A Study of Chemoreception in the Miracidia of *Schistosoma mansoni*

by

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Abstract

Miracidia of Schistosoma mansoni were tested as to their behavioral response to snail conditioned water (=SCW) and to methylene dichloride extracted SCW. Samples tested were injected into the arm of a four-arm test chamber and responses of miracidia placed in the center well were recorded. Both SCW and fractionated SCW attracted miracidia into the arms.

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Introduction

Schistosomes are digenetic trematodes responsible for the parasitic disease schistosomiasis. It is estimated that throughout the world, 200 million people are infected with these blood flukes. Schistosomes have a complex life cycle. In addition to their definitive host, they have an intermediate molluscan host. The intermediate larva or miracidia has been shown to utilize several sensory mechanisms in order to locate its snail host. Beyond the environmental cues such as light, temperature, and water velocity, it has long been known that miracidia are sensitive to chemicals present in the surrounding water. Both Thomas (1883) and Leuckart (1894) hypothesized that some component of the mucus of the snail host, Lymnaea truncatula, causes a response by Fasciola hepatica miracidia. MacInnis (1965) demonstrated that certain amino acids and short chain fatty acids attracted the miracidia of Schistosoma mansoni when the compounds were embedded in agar pyramids. It was later shown that substances in the exudates of snails alter the behavior of miracidia (Chernin, 1970; Shiff and Kriel, 1970; Ulmer, 1971). Subsequently it was shown that water conditioned by snails (=SCW) contained amino acids (Wright and Ronald, 1972; MacInnis et al., 1974). MacInnis also reported the presence of a lipid fraction of SCW, which when transesterified with methyl alcohol and analyzed by gas-liquid chromatography, indicated the presence of at least six peaks of unknown nature. Recently it has been revealed that a low ratio of calcium to magnesium ions, as is found in SCW, elicits an attractive response by miracidia (Sponholtz and Short, 1976; Stibbs et al., 1976). Among the methods used to quantitate miracidial attraction have been a calculation of percent contact with return to chemical containing agar pyramids, a 4-arm chamber Etges and Decker, 1963; Shiff and Kriel, 1970; Wright and Ronald, 1972), a Y-maze (Shiff and Kriel, 1970), a phi-chamber (Roberts et al., 1978), a photographic method of determining turning rates (Wilson and Denison, 1970), a dark field photographic technique to measure speed and change of direction (Mason and Fripp, 1976), and the percent contact with return to a dialysis membrane (Sponholtz and Short, 1975, 1976).

In this study, the 4-arm chamber method of Wright and Ronald (1972)

was adapted to quantitate the reaction of Schistosoma mansoni miracidia to Biomphalaria glabrata SCW. The response of miracidia to the SCW lipid fraction was investigated.

Materials and Methods

Miracidia

Miracidia were hatched from eggs excreted in the feces of albino mice infected with a Kenyan strain of S. mansoni passaged through a Kenyan strain of Biomphalaria pfeifferi. The eggs were washed and concentrated by sedimentation in 0.85% saline at 5C and 0 lux. Eggs were hatched in deionized tap water at pH 8 under white light. Miracidia were counted by pipetting them into a small volume of deionized tap water. The hatched miracidia were used with in 2 hours.

Snail Conditioned Water

The snail conditioned water was prepared using Biomphalaria glabrata snails maintained on a diet of lettuce and not starved before the experiments. Six snails with an average size of 0.9 cm were prepared by washing in 1 liter deionized tap water for two hours. The snails were then placed in a glass homogenizing tube containing 25 ml deionized tap water for a period of 110 minutes. The tube was illuminated by a dual flourescent fixture positioned 0.5 m overhead. Circulation was provided by an air supply connected to a capillary pipet submerged in the water and flowing at a rate of one bubble per second. After exposure to the snails, the water (including feces) was homogenized using a teflon pestle to ensure detachment of mucus from the glass surface. The contents were then centrifuged at 30,000 g for 20 minutes to remove cellular material and the supernatant stored at 5C until used.

SCW was fractionated using a 5:1 ratio of methylene dichloride : SCW. The organic fraction was then cleaned by exposing to glass distilled water. This fraction was then dried with N_2 and rehydrated with glass distilled water to the same volume as the initial SCW. Control water was treated in a similar manner.

Assay for the Effect of Snail Conditioned Water on Miracidia

Behavioral responses of miracidia of S. mansoni were tested using a chamber similar in design to that of Etges and Decker (1963) and Wright and Ronald (1972). The circular chamber was constructed of plexiglass tubing. The center well of the chamber was 4.1 cm in height and had an inside diameter of 3.2 cm. The four side arms spaced equidistant from one another around the base, were 4.2 cm long and had an inside diameter of 1.0 cm.

Responses of miracidia to treated and untreated SCW were tested with 50 miracidia in the chamber per trial. The apparatus was rinsed thoroughly between trials to prevent contamination. Two 15 watt incandescent bulbs were positioned 30 cm from the chamber in such a manner that each arm received equal illumination. All solutions were allowed to come to room temperature (25C) before testing. Samples were injected into a rubber injection port at the end of one arm using a 1 ml tuberculin syringe. Control water was likewise injected into the opposite arm. The miracidia in each arm were counted after 2.5, 5.0, and 7.5 minutes. Only miracidia that entered the side arms were considered when Chi-square tests were used to assess departures from random expectation of the numbers of miracidia that entered the four side arms.

Results

Results of the effect of fractionating B. glabrata SCW with methylene dichloride are given in Table I. The time period for counting the number of miracidia in each arm was 2.5 minutes after injection of the sample. the expected random distribution was found to be approximately 50% by comparing the number of miracidia entering the control arm injected with untreated water with the number in the arms receiving no injection.

Discussion

The findings given in this work indicate that the lipid fraction of SCW is in fact responsible for at least part of the miracidial attraction by SCW. There seems to be some background attraction created by the solvent, but this effect could probably be eliminated through the use of

another solvent system.

Stibbs (1976) stated that the principle attractive agent was magnesium. The evidence he gave for this was the sustained activity of the polor fraction of SCW which was subsequently ashed. Their experiments neglected the non-polor fraction as insignificant. But Saladin (1979) postulates that the attraction of SCW may be caused by a mixture of several compounds. For instance, the low molecular weight ions, Mg^{++} and NH_4^{++}, would be most effective at relatively large distances from the target snail. Larger weight carboxylic acids on the other hand, with non-polor carbon chains, would be more effective in close range attraction.

The base-line study submitted here supports the latter statement. Further work must be done to purify and characterize the lipoidal attractant. As suggested by MacInnis (1974), It is possible that an appropriate attractant may someday be incorporated into a larvacide, thus increasing its miracidia killing potential.

Table I

Responses of S. mansoni miracidia

A. Snail Conditioned Water

Trial	% in test arm (vs. control arm)
I	72
II	66

(P greater than .05)

B. Methylene dichloride Extracted Snail Conditioned Water

I	83
II	70
III	78

(P greater than .05)

C. Methylene dichloride Extracted Water (Control)

I	66
II	54

(P greater than .05)

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