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THE EFFECTS OF VARIOUS NATURALLY-OCCURRING SUBSTANCES ON THE SURVIVAL OF *SCHISTOSOMA MANSONI* MIRACIDIA

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ABSTRACT

Miracidia of the blood fluke, *Schistosoma mansoni*, were exposed to three naturally-occurring substances to determine the effects on their behavior and longevity. Cultures of the bacteria *Bacillus sphaericus* and *B. moratai* diluted 1:10 and 1:50 markedly shortened the normal life span of the miracidia. Miracidia lived longer than pond water controls when subjected to the media in which the bacteria were grown suggesting they are capable of absorbing energy-providing substances from their environment. Water in which snails, the natural hosts for the miracidia, had been sequestered for various periods of time snail-conditioned water (SCW) had no effect on miracidia survivorship but did cause a short-lived, non-directional turning activity. The lethal action of the bacterial cultures was considered too slow for practical use as biological control agents.

INTRODUCTION

The blood fluke, *Schistosoma mansoni*, is one of the causative agents of schistosomiasis, a disease affecting millions of people in the world. Various methods for controlling the disease have been suggested, most of which are aimed at eliminating the adult stage in humans by chemotherapeutic drugs or killing the snail intermediate host with molluscicides. Schistosomicidal drugs have been used with some degree of success but none have proved ideal in ease of administration and lack of side effects. Molluscicides also have drawbacks, which include non-specificity in their toxicity and less than complete control of snails when applied in endemic areas.

Recently, the cercarial and miracidial stages have been studied as possible places to interrupt the life cycle of schistosomes. With this in mind, we focused on

miracidial control by biological means. Several studies have indicated that water containing substances emitted from snails will alter straight-line swimming behavior of miracidia and concentrate them in a small area by a non-directional turning activity (Chernin, 1970; Brown, 1975; Prechel, Cain, and Nollen, 1976; Keshavarz-Valian, Nollen, and Maynard, 1981). Chernin (1970) called the aqueous medium in which snails had been sequestered for various periods of time snail-conditioned water (SCW) and coined the word "miraxone" for the miracidial stimulant(s). Although several studies have identified specific miraxones in SCW, to our knowledge no one has reported on the effects of constant exposure of miracidia to SCW on survival time and infectivity.

Selected species of sporulating bacteria of the genus *Bacillus* produce toxins which have larvicidal activity toward various species of mosquitoes (Singer, 1973, 1974; Davidson, Singer, and Briggs, 1975). These toxins have also been tried as control agents against black flies (Jaska, 1977) and in preliminary experiments on the snail vector for *S. mansoni* (Singer, personal communication). Their possible use as control agents against the miracidial stages of digenetic trematodes has not been attempted.

In this study we investigated the efficacy of three compounds which are produced by living organisms that might serve as biological control agents for miracidia. We assessed the effects of two strains of *Bacillus* and SCW produced by the snail *Biomphalaria glabrata* on the survivorship and behavior of the miracidia of *S. mansoni*.

MATERIALS AND METHODS

The *S. mansoni* life cycle was maintained in mice and the Puerto Rican strain of *B. glabrata*. Eggs were obtained from homogenized liver tissue taken from mice having 45- to 70-day-old infections. Hatching of miracidia was accomplished by suspending the pellet of the centrifuged liver homogenate in filtered pond water in a 125-ml erlenmeyer flask covered with black tape and topped by a rubber stopper fitted with a vertical glass tube approximately 10 cm long. Miracidia that hatched in the flask were collected with a micropipette from the open end of the tube.

SCW was prepared by sequestering 2 snails/ml of filtered pond water for 5 hr. (Chernin, 1972) or 1 snail/5 ml of water for 17 hr. (MacInnis, et al., 1974). Two species of toxin-producing bacteria, *B. moratai* (ATCC #21282) and *B. sphaericus* (SS/II-1) were obtained from Dr. Sam Singer, Western Illinois University, and the final test cultures made by the method of Singer (1974).

Miracidia were counted into groups of 20 to 30 in a small volume of water in the test vessels, 75 mm x 25 mm elliptical depression slides with a well volume of 1 ml. The test solution was added and a cover slip placed over the depression to eliminate evaporation. The exact number of active miracidia in each slide was counted at time zero and at hourly intervals thereafter until no miracidia were mobile. Four slides were prepared for each test solution and 2 slides each for pond water and medium controls were observed concurrently. Trials on SCW and *B. moratai* were repeated twice and *B. sphaericua* 5 times. Each trial included dilutions of 1:10 and 1:50 of bacterial culture with filtered pond water while SCW was used undiluted.

To test for oxygen consumption by the bacterial cultures during a time span

similar to the trials, 1:10- and 1:50-dilutions of the *B. sphaericus* culture were analyzed with a galvan oxygen analyzer. Several coplin jars of culture were sealed at time zero and the oxygen content determined at hourly intervals for 5 hr.

Evidence for visible effects of the bacteria on miracidia was obtained by sectioning fixed, paraffin-embedded material after one-half the miracidia were inactivated by a 1:10 dilution of *B. sphaericus* (not longer than 5 hr.). Sectioned miracidia were stained by a modified MacCallum-goodpasture stain and prepared for permanent mounts.

RESULTS

A normal survivorship curve constructed with data obtained from 15 trials of *S. mansoni* miracidia swimming in filtered pond water is given in Figure 1. Untreated miracidia show a mortality of approximately 25% during the first six hours and thereafter die off rapidly. Mortality dropped to 50% between hours 8 and 9 and was more than 90% by 12 hours. A few hardy individuals survived as long as 14 hours, but in these trials none lived past the 15-hour mark.

Tests with the two types of SCW revealed essentially no difference in miracidial survival when compared to the pond water controls (Figure 2). Only during the early observational periods of the trials were any differences noted between experimental and control slides and these were miniscule. When miracidia were first introduced into the test vessels, they exhibited the typical frenzied, non-directional turning activity previously described by several investigators for *S. mansoni* miracidia. This activity soon subsided and the miracidia resumed their normal swimming activity.

Of the two bacterial cultures tested, *B. moratai* was the most active miracidicidal agent. When compared to pond water controls, a dilution of 1:10 of the test culture was more lethal than the 1:50 dilution (Figure 3). The 1:10 dilution reduced survival to less than 10% after five hours but the 1:50 dilution did not show a deleterious effect when compared to the pond water control until six hours. On the other hand, the miracidia in the media controls had relatively longer survival times than the pond water controls. Thus the growth medium alone enhances miracidial longevity but bacteria in the medium shorten the normal life span even overcoming the supportive effect of the medium in which the bacteria are grown.

B. sphaericus cultures proved to be slightly less lethal than *B. moratai* (Figure 4). Percent survival dropped below 10% after six hours when miracidia were exposed to a 1:10 dilution of bacteria compared to 11 hours for miracidia in the pond water control. All media controls prolonged miracidial life slightly, but not to the extent seen with the media controls for *B. moratai*.

Dissolved oxygen (DO) determinations on 1:10 and 1:50 dilutions of a *B. sphaericus* culture are given in Table 1. After one hour, the DO content of the 1:10 dilution reached a low level (1.3 to 1.5 mg/liter) with little variation for the duration of the testing. One hour was also needed for a plateau to be reached for the 1:50 dilution (1.8 to 2.7 mg/liter).

Photomicrographs of miracidia exposed to *B. sphaericus* for five hours showed no bacteria had penetrated the miracidia. However, clumping of bacteria on the outside, especially on the anterior end, was observed on all miracidia sectioned and stained.

TABLE 1. Dissolved Oxygen in mg/liter Observed in Two Dilutions of a *Bacillus sphaericus* Culture at One-hour Intervals.

Time in Hours	Dissolved Oxygen in mg/liter	
	1/10 Dilution	1/50 Dilution
0	6.5	7.9
1	1.5	2.9
2	1.3	1.9
3	1.4	2.7
4	1.3	2.1
5	—	1.8

DISCUSSION

The normal survivorship curve for *S. mansoni* miracidia observed in this study (Figure 1) indicated a slow mortality reaching 25% by six hours after hatching followed by a more precipitous die off. Maldonado (1954) described a similar early death of miracidia and considered it a normal component of mass miracidial hatchings. Those miracidia showing early mortality were considered incompetent at hatching or old mature eggs that had been near death within the host tissues before being liberated in the hatching process. The decline of living miracidia from 7 to 13 hours corresponds to a depletion of energy reserves within the miracidia. This pattern differs slightly from that described by Hairston (1973) where 75% of *S. mansoni* miracidia were alive at eight hours followed by a rapid die off. However, different studies have reported varied survival rates for *S. mansoni* miracidia ranging from an average life span of 5 to 6 hours by Maldonado (1967) to 80% survival after 7 hours by Chernin (1968). For this reason pond water controls were included in each trial for comparison to experimental results.

Our results indicated that the presence of either *B. sphaericus* or *B. moratai* in 1:10 or 1:50 dilutions shortened the life span of *S. mansoni* miracidia (Figures 3 and 4), while SCW had no adverse effect on the survivorship of the miracidia (Figure 2). This raised the question of whether the lethal qualities of the bacterial cultures were due to bacterial toxins or the result of some other environmental change brought about by the presence of bacteria.

The most obvious environmental change imposed upon the miracidia was the presence of the media in which the bacteria were grown. Results obtained from the media controls clearly show that the media did not harm the miracidia in terms of survivorship and in the tests with *B. moratai* the medium greatly enhanced the survival of the miracidia. This medium, Bacto brain-heart infusion, contains glucose, which has been found by Bruce et al. (1971) and Lewert, Para, and Ozeet (1970) to be taken up by miracidia and thus prolong their life span. Therefore, culture media can be ruled out as a source of lethal agents in the test solution.

Because the two species of bacteria tested were aerobic, the lack of oxygen was suspected as a factor causing miracidial death. Kawata and Kruse (1966) reported the survival of miracidia was impaired by anaerobic conditions of sewage stabilization ponds. Bruce et al. (1971) studied the respiratory requirements of *S. mansoni* miracidia and found that as their glycogen reserves are depleted a greater portion of

their energy requirements is met by aerobic respiration, resulting in a higher O_2 consumption in older miracidia (5 hours old).

In our study oxygen depletion does not seem to be an important factor in miracidial mortality. DO determinations in the bacterial cultures (Table 1) reached a low level by one hour and remained there for the duration of the testing. In none of the tests with bacterial cultures did miracidia show a die off attributed to oxygen depletion. As determined by Bruce et al. (1971) the oxygen requirements for miracidia are small (6 liter/hr/1,000 miracidia). At the lowest DO reading in the cultures (1.3 mg/liter) the amount of available oxygen was approximately 30 liters/1,000 miracidia, more than an adequate amount to sustain the small numbers of miracidia used in our trials.

The bacteria used in this study, *B. moratai* and *B. sphaericus*, are ubiquitous in nature and are easy to grow on ordinary laboratory media. Certain strains of *B. sphaericus* kill mosquito larvae even after broth cultures had been diluted 10 million times (Singer, 1974). Although some endospore-forming bacteria can invade tissues, *B. sphaercis* is known to kill mosquito larvae by means of cell wall associated toxins only after ingestion and digestion in the mid-gut (Singer, 1980). Evidence presented here indicated *B. sphaericus* does not invade miracidia. Thus, it seems unlikely that *S. mansoni* miracidia died as a result of a bacterial toxin. However, the coating of miracidia by bacteria which was observed in sectioned material may have slowed them down and caused miracidial immobilization. *B. moratai* was more active against *S. mansoni* miracidia than *B. sphaericus*. Since this species has not shown activity against mosquito and black fly larvae (Jaska, 1977), little is known about its ability to produce toxins. It is possible both species of bacteria change some environmental parameter other than those investigated here to cause the early death of miracidia.

The other type of naturally-occurring substance tested, SCW, was observed to elicit the typical erratic swimming behavior of miracidia but the reaction was short-lived. Chernin and Perlstein (1972) reported miracidia that become detached from snails during the penetration process die rapidly, presumably because an irreversible expenditure of energy reserves and glandular stores was initiated. Further studies by Chernin (1972) reported that *S. mansoni* miracidia only attempt penetration of host tissue in the presence of SCW. We thought that SCW might trigger either a similar irreversible response and cause miracidia to die or through stimulation of hyperactivity cause a very rapid depletion of energy stores. However, our data (Figure 2) show neither pattern of activity occurred during the long-term tests. Schistosome miracidia seem to require repeated stimulation with SCW to sustain constant non-directional turning activity (Shiff and Kriel, 1970).

Before bacterial cultures could be used as miracidicidal agents, more lethal strains will have to be found. Any control agent used in nature must act swiftly to kill the target organism and not produce adverse effects in the environment. Although 1:10 and 1:50 dilutions of bacteria kill miracidia, they do not kill them fast enough. Furthermore, the effect of administering bacterial cultures in the environment are unknown. However, some studies are being carried out along these lines with *B. sphaericus* at levels used for control of mosquito larvae (Singer, 1980).

LITERATURE CITED

Brown, R. A. 1976. The hydrogen ion as an attractant for *Schistosoma mansoni* miracidia. Rev. Latinoam. di Microbiol. 18:217-219.

Bruce, J. I., M. D. Ruff, J. K. Chin, and L. Howard. 1971. *Schistosoma mansoni* and *Schistosoma japonicum*: Oxygen uptake by miracidia. Exp. Parasitol. 30:124-131.

Chernin, E. 1968. Interference with the capacity of *Schistosoma mansoni* miracidia to infect the molluscan host. J. Parasitol. 54:509-516.

Chernin, E. 1970. Behavioral responses of miracidia of *Schistosoma mansoni* and other trematodes to substances emitted by snails. J. parasitol. 56:287-296.

Chernin, E. 1972. Penetration activity of *Schistosoma mansoni* miracidia stimulated by exposure to snail-conditioned water. J. Parasitol. 58:209-212.

Chernin, E., and J. M. Perlstein. 1971. Protection of snails against miracidia of *Schistosoma mansoni* by various aquatic invertebrates. J. Parasitol. 57:217-219.

Davidson, E. W., S. Singer, and J. D. Briggs. 1975. Pathogenesis of *Bacillus sphaericus* strain SS II-1 infection in *Culex pipiens quinquefasciatus* (=*C. pipiens fatigans*) larvae. J. Invert. Path. 25:179-184.

Hairston, N. G. 1973. The dynamics of transmission, p. 250-336. *In* N. Ansari (ed.), Epidemiology and Control of Schistosomiasis. Univ. Park Press, Balt.

Jaska, J. M. 1977. The larvicidal effects of various bacilli against field-collected *Simulium vittatum*. Masters thesis, Western Illinois University, 74 p.

Kawata, K., and C. W. Kruse. 1966. The effect of sewage stabilization ponds on eggs and miracidia of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. 15:896-901.

Keshavarz-Valian, H., P. M. Nollen, and G. Maynard. 1981. *Philophthalmus gralli*: chemosensitivity of miracidia. J. Parasitol. 67:527-530.

Lewert, R. M., J. Para, and M. A. Ozcet. 1970. Miracidial uptake of glucose in intact eggs of *Schistosoma mansoni*. J. Parasitol. 56:1250-1251.

Macinnis, A. J., W. M. Bethel, and E. M. Cornford. 1974. Identification of chemicals of snail origin that attract *Schistosoma mansoni* miracidia. Nature 248:361:362.

Maldonado, J. F. 1967. Schistosomiasis in America. Editorial Cientifico-Media, Barcelona, Spain.

Prechel, D. P., G. D. Cain, and P. M. Nollen. 1976. Responses of *Megalodiscus temperatus* miracidia to amino and sialic acids found in snail-conditioned water. J. Parasitol. 62:693-697.

Shiff, C. J., and R. L. Kriel. 1970. A water soluble product of *Bulinus (Physopsis) globosus* attractive to *Schistosoma haematobium* miracidia. J. Parasitol. 56:281-286.

Singer, S. 1973. The insecticidal activity of recent isolates and their toxins against mosquito larvae. Nature 244:110-111.

Singer, S. 1974. Entomogenous bacilli against mosquito larvae. Chapt. 18, p. 187:194. *In* Developments in Industrial Microbiology 15. AIBS, Washington, D.C.

Singer, S. 1980. *Bacillus sphaericus* for control of mosquitoes. Biotech. and Bioengin. 22:1335-1355.

Figure 1. Composite Pond Water Controls (15 Trials)

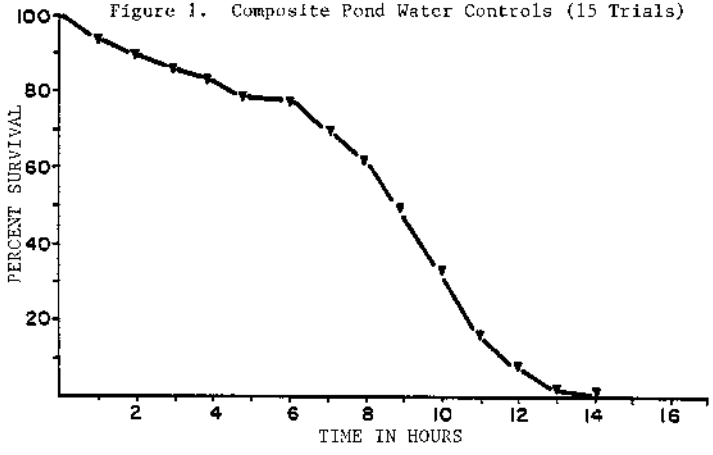
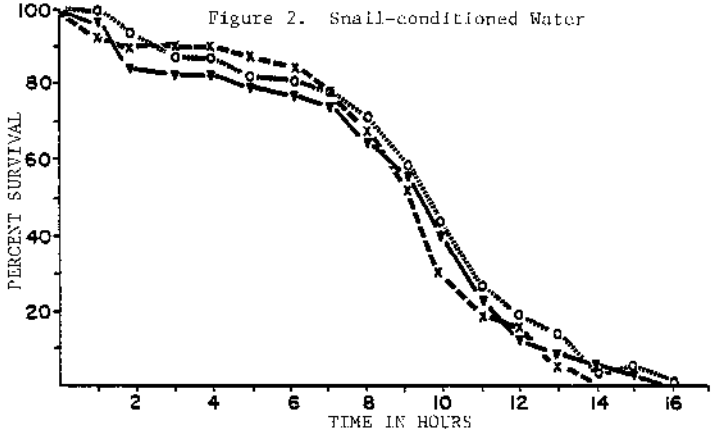


Figure 2. Snail-conditioned Water



Figures 1 and 2. Average percent survival of Schistosoma mansoni miracidia in pond water (▼) and two types of snail-conditioned water (SCW): 2 snails/ml for 5 hours (X) and 1 snail/5 ml for 17 hours (0).

Figure 3. Bacillus moratai

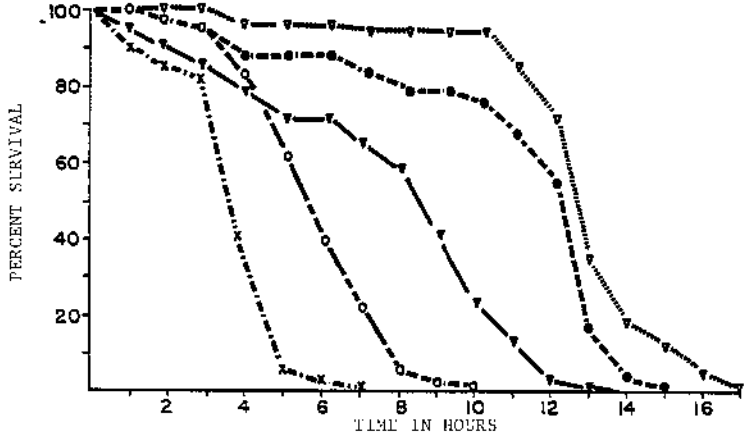
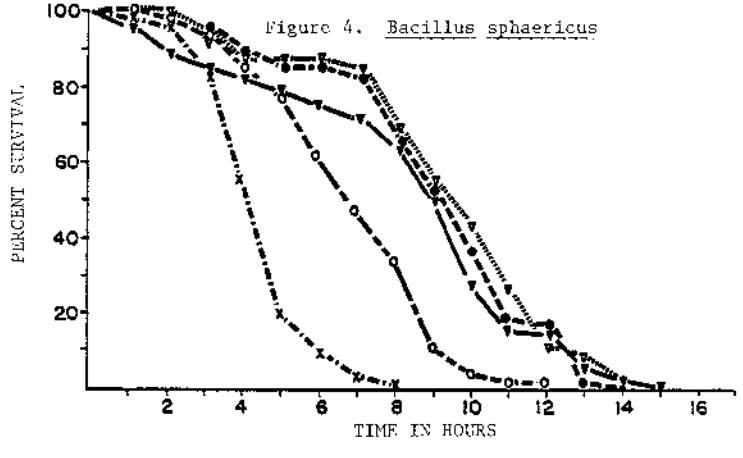


Figure 4. Bacillus sphaericus



Figures 3 and 4. Average percent survival of Schistosoma mansoni miracidia exposed to 1:10 (X) and 1:50 (0) dilutions of bacteria. Controls are: pond water (▼), 1:10 media (▽) and 1:50 media (●).