In Vitro Excystment of the Metacercaria of 
*Acanthoparyphium spinulosum* (Trematoda: Echinostomatidae)

H. S. Bass\(^1\) and W. B. LeFlorence\(^2\)

\(^1\) Department of Biology, Atlanta University, Atlanta, Georgia 30314 and

\(^2\) Department of Biology, Spelman College, Atlanta, Georgia 30314

**ABSTRACT:** In vitro excystment studies on the metacercaria of *Acanthoparyphium spinulosum* indicated that optimal excystment occurred when the cysts were incubated in either a complete medium containing trypsin and bile salts, or bile salts alone following acid pretreatment, and the reductant (sodium dithionite) at a temperature of 42°C and a pH of 7.8. A relatively lower percentage of excystment was obtained following pretreatment with acidified trypsin or Hanks' BSS, the reductant, and incubation in trypsin at a pH of 7.8. Worms that excysted in any medium containing trypsin became sluggish after 1 hr in comparison to worms that excysted in bile salts, indicating that trypsin may have an inhibitory effect. Worms excysted in bile salts medium after pretreatment in acid and the reductant were more active, suggesting that bile salts may act as a muscular stimulant. Pretreatment with acidified pepsin followed by incubation in the complete medium, in the absence of the reductant, resulted in a low percentage of excystment; whereas, pretreatment with acidified Hanks' BSS followed by incubation in the complete medium in the absence of the reductant resulted in no excystment. The in vitro excystment process was initiated by acid pretreatment that resulted in vigorous muscular activity of the enclosed larva that eventually produced the escape aperture for emergence. A synergistic effect between trypsin and bile salts does not appear to occur in the excystment of this parasite.

Several reports have been concerned with the in vitro excystment of metacercarial cysts of echinostomes. Howell (1970) studied the conditions necessary for the excystment of *Echinostomum serratum*; Fried and Grigo (1975) reported on *E. flexum*; Fried and Butler (1978) observed the excystment of *Echinostoma revolutum*; Kirschner and Bacha (1980) described factors involved in the excystment of *Himasthla quissetensis*; and LeFlorence and Bass (1982) investigated the conditions responsible for the excystment of *H. rhigedana*. There have been no similar studies on the metacercaria of *Acanthoparyphium spinulosum*. The first and second intermediate host of *A. spinulosum* is the brackishwater snail *Cerithidea hegewischii californica*. Cercariae that develop in daughter rediae excyst in the radular mass of the snail. The natural adult hosts are the black-bellied plover, *Pluvialis squatarola*, and the American avocet, *Recurvirostra americana*, which acquire the parasite by eating infected snails. Adults have been grown experimentally in the domestic chick by Martin and Adams (1961). The present study was undertaken to examine the factors that bring about excystment of *A. spinulosum* in vitro and to describe the behavior of the metacercaria during excystment.

**Materials and Methods**

Metacerciae of *Acanthoparyphium spinulosum* were obtained from the mudflat snail *Cerithidea californica* purchased from Jones Biomedicals and Laboratory, Long Beach, California, and Pacific Bio-Marines Laboratories, Venice, California. Several snails were placed in a finger bowl containing 50 ml of artificial seawater and maintained at room temperature. To obtain metacercariae, snails were crushed and the radular mass was removed and washed in artificial seawater. Cysts were dissected from the radular tissue and then washed in 0.85% saline prior to use.

Experiments were done in 2 ml of test medium in 3.5-cm-diameter culture dishes placed in a water bath at 42°C. The treatment groups were as follows: 1) pretreatment with 0.5% pepsin (1:10,000) in Hanks' BSS adjusted to pH 2.0 with 6 N HCl (acidified pepsin) for 1 hr and incubation in 0.2% sodium taurocholate (crude ox bile) and 0.5% trypsin (1:250) in Hanks' BSS adjusted to pH 7.8 with 7.8% NaHCO₃ (complete medium); 2) pretreatment with acidified pepsin for 1 hr, the reductant 0.015 M sodium dithionite (Na₂S₂O₄) for 10 min, and incubation in the complete medium; 3) pretreatment with acidified pepsin for 1 hr, the reductant for 10 min, and incubation in 0.2% ox bile and Hanks' BSS, pH 7.8; 4) pretreatment with acidified pepsin for 1 hr, the reductant for 10 min, and incubation in 0.5% trypsin in Hanks' BSS, pH 7.8; 5) pretreatment with acidified pepsin for 1 hr, the reductant for 10 min, and incubation in Hanks' BSS, pH 7.8; 6) pretreatment with acidified pepsin for 1 hr, the reductant for 10 min, and incubation in the complete medium; 7) incubation in the complete medium; 8) pretreatment with Hanks' BSS, pH 2.0 (acidified Hanks') instead of acidified pepsin for 1 hr and incubation in the complete medium; 9) pretreatment with acidified Hanks' for 1 hr, the reductant for 10 min, and incubation in the complete medium; 10) pretreatment with acidified Hanks' BSS for 1 hr, the reductant for 10 min, and incubation in 0.2% ox bile and Hanks' BSS, pH 7.8; 11) pretreatment with acidified Hanks' for 1 hr, the reductant for 10
Effect of acidified pepsin and Hanks’ BSS on excystment

Results
Effects of treatments

Maximal excystment of Acanthoparyphium spinulosum occurred in the complete medium or in 0.2% bile salts following pretreatment with acidified pepsin or acidified Hanks’ BSS for 1 hr, and the reductant for 10 min. Figure 1 shows that the highest rate and percentage was obtained using the complete medium, whereas, either bile salts or trypsin alone produced lower percentages after pretreatment with acidified pepsin and the reductant. Figure 2 shows that after pretreatment with acidified Hanks’ BSS and the reductant, the highest rate and percentage resulted when bile salts alone were used, whereas, the complete medium and trypsin alone produced lower percentages.

Pretreatment with either acidified pepsin or Hanks’ BSS and incubation in the complete medium in the absence of the reductant resulted in 1% and 0% excystment, respectively. When cysts were pretreated with acidified pepsin for 1 hr, the reductant for 10 min followed by Hanks’ BSS at pH of 7.8, 35% of the larvae excysted, but when similar experiments were repeated using acidified Hanks’ instead of acidified pepsin, no excystment occurred. If cysts were exposed to the reductant for 10 min and incubated in either the complete excystment medium or alkaline Hanks’ BSS only 11% and 0% excysted, respectively. No excystment was observed when cysts were incubated in either the complete medium, alkaline Hanks’ BSS, and 0.85% physiological saline.

Copyright © 2011, The Helminthological Society of Washington
Figures 3-6. Excystment of Acanthoparyphium spinalosum metacercaria. 3. Metacercaria partially emerged after brief treatment with the complete medium. Scale bar = 0.025 mm. 4. Anterior portion of metacercaria partially emerged through the cyst wall. Scale bar = 0.025 mm. 5. Scanning electron micrograph of a partially excysted metacercaria. Note forebody (F) and cyst wall (C). Scale bar = 78 μm. 6. Encased posterior portion of metacercaria with concretions (arrow) in the excretory system. Scale bar = 0.025 mm.

Excystment

The untreated metacercarial cyst wall is composed of two layers, the outer rough and irregular, the inner smooth and ovoid. Both layers are transparent, enabling direct observation of the enclosed larva. The cyst wall show no distinct region through which the metacercaria could emerge during excystment. Excystment was initiated during acid pretreatment at which time the concretions within the excretory system moved back and forth and the larva swelled within the cyst wall. When the metacercaria was exposed to the reductant, the outer cyst membrane became delicate and sticky. The worm then developed an opaque color. In the complete medium the worm slowly rotated until its oral sucker was aligned against the inner cyst membrane. Then the worm vigorously pushed against the inner cyst wall, with its collar eventually producing an escape aperture (Fig. 3). Once the outer cyst membrane was broken, the larva rapidly exited to the area of the acetabulum (Fig. 4) and then beyond that area almost freeing itself (Fig. 5). The emerged anterior portion of the worm
moved back and forth and the enclosed posterior portion pushed itself against the cyst membrane (Fig. 6) until the parasite completely freed itself. The excystment process was usually completed within 1–3 min in the complete medium. Worms that excysted in bile salts alone were very active for up to 3.5 hr compared to those that excysted in media that contained trypsin. Those worms became sluggish after 1 hr postexcystment.

Discussion

The results of the present investigation reveal that maximal excystment of Acanthoparyphium spinulosum occurs at 42°C and pH 7.8 as a result of acid pretreatment, followed by incubation in the reductant, sodium dithionite, and exposure to either bile salts alone or a complete medium consisting of bile salts and trypsin. Although acid pretreatment appears to be necessary, acidified pepsin increased the rate of excystment when compared with pretreatment with acidified Hanks'. This observation is in accord with that of Howell (1970) on Echinoparyphium serratum cysts. Acid pretreatment and reducing conditions are prerequisites for the successful excystment of E. serratum and Himasthla quissetensis (Howell, 1970; Kirschner and Bacha, 1980). These two echinostomes are similar to A. spinulosum in that they also require a second intermediate host. Moreover, our results are similar to those of Kirschner and Bacha (1980) with H. quissetensis in that pretreatment with either acidified pepsin or Hanks' and the reductant are required for maximal excystment. Acid pretreatment and reducing conditions are important in achieving a high percentage of excystment of other trematodes, notably Fasciola hepatica and Zygocotyle lunata (Dixon, 1966; Fried et al., 1978; Fried and Butler, 1979). However, the excystment pattern of A. spinulosum differs from the observation of LeFlore and Bass (1982) for H. rhigedana in that acid pretreatment and the reductant are not required for excystment, although both enhanced the rate and percentage of excystment. This may be explained because of the difference in excystment of these two echinostomes. The cercaria of H. rhigedana encysts in the open and does not require a second intermediate host.

Excystment in a trypsin-bile salt medium with a synergistic effect has been reported for E. serratum by Howell (1970), Parorchis acanthus by Fried and Roth (1974), E. flexum by Fried and Grigo (1975), H. rhigedana by LeFlore and Bass (1982) and Cloacitrema michiganensis by LeFlore and Bass (1983a). Maximum excystment of A. spinulosum can be achieved with a trypsin-bile salt medium; however, a medium with bile salts alone increased the rate and percentage of excystment. Therefore, a synergistic effect between these two substances does not seem to occur in this case. Bile salts appeared to play a significant role in stimulating muscular contraction and rate of emergence of A. spinulosum. The metacercariae, of a number of other trematodes have been shown to be stimulated by the addition of bile salts, resulting in increased muscular activity and rate of emergence (Erasmus and Bennet, 1965; Dixon, 1966; Howell, 1970; Fried and Butler, 1978; Mitchell et al., 1978; Johnston and Halton, 1981). Lackie (1975) suggested that bile salts stimulate increased muscular activity of metacercaria, and this increased activity may be assumed to assist the larva in pushing its way out of the cyst membrane.

As the excystment process of the metacercaria of A. spinulosum was initiated during pretreatment, the concretions within the excretory system began moving. The function of these structures is unknown; however, Smyth (1969) suggested that they play a very important part in phosphorylation and energy transfer mechanisms. Concretions may be seen in adult trematodes of a few marine families, but are probably expelled during excystment of most other adults or shortly thereafter (Erasmus, 1967; Fried and Butler, 1978). LeFlore and Bass (1983b) observed that concretions within the excretory system of the encysted metacercaria of H. rhigedana histochemically localized alkaline phosphatase.

Incubation in any medium containing trypsin resulted in a low percentage of excystment, producing worms that became sluggish within 1 hr. Apparently, trypsin has a detrimental effect on the excysted larva of A. spinulosum. Johnston and Halton (1981) reported an inhibitory effect by trypsin on the excystment of Bucephaloides gracilescens. Various percentages of excystment have been obtained in media containing trypsin for Sphaeridotrema globulus and H. quissetensis (Macy et al., 1968; Kirschner and Bacha, 1980; Fried and Huffman, 1982).

Acknowledgments

This study was supported by Grant RR 8006 from the General Research Support Branch, Division of Research Resources, National Insti-
tutes of Health, and funds provided by Grant 78-11955 from the National Science Foundation to the Atlanta University Resource Center for Science and Engineering. This is research Publication No. 130 of the Atlanta University Center Science Research Institute. We wish to thank Dr. Bernard Fried of Lafayette College, Easton, Pennsylvania for critically reading the manuscript.

Literature Cited


