Journal of Helminthology (2004) **78,** 343–346 DOI: 10.1079/JOH2004244

Effects of *Schistosoma mansoni* infection on inorganic elements in the snail *Biomphalaria glabrata*

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Abstract

Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used to study element ions in whole bodies of uninfected *Biomphalaria glabrata* snails and those experimentally infected with larval *Schistosoma mansoni* trematodes. Infected snails were analysed 8 weeks post-infection. Cohort snails that were left uninfected were analysed at the same time as the infected snails. Sixteen elements (aluminum, boron, barium, calcium, cadmium, copper, iron, potassium, magnesium, manganese, sodium, nickel, lead, selenium, tin and zinc) were found to be present in infected and uninfected whole bodies at concentrations above the detection limit of the ICP-AES analysis. Of these, calcium, cadmium, manganese and sodium were present in significantly higher amounts (Student's *t*-test, $P < 0.05$) in whole infected versus whole uninfected snails. Variations in the present results compared with other studies reflect intrinsic differences in the larval trematode–snail systems used.

Introduction

Although considerable information is available on the organic metabolism of *Biomphalaria glabrata* infected with the intramolluscan stages of *Schistosoma mansoni*, little information is available on the inorganic metabolism of such relationships (Shaw & Erasmus, 1987; Thompson, 1997). Several studies have suggested that inorganic metabolism alterations due to infection by *S. mansoni* in *B. glabrata* occur. For instance, parasitism by larval stages of *S. mansoni* causes changes in calcium reserves, and the effects are not restricted to the digestive gland–gonad complex (DGG), the target organ of the intramolluscan stages (Shaw & Erasmus, 1987). Also, the cercariae sequester large amounts of calcium in their pre-acetabular glands, and such sequestration probably occurs at the expense of calcium in the shell and haemolymph of the snail (Davies, 1983).

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There have been many studies on the effects of parasites on metal concentrations in their hosts. Some of these have indicated that parasites are able to alter the uptake and storage of chemicals inside their hosts. For instance, Bergey *et al.* (2002) reported that the grass shrimp, *Palaemonetes pugio*, parasitized by the isopod, *Probopyrus pandalicola*, accumulated lower concentrations of mercury than unparasitized shrimps. They also noted that the mummichog, *Fundulus heteroclitus*, parasitized with the nematode, *Eustrongylides* sp., also accumulated lower concentrations of mercury than unparasitized fish. Bergey *et al.* (2002) stated that the mechanism by which parasitized hosts accumulate fewer toxicants than unparasitized hosts is unknown, but it may be due in part to a lower metabolic rate. Sures *et al.* (1999) noted that the parasites themselves, particularly acanthocephalans and cestodes of fish, can accumulate heavy metals at concentrations that are much higher than those in the host tissues or environment. Sures *et al.* (1999) have discussed the recently described phenomenon of conspicuous metal accumulation by parasites and how this might be applied to environmental monitoring. They also suggested how

https://doi.org/10.1079/JOH2004244 Published online by Cambridge University Press

environmental science and parasitology might profit from each other in the near future.

In support of Bergey *et al.* (2002), mentioned in the previous paragraph, Evans *et al.* (2001) showed that the marine periwinkle, *Littorina littorea*, parasitized with several larval trematodes had significantly lower levels of iron, copper, and nickel than uninfected snails. Based on the study of Evans *et al.* (2001), we initiated the present study on infected and uninfected *B. glabrata* snails to determine if infection with larval *S. mansoni* would significantly lower the levels of certain elements in the infected snails. This study used inductively coupled plasma atomic emission spectrometry (ICP-AES) to examine the effects of larval parasitism by *S. mansoni* on the element ion content of experimentally infected *B. glabrata* snails.

Materials and methods

Snail collection and sample preparation

Biomphalaria glabrata snails, about 7 mm in shell diameter, were experimentally infected with *S. mansoni* by exposure en masse to approximately eight miracidia per snail (Fried *et al.*, 2001). Infected snails along with uninfected controls were maintained at 22–24°C in artificial spring water (ASW; Ulmer, 1970) in glass vessels and used 8 weeks post-infection. Snails were isolated individually to determine larval infection with *S. mansoni* and then subsequently dissected to confirm the infection as described by Fried *et al.* (2001). Samples, each of ten infected or uninfected snail bodies, were pooled to achieve a wet weight of about 1 g. Three pools each of infected and uninfected snails were prepared for analysis. Prior to use, each sample was rinsed several times with deionized water, digested in 10 ml of boiling concentrated nitric acid, and diluted to 25.0 ml with 2% (v/v) nitric acid.

Elemental analysis by ICP-AES

A Thermo Jarrell Ash simultaneous-reading ICP-AES instrument equipped with an autosampler was used to measure the concentrations of 28 elements in the sample solutions. The instrument was calibrated following EPA method 6010B, which uses a three-point calibration curve generated from measurement of a calibration blank and two multi-element standards. Interelement correction factors were employed to minimize interference between elements in the samples. A reagent blank sample was also analysed. The samples, standards, and a blank were analysed using three 30 s integrations. The results for each element in each sample were averaged to calculate the final concentration value (mg g^{-1}), using the equation:

$$\text{Concentration of element} = \frac{\text{C*25 ml}}{\text{(sample mass in g)*1000}}$$

where C is the concentration value from the instrument (in ppm) with the blank subtracted. A number of quality control checks were made during the analyses to verify the calibration curve, blank and interelement correction factors.

Results

Table 1 presents data obtained from the ICP-AES analysis of three samples of infected and uninfected snails, each sample containing ten snails with a combined wet weight of approximately 1 g. Values in the table represent the arithmetic mean and standard deviation of the three samples. Of the 28 elements analysed by the instrument, the following 12 were found to be present at concentration levels below the detection limits of the instrument and were not considered further: Ag, As, Be, Bi, Co, Cr, Hg, Mo, Sb, Ti, Tl and V. The 16 elements that were detected at concentrations above their detection limits are listed in table 1. For each of these elements, the means were compared for significant differences between infected and uninfected snails using Student's *t*-test, calculated by means of a data analysis tool in Microsoft's Excel spreadsheet program. Four elements (Ca, Cd, Mn and Na) were found to be present at significantly higher concentrations ($P < 0.05$) in infected compared with uninfected snails. While the amounts of the other 12 elements showed variation, their concentration differences were not significant ($P > 0.05$).

Discussion

Gabrashanska *et al.* (1991), using neutron activation analysis in studies of *Lymnaea stagnalis* snails infected with the larval trematode *Echinostoma revolutum*, found significantly higher concentrations of Ca, Na, Rb and Sb, and significantly lower concentrations of Ce, Cr, Cs, Cu, Fe and Zn in the digestive glands of infected snails. Layman *et al.* (1996a), using flame and graphite furnace atomic absorption spectrometry (AAS) and ICP-AES, showed changes in certain element ions as a result of larval *E. trivolvis* infection in the DGG of *Helisoma trivolvis*.

Table 1. Mean values ± standard devation in $\mu g\, g^{-1}$ of wet tissue obtained for elements in *Biomphalaria glabrata* infected with *Schistosoma mansoni* and uninfected controls using inductively coupled plasma atomic emission spectrometry.

Element	Uninfected*	Infected*	Value of *P*
Al	4.22 ± 1.4	1.88 ± 1.6	0.132
B	1.64 ± 1.2	1.66 ± 2.2	0.989
Ba	0.996 ± 0.60	0.479 ± 0.056	0.277
Ca	4660 ± 1800	8820 ± 1300	0.0315**
Cd	0.648 ± 0.22	1.24 ± 0.21	0.0275**
Cu	2.36 ± 0.69	4.10 ± 0.12	0.0504
Fe	37.3 ± 11	39.4 ± 5.9	0.791
K	621 ± 180	963 ± 12	0.0820
Mg	345 ± 110	387 ± 23	0.586
Mn	3.51 ± 0.87	7.34 ± 0.40	0.00622**
Na	130 ± 47	274 ± 17	0.0157**
Ni	0.250 ± 0.15	0.163 ± 0.033	0.432
Pb	0.338 ± 0.36	0.175 ± 0.24	0.548
Se	0.667 ± 0.18	1.05 ± 0.11	0.0528
Sn	0.409 ± 0.11	0.596 ± 0.048	0.0751
Zn	18.0 ± 7.1	16.2 ± 1.1	0.706

* $n = 3$ samples with each sample containing 10 snails with a combined wet weight of approximately 1 g.
** Differences significant at $P < 0.05$.

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They found significantly higher amounts of Na and significantly lower amounts of Mg and Mn in the DGG of infected snails. Kaufer *et al.* (2002), using graphite furnace AAS and ion chromatography, showed changes in certain element ions as a result of larval *Euhaplorchis californiensis* infection in the DGG of *Cerithidea californica*. They found significantly higher amounts of Ca, and significantly lower amounts of Mg, in the DGG of infected snails. Differences in metallic ions as a result of larval parasitism in the aforementioned studies reflect alterations in ionic balance and an influx of certain ions and an outflux of other ions from the larval trematodes to the snail host.

The present results show that there are also significant changes in certain element ions as a result of larval *S. mansoni* infection of *B. glabrata*. We found significantly higher amounts ($P < 0.05$) of Ca, Cd, Mn and Na in infected as compared with uninfected snails. In our study, we analysed whole bodies (minus the shells) of the snails, although previous studies have suggested that the effects of larval trematode parasitism are mainly confined to the DGG of snails. However, there are studies that have demonstrated that the effects of larval trematode parasitism are not restricted to the DGG. For instance, Davies & Erasmus (1984) and Shaw & Erasmus (1987) found that the tissues of *B. glabrata* contain three types of calcium cells, A, B and C, distributed throughout the body, and that these cells are damaged as a result of parasitism by larval *S. mansoni.*

In contrast to the findings of Evans *et al.* (2001) and Bergey *et al.* (2002) in which parasitism significantly lowered the amounts of certain elements in infected hosts, we found a significant elevation of calcium, cadmium, manganese and sodium in *B. glabrata* infected with larval *S. mansoni* as compared to the uninfected snails. Thus, the phenomenon of parasitism significantly lowering the metal content of the infected host should not be considered as universal. We are not certain why the concentrations of certain elements are significantly increased in *B. glabrata* infected with larval trematodes. As discussed by Evans *et al.* (2001) for their snail–larval trematode model, a major reason for the difference in elements is the pathologic effect of larval trematodes on the digestive gland cells of the infected snails. As discussed by Bebianno & Langston (1995), an important function of digestive gland cells is the storage of various elements, where the elements are held in membrane-insoluble granules in the cells. Destruction of the digestive gland cells by larval trematodes probably reduces the storage volume and holding capacity of elements in the infected snails.

For calcium in particular, the present study shows that *S. mansoni* infection significantly changes the concentration levels, with infected snails having nearly twice the amount of calcium compared to uninfected snails. Shaw & Erasmus (1987) found that parasitism by larval *S. mansoni* induced morphological changes to the internal calcium reserves of *B. glabrata*. Following parasitism, the metabolism of the host changed presumably by the production and release of CO_2 and waste metabolites into the haemolymph. In a study by Davies (1983), the pre-acetabular gland cells of *S. mansoni* cercariae, still within sporocysts, contained abundant supplies of calcium. It is suggested that the calcium is absorbed through the molluscan haemolymph, thereby causing changes to the calcium metabolism of the snail.

There are few reports of the quantitative analysis of element ions in *B. glabrata*. Nduka & Harrison (1980) determined the concentrations of Ca, Mg, Na and K in various planorbid snails, including *B. glabrata*, by AAS. Another study by Layman *et al.* (1996b) reported, for the first time, the trace-element profile of *B. glabrata* determined by ICP-AES, but no differences with respect to element ion concentrations between *B. glabrata* infected with *E. caproni* and uninfected controls were found.

Acknowledgements

The work of J. Ong was supported by a grant from the Lafayette College EXCEL Program. We are grateful to Dr Fred A. Lewis, Head, Schistosomiasis Laboratory, Biomedical Research Institute, Rockville, Maryland, USA, for supplying the *Biomphalaria glabrata* snails used in this work through NIH-NIAID contract NO1-AI-55270.

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(Accepted 17 March 2004)
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