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☐ **Brief Communication** ☐

Effects of exogenous glucose on survival and infectivity of *Schistosoma mansoni* cercariae

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Abstract: The effects of exogenous glucose in artificial spring water (ASW) were studied on the survival and infectivity of *Schistosoma mansoni* cercariae. The mean percent survival of cercariae maintained in 1% glucose in ASW for 36 and 48hr was significantly greater than that of cercariae maintained identically in ASW. Cercariae maintained in ASW with or without glucose for 24hr, fixed in neutral buffered formalin, and stained in Oil Red O, showed an accumulation of neutral lipid in the tail. Cercariae maintained as described above and stained in periodic acid-Schiff exhibited depleted glycogen, mainly from the tail. Cercariae maintained in ASW with glucose for 24hr did not resynthesize glycogen. Cercariae maintained in ASW with glucose for 24hr were as capable of infecting male FVBN202 mice as were freshly emerged cercariae, and increased the percent of worm recovery. Exogeneous glucose added to ASW prolonged the survival of *S. mansoni* cercariae and increased infectivity in terms of worm recovery.

Key words: *Schistosoma mansoni*, trematodes, cercariae, glucose, survival, infectivity

Glucose appears to enhance the survival of *Schistosoma mansoni* cercariae in aquarium water or artificial spring water (ASW), although detailed experiments on this topic are not available (Smyth, 1966; Sturrock, 1993). A recent study (Fried et al., 1998) showed that exogenous glucose enhanced the survival of *Echinostoma trivolis* cercariae in ASW.

The purpose of this study was to determine if glucose enhanced the survival of *S. mansoni* cercariae in ASW with glucose (ASWG). We also used histochemical methods to determine the presence of glycogen and neutral lipid in cercariae maintained in ASW or ASWG. Observations were also made on the infectivity of cercariae maintained in ASW or ASWG that were used to infect laboratory mice.

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In preliminary experiments, we determined that cercarial survival was greater in 1% ASWG compared with that in 0.5% or 0.1% ASWG. The ASW was prepared as described in Ulmer (1970). Likewise, preliminary experiments showed that cercarial survival in 1% maltose or 1% sucrose in ASW was no different than that in ASW. All sugars were purchased from Sigma Co. (St. Louis, Mo., USA).

Preliminary experiments used large numbers of cercariae (> 2000/50 ml of ASW or 1% ASWG). These experiments showed that whereas more than 10% of the cercariae were alive and active (showed vibratory movements of the tail and body) at 48hr post-cercarial emergence in 1% ASWG, most were dead by 48 hr in ASW. All cultures were maintained at 23-24°C.

S. mansoni cercariae were obtained from experimentally infected *Biomphalaria glabrata* (NM RI strain) snails and used within 1hr

post-emergence as described in Fried et al. (2001). Based on the preliminary qualitative experiments on large numbers of cercariae, the quantitative experiment reported below was done.

Freshly emerged cercariae (1hr old) were placed 10 per well in 0.5 ml of ASW or 1% ASWG in a multiwell chamber. A total of 12 wells was used for cercariae maintained in 1% ASWG and an additional 12 wells were used for cercariae maintained in ASW. The multiwell chamber was maintained at 23 to 24°C for 72hr. The number of live cercariae was determined at 12hr intervals up to 72hr post-cercarial emergence. Live cercariae showed vibratory body and tail movements or reacted to probing with a needle.

The results of the quantitative experiments are seen in Fig. 1. There was a significant increase in cercarial survival in 1% ASWG at both 36 and 48hr post-cercarial emergence compared to cercariae maintained in ASW (one way ANOVA, $P < 0.05$).

Histochemical observations were made on neutral lipid using Oil Red O (ORO) staining on whole cercariae as described in Fried et al. (1998). Freshly emerged cercariae (1hr old) or those maintained in 1% ASWG for 24hr and some maintained in 1% ASWG for 48hr were fixed in cold 10% neutral buffered formalin, stained in ORO, and mounted in glycerin jelly. Observations were made on 25 to 50 cercariae maintained under the conditions described above. Histochemical whole mount observations were also made on glycogen based on similar numbers of cercariae described above. The procedure used was that of periodic-acid Schiff (PAS) on whole cercariae fixed in absolute ethanol-formalin (9:1). Control cercariae were treated in 1% malt diastase prior to PAS treatment as described in Fried et al. (1998).

Histochemical ORO staining showed that more than 50% of the freshly emerged cercariae did not contain lipid droplets. The remaining fresh cercariae each showed less than 25 small ORO positive droplets (< 1 μm diameter) in the tail stem and furcae. These droplets may reflect lipid byproducts resulting from carbohydrate metabolism. Our observations were similar to what was seen in fresh cercariae of the avian schistosome *Ornithobilharzia canaliculata* (see Fig. 1 in Fried and McFalls, 1975). Cercariae aged for 24hr in ASW or for 24 and 48hr in ASWG showed variable results. Approximately 50% of these cercariae were ORO negative but the remaining cercariae aged with or without glucose showed a large number of droplets (between 25 and 100), about 0.5 to 10 μm in diameter in the tail stem and furcae. Cercariae that were lipid positive looked similar to what

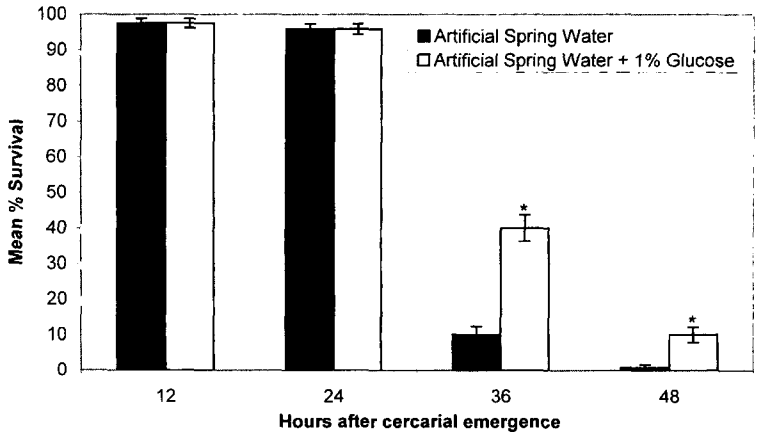

Fig. 1. Mean percent survival ± SE of *Schistosoma mansoni* cercariae aged in artificial spring water (shaded) versus artificial spring water plus 1% glucose (unshaded) for 12 to 48 hr post-cercarial emergence. Asterisk indicates a significant ($P < 0.05$) enhancement in survival at 36 and 48 hr of cercariae maintained in the glucose solution.

was seen in Fig. 2 of Fried and McFalls (1975) for aged cercariae of *O. canaliculata*.

The histochemical reactions of cercariae prepared in PAS were very consistent. Freshly emerged cercariae showed PAS positive reactions in the body and tail and diastase eliminated the reaction in the tail and furcae and in the parenchyma between the tegument and cercarial glands in the body. Most cercariae aged for 24 and 48hr with or without glucose showed reduced or nil reactivity to PAS in the tail stem and furcae. The reaction was less consistent in the body, but some loss of reactivity in the parenchyma was noted.

Fifteen 6-wk-old male mice (FVBN202 strain) each weighing about 20 g were individually exposed for 1 hr by the percutaneous tail method (Lewis, 1998) to 60 cercariae. These mice are transgenic for the rat neu gene, and spontaneously develop mammary carcinomas between 6 and 7 months of age (Kurt et al., 2000); they have not been used previously in schistosomiasis research. Five mice each received freshly emerged cercariae (1hr old); another five each received cercariae maintained for 24hr in ASW and five others each received cercariae maintained in 1% ASWG for 24 hr. All mice were necropsied at 6 wk post-infection (p.i.) following light ether anesthetization and cervical dislocation. Adult schistosomes were recovered by dissection mainly in pairs from the mesenteric veins. Some worms were also recovered via liver washing. The results of this experiment are shown in Table 1. Almost twice as many worms were recovered from mice exposed to cercariae maintained in 1% ASWG for 24hr compared to mice exposed to the freshly emerged cercariae. However, there was no significant difference in worm recoveries between any of the groups ($P > 0.05$).

Table 1. Infectivity of 5 mice per group[a] with each mouse exposed to 60 cercariae of *Schistosoma mansoni* via the tail route

Group[a]	Total number of worms recovered	% of worm recovery (Mean ± SE)
A	34/300	11.3 ± 1.2
B	40/300	13.3 ± 1.8
C	65/300	21.7 ± 4.0

[a]Group A = fresh cercariae, used within 1 hr post-cercarial emergence; B = cercariae aged for 24 hr in artificial spring water (ASW) without glucose; C = cercariae aged for 24 hr in ASW plus 1% glucose.

In conclusion, glucose enhanced the survival of *S. mansoni* cercariae maintained in artificial spring water. Some accumulation of neutral lipid occurred in cercariae aged in artificial spring water with or without glucose. Glycogen was found mainly in the cercarial tail. By 24hr post-emergence, cercariae maintained in artificial spring water with glucose showed no histochemical evidence that glycogen was resynthesized. Cercariae maintained in artificial spring water with glucose for 24hr were capable of infecting mice with increased worm recovery.

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REFERENCES

Fried B, Eyster LS, Pechenik JA (1998) Histochemical glycogen and neutral lipid in *Echinostoma trivolvis* cercariae and effects of exogenous glucose on cercarial longevity. *J Helminthol* **72:** 83-85.

Fried B, McFalls EO (1975) Histochemical observations on neutral fat in newly emerged and day-old cercariae of the avian schistosome, *Ornithobilharzia canaliculata* (Rudolphi, 1918) *Proc Helm Soc Wash* **42:** 57-58.

Fried B, Muller EE, Broadway A, Sherma J (2001) Effects of diet on the development of *Schistosoma mansoni* in *Biomphalaria glabrata* and on the neutral lipid content of

the digestive gland-gonad complex of the snail. *J Parasitol* **87:** 223-225.

Kurt RA, Whitaker R, Baher A, Seung S, Urba W (2000) Spontaneous mammary carcinomas fail to induce an immune response in syngenic FVBN202 neu transgenic mice. *Int J Cancer* **87:** 688-694.

Lewis FA (1998) Schistosomiasis. In: Coico R (ed) Current protocols in immunology. John Wiley & Sons, New York, pp 19.1.1-19.1.28.

Smyth JD (1966) The physiology of trematodes, WH Freeman and Company, San Francisco, p 256.

Sturrock RF (1993) The parasites and their life cycles. In: Jordan P, Webbe G, Sturrock RF (eds) Human schistosomiasis. CAB International Press, Wallingford, pp 1-32.

Ulmer MJ (1970) Notes on rearing snails in the laboratory. In: MacInnis AJ, Voge M (eds) Experiments and techniques in parasitology. WH Freeman and Company, San Francisco, pp 143-144.