column (■) represents mean ± SE in ASWG.

https://doi.org/10.1079/JOH2003208 Published online by Cambridge University Press

Table 1. Percent of *Echinostoma caproni* cercariae showing linear movement*.

	% cercariae showing linear movement	
Age of cercariae (h)	in ASW	in 1% ASWG
0	100	–
4	100	100
8	100	100
12	80	70
16	40	0
20	0	–

n = 10 cercariae for each observation.
*Cercariae that did not cross a 1 cm radius within 5 min were considered to have lost the ablility to move in a linear direction. ASW, artificial spring water; 1% ASWG, 1% glucose in artificial spring water.

was enhanced, infectivity of *E. caproni* cercariae to *H. trivolvis* (Co strain) snails decreased when maintained in 1% ASWG. The findings of decreased infectivity of *E. caproni* cercariae to a host in the presence of exogenous glucose are similar to that of Fried *et al.* (2002) who concluded that infectivity of *S. mansoni* to mice was not increased significantly when cercariae were maintained in 1% glucose for 24 h based on the number of adult worms recovered in mice.

Cercariae of *E. trivolvis* survived longer in 1% ASWG than in ASW alone, but maximum time of survival in either medium was not given (Fried *et al.*, 1998). In that study there was a significant increase in *E. trivolvis* cercarial survival in ASWG versus ASW at 23 h. In the present study, there was no significant difference in cercarial survival at any time.

Newly emerged echinostome cercariae show a continuous swimming mode with locomotion in all directions (Haas, 2000). In an attempt to quantify cercarial movement, Meyrowitsch *et al.* (1991) measured linear swimming speed of *E. caproni* maintained at various temperatures in a standard water type. They developed a method for measuring linear (horizontal) movement by maintaining a constant water height of 1.5 mm. Using this method, the cercariae moved in a mainly horizontal direction, so that linear movement in $mm\,s^{-1}$ could be measured. Meyrowitsch *et al.* (1991) reported a linear swimming speed of about 2 $mm\,s^{-1}$ at 0 h to about 1.5 $mm\,s^{-1}$ at 9 h in *E. caproni* cercariae maintained at 25°C in a standard water. Even with the restriction of water height, the cercariae observed in our study exhibited some non-linear swimming behaviour. Because of non-linear components involved in cercarial swimming, we did not analyse swimming speed in $mm\,s^{-1}$ as in the Meyrowitch *et al.* (1991) study. While swimming speed was not compared, cercariae in our study continued to show linear movement for up to 12 h after maintenance in 1% ASWG and for up to 16 h after maintenance in ASW while those observed by Meyrowitch *et al.* (1991) apparently ceased movement beyond 9 h. The discrepancies between the two studies may be accounted for, in part, by differences in the strain of *E. caproni*, water height, the media and temperature. In the absence of equipment to measure the complex swimming path of cercarial movement, the method for determining the presence or absence of linear movement of echinostome cercariae as described herein is a useful alternative.

We are not sure why both cercarial infectivity and linear movement were decreased in the glucose solution compared to the controls. Perhaps the weight of the glucose impeded the linear movement of the cercariae. Such impedance may have resulted in decreased cercarial infectivity.

References

Fried, B. & LaTerra, R. (2002) *In vitro* and *in vivo* encystment of the cercariae of *Echinostoma caproni*. *Journal of Parasitology* **88**, 1124–1129.

Fried, B., Eyster, L.S. & Pechenik, J.A. (1998) Histochemical glycogen and neutral lipid in *Echinostoma trivolvis* cercariae and effects of exogenous glucose on cercarial longevity. *Journal of Helminthology* **72**, 83–85.

Fried, B., LaTerra, R. & Kim, Y. (2002) Effects of exogenous glucose on survival and infectivity of *Schistosoma mansoni* cercariae. *Korean Journal of Parasitology* **40**, 55–58.

Ginetsinskaya, T.A. (1988) *Trematodes, their life cycles, biology and evolution*. Amerind, New Delhi.

Haas, W. (2000) The behavioral biology of echinostomes. pp. 175–197 *in* Fried, B. & Graczyk, T.K. (*Eds*) *Echinostomes as experimental models for biological research*. Dordrecht, Kluwer.

Haas, M.R. & Fried, B. (1974) Observations on cephalic glands in *Philophthalmus hegeneri*. *Journal of Parasitology* **60**, 1041–1043.

Meyrowitsch, D., Christensen, N.Ø. & Hindsbo, O. (1991) Effects of temperature and host density on the snail-finding capacity of cercariae of *Echinostoma caproni* (Digenea: Echinostomatidae). *Parasitology* **102**, 391–395.

Ulmer, M.J. (1970) Notes on rearing of snails in the laboratory. pp. 143–144 *in* MacInnis, A.J. & Voge, M. (*Eds*) *Experiments and techniques in parasitology*. San Francisco, Freeman.

(Accepted 12 August 2003)