Attachment of *Cyclocoelum ocaleum* Miracidia to Snails and Subsequent Penetration by Their Redial Stage

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**ABSTRACT:** The miracidium of *Cyclocoelum ocaleum* contains a fully formed redia. After attachment, the snail’s epithelial cells are breached by the distal portion of the apical papilla. Retraction of its tip pulls a plug of snail tissue into the papillar cavity, thus forming a stable attachment between miracidium and snail. During this process secretions from the miracidium’s apical and lateral glands are released, presumably causing lysis of snail tissues. Then, miracidial membranes near the apical papilla are digested by secretions from the radial esophageal glands, resulting in an opening through which the redia passes into the snail when the apical papilla of the miracidium retracts. Upon locating a hemolymph vessel the redia migrates to the hemocoel surrounding the buccal bulb.

Cyclocoelid miracidia are unusual, each containing a fully formed redia. When contact is made with a suitable snail, they attach and the redia penetrates, leaving the empty miracidia behind.

Even though a number of authors have observed this phenomenon, including Szidat (1932), Stunkard (1934), Ginetsinskaia (1949), Taft (1973, 1975) and Taft and Heard (1978), a detailed study of miracidial attachment and subsequent penetration by the redia has not been published.

This paper presents aspects of miracidial attachment, escape of the redia from the miracidium and early migration of the redial stage of *Cyclocoelum ocaleum* through snail tissues.

**Materials and Methods**

Gravid adults of *C. ocaleum* were removed from the orbits of American coots, *Fulica americana*, and were dissected in previously boiled aquarium water. Miracidia thus obtained were exposed to snails (*Gyraulus hirsutus*) for 10 min to 1.5 hr. Exposed snails were fixed in Gendre’s fluid, embedded in paraplast, and sectioned at 5–8 μm. Sections stained with mercuric bromphenol blue (Mazia et al., 1953) a general protein stain, allowed observation of general radial morphology, and in particular, musculature of the miracidial apical papilla. The PAS technique (Pearse, 1960) for polysaccharides and polysaccharide complexes delineated the lateral and apical glands of the miracidium and, to a lesser extent, the radial esophageal glands. Aldehyde fuchsin (Cameron and Steele, 1959), considered a neurosecretory stain, proved good for general morphology and also stained contents of the apical and esophageal glands purple, indicating the time of release of their contents.

The examination of over 200 sectioned snails with miracidia and rediae in various stages of attachment and penetration provided clarification of many aspects of the attachment and penetration process.

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1 Supported in part by research grant 8408 from University of Wisconsin Regents.
Results and Discussion

Initial attachment of miracidia to the molluscan host is difficult to observe because of their tendency to fall off at the stage when the snail is placed in fixative. However, several authors working at both light and EM levels have speculated on that process. Dawes (1960) suggested that the apical papilla of *Fasciola hepatica* miracidia retracts on contact with the snail, resulting in negative pressure which assures adhesion to the host at that point. Studying schistosomes, Wadji (1966) assumed that two mechanisms were involved: beating of the cilia to keep the miracidium pushed against the snail; and use of a mucoid substance secreted by the lateral (adhesive) glands of the miracidium causing it to adhere to the snail. Wilson et al. (1971) also postulated an adhesive role for the lateral glands of *F. hepatica* miracidia.

Wilson (1969), Kinoti (1971), LoVerde (1975), and Blankespoor and van der Schalie (1976) postulated that filaments or corrugations on the apical papilla of various miracidia aid in attachment. However, Køie and Frandsen (1976) theorized that folds on the apical papilla act as a “rasp,” or play some role in the discharge of glandular secretions. Coil (1977), and Wilson et al. (1971) suggest that formation of a sucker caused by invagination of the tip of the apical papilla may also be responsible for initial miracidial attachment.

Living *C. oculum* miracidia are approximately 0.192 × 0.069 mm. They are covered externally with cilia, except for the apical papilla. Internally, an apical gland, flanked by lateral glands, is located immediately posterior to the apical papilla. During penetration, all three glands empty via ducts opening on the apical papilla. Behind these glands is a cavity containing a fully formed redia. Anteriorly, the redia bears two minute appendages; posteriorly, there are two larger appendages and a tail. Internally, the redia possesses a pharynx, with a so-called esophageal gland on either side, and a sacciform intestine.

Like other miracidia which have been studied, initial attachment of *C. oculum* miracidia to the snail’s surface is still unclear. However, once the surface is breached, attachment and penetration becomes easier to observe. Retraction of the apical papilla at the tip forms a cavity into which a plug of the snail’s tissue is pulled (Figs. 1, 2). This connection is firm between miracidium and snail, as evidenced by numerous observations of it in tissue sections. By the time the miracidium has penetrated the snail’s epithelium, a portion of the apical and lateral gland contents has been voided (Fig. 3). Whether secretions of these glands have more than one function is still debatable, but circumstantial evidence indicates that cytolysis is involved.

Concomitant with miracidial gland emptying is the release of redial esophageal gland contents (Fig. 4). If one studies these redial glands from the time the miracidium breaches the snail’s epithelium until the redia enters the snail, a rapid decrease in the contents of the glands can be observed. Occasionally, a small amount of glandular material was seen near the anterior edge of the redial pharynx (Fig. 8). From these observations it is presumed the function of the redial glands is to lyse the wall of the miracidium between the first tier of epidermal plates and the apical papilla, thus allowing the redia to escape. Ginetsinskaia (1949) figured what appears to be esophageal glands in a redia of *Cyclocoelum microstomum* just after it had recently entered snail mantle tissue. From her figure it is difficult to tell whether these glands are empty or full.
Figures 1–4. *Cyclocoelum oculum* miracidia and rediae. Scale = 95 μg. 1. Plug of snail tissue drawn into a suckerlike cup formed at the end of the greatly extended apical papilla (ap) of the miracidium containing a redia (r). 2. Plug of snail muscular and connective tissue drawn into invaginated distal end of apical papilla (ap) of a miracidium (m) with a redia (r). 3. Attached miracidium with contained redia (r), showing partially empty apical (ag) and lateral glands (lg). 4. Esophageal glands (eg) of the redia (r) within miracidium (m).
Figures 5–9. *Cyclocoelum oculeum* miracidia and rediae. 5. Redia (r) passing from the miracidium (m) into snail tissue. Note the redial pharynx (p), and the retraction of the muscular apical papilla (ap) into the miracidium. Scale = 95 μm. 6. Redia (r) passing from the miracidium (m) into snail tissue. Note the pharynx (p) of the redia (r) and apical gland (ag) of the miracidium. Scale = 95 μm. 7. Empty miracidium (m), with retracted apical papilla (ap) and apical gland, still attached to snail. Observe the opening (o) through which the redia passed. 8. Redia (r) within hemolymph vessel (hv) of snail. Note residual material (arrow) from the esophageal glands at the anterior end of the redia. Scale = 200 μm. 9. Redia (r) near snail’s salivary glands (sg). Scale = 200 μm.
Once the miracidial membranes are dissolved, the apical papilla retracts (Fig. 5) and the redial stage squeezes through the opening. At this time, the redia is two to two and one-half times as long as the miracidium and is constricted as it passes through the opening (Figs. 5, 6).

Once the redial stage has left, the remnant of the miracidium remains attached. The opening through which the redia passed is evident (Fig. 7). Eventually, the miracidium falls from the snail.

Immediately upon leaving the miracidium the redia enters a hemolymph vessel. At that time, the esophageal glands are empty, except for occasional residues of material near the anterior end of the pharynx (Fig. 8). The redia migrates via these vessels to a sinus surrounding the snail’s buccal bulb and salivary glands (Fig. 9). Recently liberated rediae always migrate to this area regardless of the point of entry.

Sixteen-millimeter cinephotomicrography of living miracidia, as well as TEM and SEM studies are currently under way to further elucidate the miracidial attachment process and subsequent release and migration of the rediae.

Acknowlegments

I would like to thank Dr. Lyle Nauman, Jerry Bartelt, and Cindy Swanberg for aid in collecting American coots, Drs. William LeGrande and Martin J. Ulmer for critically reading the manuscript, and Dr. Robert Price for translating a Russian article.

Literature Cited


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**Report on the Brayton H. Ransom Memorial Trust Fund**

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Receipts: Net interest received in 1981 .................................. 608.10

$6203.03

Disbursements: Grant to The Helminthological Society of Washington for 1980 (made on February 3, 1981) ................... 50.00
Grant to The Helminthological Society of Washington for 1981 ...................................................... 50.00
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