Chromatography **2015**, *2*, 118-124; doi:10.3390/chromatography2010118

OPEN ACCESS

chromatography

ISSN 2227-9075

www.mdpi.com/journal/chromatography

Review

Recent High Performance Thin Layer Chromatographic Studies on *Biomphalaria glabrata* (Gastropoda)

Bernard Fried and Aditya Reddy *

Department of Biology, Lafayette College, Easton, PA 18040, USA; E-Mail: adireddymd@gmail.com

* Author to whom correspondence should be addressed; E-Mail: friedb@lafayette.edu.

Academic Editor: Mark Devlin Maloney

Received: 9 February 2015 / Accepted: 5 March 2015 / Published: 10 March 2015

Abstract: This review examines the recent high performance thin layer chromatography (HPTLC) literature on the effects of biotic and abiotic factors on certain analytes in the medically important freshwater snail, *Biomphalaria glabrata*. The analytes studied were lipids, lipophilic pigments, amino acids, and carbohydrates. As determined by HPTLC, various factors, such as larval parasitism, estivation, temperature changes, and others, alter the metabolism of the snail and cause significant changes in the chemical contents of the analytes under study.

Keywords: high performance thin layer chromatography (HPTLC); *Biomphalaria glabrata*; *Schistosoma mansoni*; miracidia; lipids; lipophilic pigments; carbohydrates; amino acids

1. Introduction

Biomphalaria glabrata is a medically important air-breathing freshwater snail and a model used for studies in biological and chemical research. It is also the major snail vector of *Schistosoma mansoni*, an important trematode parasite that causes a debilitating water bone infectious disease known as schistosomiasis. This mainly tropical-neotropical disease results in significant morbidity and mortality to millions of people globally. Much work of late has been done to analyze the chemical and biochemical effects of abiotic and biotic factors on the snail. These recent endeavors have primarily used the modern chromatographic technique, known as high performance thin layer chromatography (HPTLC). Sherma and Fried [1] published a comprehensive review on the topic in 2011 and discussed the advantages of using modern HPTLC in this type of work. That review also covered relevant literature on this topic until about 2010. The analytes covered in that review were lipids, lipophilic pigments, carbohydrates,

and amino acids. Herein, we have included a total of 11 new papers since that earlier Sherma and Fried [1] review, and the purpose of this short review is to document the salient findings of the new papers. All studies mentioned in this review used HPTLC-densitometry and are covered in reverse chronological order.

2. Literature Review

Hunsberger *et al.* [2] studied the effects of both high and low temperatures on the neutral and polar lipid content of the digestive gland-gonad (DGG) complex of *Biomphalaria glabrata* snails. Low temperature observations were performed at −10 °C, 6 °C, 14 °C, and ambient (22–24 °C) temperatures. The high temperature observations were performed at ambient (22–24 °C), 28 °C, and 32 °C. Snails were placed at these temperatures for various durations; their DGGs were subsequently analyzed to quantify any changes that occurred in their lipid content as a function of these varied temperatures. In this study and in the Beideman *et al.* [3] study discussed below, neutral lipids were separated on silica gel plates in a mobile phase of petroleum ether-diethyl ether-acetic acid (80:20:1). All ratios mentioned in this review indicate volumes. Phospholipids were separated on silica gel plates in a chloroform-methanol-deionized water (62:25:4) mobile phase. Neutral lipids were detected by charring with the phosphomolybdic acid detection reagent for lipids, whereas phospholipids were detected by charring with 50% sulfuric acid. In the low temperature experiment, after 30 min, the snails maintained at the ambient temperature had significantly greater amounts of phosphatidylcholine and phosphatidylethanolamine than those maintained at colder temperatures. Cold temperatures therefore had a detrimental effect on the structural lipids of the snails. In the high temperature experiment, at four weeks after study initiation, snails maintained at the ambient temperatures had significantly greater amounts of free sterols than those maintained at the higher temperatures. These findings suggest that high temperatures have a deleterious effect on the lipid metabolism of this aquatic pulmonate.

Beideman *et al.* [3] characterized and quantified neutral and polar lipids in *B. glabrata* snails subjected to either *Echinostoma caproni* and *S. mansoni* miracidia coexposure, or single exposure to each of these trematode parasites. Observations on survival and fecundity were made on an unexposed population (two cultures of 25 snails each); a population exposed exclusively to *S. mansoni* (two cultures of 25 snails, each exposed to six miracidia per snail); a population exposed exclusively to *E. caproni* (two cultures of 25 snails, each exposed to 10 miracidia per snail); and a population exposed to *S. mansoni* and one week later exposed to *E. caproni* (two cultures of 25, each exposed to 10 *E. caproni* miracidia and six *S. mansoni* per snail). Each culture contained 800 mL of artificial spring water; snails were maintained at 25 ± 1 °C and fed boiled romaine lettuce ad libitum. A sample of each population was necropsied at two, four, six, and eight weeks post exposure to *E. caproni*. Significant differences in lipid concentrations were observed for the free fatty acids. Survival of snails declined mainly in the group exposed only to *E. caproni* miracidia and in the coexposed group. Fecundity (based on egg laying) increased in the group exposed only to *E. caproni* miracidia, but declined in the other groups.

Hunsberger *et al.* [4] studied the effects of a five *versus* 25 miracidia exposure of *E. caproni* on the lipid composition of *B. glabrata*. A 50 miracidia dose was not used because such a high level of exposure caused severe snail mortality by three weeks post-exposure (PE). Lipid concentrations were determined in the digestive-gland gonad complex (DGG) of the exposed snails and in uninfected matched controls at two and four weeks PE. Lipids were extracted from the DGGs and chromatographed as described in

both Hunsberger *et al.* [2] and Beideman *et al.* [3]. No significant differences in the concentrations of free sterols, free fatty acids, triacylglycerols, phosphatidylcholine, and phosphatidyethanolamine were seen at two weeks PE in any of the groups. At four weeks PE, the free fatty acid concentration increased significantly in the snails exposed to 25 miracidia when compared to that of either the five miracidia/snail group or to the control group. Elevation of the free fatty acid fraction in the high dose snail group suggested that some changes occurred in the lipid metabolism of the snails in that group as a function of the miracidial dose.

Dieterich *et al.* [5] determined the concentration of β-carotene and lutein in the whole body and the DGG of uninfected *B. glabrata* snails and those infected with *S. mansoni* for six and eight weeks. Statistical analysis of the weight percentages of each pigment showed a significant decrease ($P < 0.05$) in the concentration of β-carotene in the DGGs of infected *B. glabrata* at six and eight weeks post-infection compared to the uninfected snails. No significant differences were seen in the concentrations of β-carotene in the whole body of the uninfected versus infected snail samples. Changes in the lutein concentration of the infected DGG and whole snail bodies were not significant when compared to the uninfected controls. In conclusion, larval *S. mansoni* infection caused a significant decrease in the β-carotene concentration of the DGG at six and eight weeks post infection.

Dieterich *et al.* [6] characterized and quantified neutral and polar lipid classes present in *B. glabrata* snails subjected to crowding. Observations on survival and fecundity were made on a non-crowded population (10 snails per culture), a moderately crowded population (20 snails per culture), and a heavily crowded population (40 snails per culture). Each culture contained 800 mL of artificial spring water, and snails were maintained at 25 ± 1 °C and fed romaine lettuce *ad libitum*. After four weeks of culture, the snails were necropsied. No significant differences in any of the neutral and polar lipid classes were found among the three aforementioned snail populations.

Popovic *et al.* [7] analyzed the glucose and maltose concentrations of the DGG of mature adult uninfected *B. glabrata* snails maintained in laboratory cultures containing various dilutions of artificial ocean water (AOW), as well as the whole bodies of young adult *B. glabrata* snails maintained in laboratory cultures at the same dilutions of AOW for up to four weeks. Controls for both groups consisted of snails of the same size maintained identically in deionized water and artificial spring water. Sugars were extracted from the snails in the ethanol-water (70:30). Random samples from both experimental groups were tested to detect the presence of different types of carbohydrates; the major sugars detected in the snails were glucose and maltose. These sugars were separated on EMD Millipore silica gel preadsorbent plates with mobile phase 1-butanol-glacial acetic acid-diethyl ether-deionized water (27:18:5:3), detected using α-naphthol-sulfuric acid reagent, and quantified by densitometry with a CAMAG TLC Scanner 3 at 515 nm. The mature adult snails showed high survival rates in most salinities, and only in 25% salinity was the survival lower than in other groups. In the experimental group of young adult snails, the survival was lower, especially in 25% salinity where only 40% of the snails survived up to four weeks. No significant statistical differences in the carbohydrate composition as a function of salinity were found in either of the two experimental groups.

O'Sullivan *et al.* [8] determined the glucose and maltose content of the DGG of adult *B. glabrata* snails maintained at 17 °C, 24 °C, and 31 °C, as well as the whole bodies of juvenile *B. glabrata* snails maintained at these same temperatures. Sugars were extracted in ethanol-water (70:30) from the snails and separated on silica gel plates in a mobile phase of 1-butanol-acetic acid-diethyl ether-deionized water

(27:18:5:3). The major sugars detected in both experimental groups were glucose and maltose. One week after the study was initiated, adult snails maintained at 31 °C had a significantly higher glucose content than those maintained at lower temperatures. An elevated metabolic rate of the adult snails maintained at the highest temperature probably accounted in part for the high glucose content measured in the DGG. The juvenile snails showed considerable variation in sugar content as a result of snail maintenance at the different temperatures. For each week that data were collected, there were significant differences in both the glucose and maltose content of the juveniles as a factor of temperature. This difference in sugar content in juveniles relative to the adults as a response to temperature extremes can probably be attributed, in part, to the higher metabolic rate of juveniles compared to adult snails. Moreover, whole bodies of the juveniles were used for sugar analysis compared to only the DGG of the adult snails. Sugar differences in sample type could explain, in part, the difference in sugar content between the two groups as a factor of temperature.

Holman *et al.* [9] determined the free-pool amino acid content of whole bodies of sexually immature *B. glabrata* snails maintained at 15 °C, 24 °C, or 31 °C. Amino acids were extracted from whole snail bodies in 70:30 ethanol-water and separated on either silica gel or cellulose stationary phases with 2-butanol-pyridine-glacial acetic acid- deionized water (39:34:10:26) or 2-butanol-pyridine-25% ammonia-deionized water (39:34:10:26). Amino acid zones were visualized by postchromatographic derivatization with ninhydrin and quantified by visible mode slit-scanning densitometry at 610 nm. Comigration of bands with amino acid stock solutions confirmed the presence of alanine, arginine, asparagine, aspartate, glutamate, glutamine, glycine, lysine, threonine, and valine in all samples. Multiple t-test comparisons showed a significant increase in valine in snails maintained at 15 °C compared to those at 31 °C at three weeks post-treatment ($P < 0.015$). This finding could suggest that there are inherent variations in the metabolism of snails cultured at different temperatures.

O'Sullivan *et al*. [10] studied the pH tolerance of freshwater snails with particular emphasis upon the *B. glabrata* snail. They noted that the environment in which animals can live is dictated by multiple abiotic and biotic factors. One important factor, especially for aquatic animals, is pH. Optimal pH is vital to ensure the occurrence and homeostasis of important biological processes. Enzymes work best at certain pH values, and the environmental pH may affect the enzyme effectiveness. The medically important, aquatic snail *B. glabrata* has been studied extensively in regards to its behavior and biochemistry. This review examines the pH range at which *B. glabrata* can survive optimally and the effect pH has on the metabolism of *B. glabrata*. Based on previous studies on the pH tolerance of *B. glabrata* both in the field and the laboratory, these snails tolerate a relatively broad pH range. The paper also examines the literature on pH tolerance in laboratory and experimental studies on selected freshwater snails with major emphasis on *B. glabrata*. Lastly, a biochemical study on the effects of subjecting the *B. glabrata* snail to a wide range of pH values showed that such changes did not alter the composition of the snail lipids.

Bolstridge *et al.* [11] examined the effects of extreme temperature on the neutral lipid content of *B. glabrata*, and observations on fecundity were made at these extreme temperatures of 32 °C, 28 °C, and 14 °C. Fecundity results showed that egg laying at 32 °C was higher than in the lower temperature groups. Free sterols, free fatty acids, and triacylglycerols were quantified at two and four weeks after the initiation of the experiment using Analtech channeled high performance silica gel plates with a preadsorbent zone. Plates were developed using the Mangold mobile phase, petroleum ether-diethyl

ether-glacial acetic acid (80:20:1), and after treatment with 5% ethanolic phosphomolybdic acid spray reagent and 10 min of heating at 110 °C, neutral lipids appeared as blue zones on a yellow background. Quantitative densitometric analysis was performed using a CAMAG TLC Scanner II with the tungsten light source set at 610 nm. Quantitative identification was based on correspondence of Rf value of sample and neutral lipid standard zones. At week 2 after the initiation of the experiment, there were significant differences in triacylglycerol content between all three temperature groups, suggesting that snails at the higher temperatures were building reserves of depot fat. Global climate changes have led to concerns about the spread of infectious disease, including snail born infections such as schistosomiasis; some of these concerns relate to the ability of vector snails to adapt to climate change, making studies on lipid use of vector snails at temperature extremes crucial to a better understanding of the potential spread of snail-borne disease.

Vasta *et al.* [12] determined changes in the amino acid content of the *B. glabrata* snail DGG as a function of estivation. Amino acids were extracted in ethanol-water (70:30) from the DGG of *B. glabrata* snails estivated for seven days and determined on silica gel or cellulose layers developed with either 2-butanol-pyridine-glacial acetic acid-deionized water (39:34:10:26) or 2-butanol-pyridine- 25% ammonia-deionized water (39:34:10:26). Separated zones were detected by postchromatographic derivitization with ninhydrin and quantified by visible mode slit-scanning densitometry at 610 nm. Alanine, arginine, glycine, leucine, isoleucine, lysine, serine, and valine were all unequivocally identified in chromatograms from both estivated and unestivated snails. HPTLC-dens showed a significant increase in alanine in estivated snails compared to unestivated snails. HPTLC-dens showed a significant increase in alanine in estivated snails compared to unestivated controls. The finding of increased alanine levels in the DGG during estivation is in agreement with the hypothesis that anaerobic pathways are an important part of overall metabolism in *B. glabrata* during estivation.

3. Conclusions

This paper has provided an update on the recent HPTLC studies on various analytes in the *B. glabrata* snail. It is concluded that various abiotic and biotic factors can cause significant changes in the chemical content of this medically important snail. For the analytes under study, *i.e.*, lipids, lipophilic pigments, amino acids and carbohydrates, HPTLC is a very useful method for their quantification.

Acknowledgements

The authors would like to thank Vivienne Felix for her editorial support in the preparation of this review manuscript.

Author Contributions

Bernard Fried: concept approval, research and interpretation of key papers; Aditya Reddy: summating key findings, manuscript development and submission.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Sherma, J.; Fried, B. Thin-Layer and High Performance Thin Layer Chromatographic Analysis of Biological Samples. In *Advances in Chromatography*, 49th ed.; Grushka, E., Gringley, N., Eds.; CRC Press: Boca Raton, FL, USA; 2011; pp. 401–422.
2. Hunsberger, A.; DeGrandchamp, D.; Fried, B.; Sherma, J. Effects of high and low temperatures on the lipid content of the digestive gland-gonad complex of *Biomphalaria glabrata* as determined by high performance thin layer chromatography. *J. Liq. Chromatogr. Relat. Technol.* **2014**, *37*, 2989–2999.
3. Beiderman, D.; Fried, B.; Sherma, J. Effects of coexposure with *Echinostoma caproni* and *Schistosoma mansoni* miracidia on neutral and polar lipids of *Biomphalaria glabrata* as determined by high-performance thin-layer chromatography-densitometry and observations on snail survival and fecundity. *J. Liq. Chromatogr. Relat. Technol.* **2013**, *17*, 2489–2496.
4. Hunsberger, A.; Fried, B; Sherma, J. Effects of *Echinostoma caproni* miracidia dose on the neutral and polar lipids of *Biomphalaria glabrata* as determined by high-performance thin-layer chromatography. *Acta Parasitol.* **2013**, *58*, 615–618.
5. Dieterich, N.; Fried, B.; Sherma, J. Effects of *Schistosoma mansoni* infection on lutein and β-carotene concentrations in *Biomphalaria glabrata* snails as determined by quantitative high performance reversed phase thin-layer chromatography. *Acta Parasitol.* **2013**, *58*, 259–262.
6. Dieterich, N.; Fried, B.; Sherma, J. Effects of crowding on the neutral and polar lipids of *Biomphalaria glabrata* as determined by high-performance thin-layer chromatography-densitometry and observations on snail survival and fecundity. *J. Liq. Chromatogr. Relat. Technol.* **2012**, *35*, 1444–1451.
7. Popovic, N.; Fried, B.; Sherma, J. Effects of increased salinity on glucose and maltose composition of *Biomphalaria glabrata* snails as determined by high performance thin-layer chromatography-densitometry. *J. Liq. Chromatogr. Relat. Technol.* **2012**, *35*, 1452–1458.
8. O'Sullivan, C.; Sherma, J.; Fried, B. Effects of maintenance temperature on the glucose and maltose content of *Biomphalaria glabrata* snails as determined by the high performance thin-layer chromatography-densitometry. *J. Liq. Chromatogr. Relat. Technol.* **2011**, *34*, 946–953.
9. Holman, N.S.; Vasta, J.D.; Fried, B.; Sherma, J. Effects of maintenance temperature on the amino acid content of *Biomphalaria glabrata* snails as determined by high performance thin-layer chromatography-densitometry. *J. Liq. Chromatogr. Relat. Technol.* **2011**, *34*, 936–945.
10. O'Sullivan, C.; Fried, B.; Sherma, J. Studies on pH tolerance of freshwater snails. *Trends Comp. Biochem. Physiol.* **2011**, *15*, 63–66.

11. Bolstridge, J.; Fried, B.; Sherma, J. Effects of temperature on the neutral lipid content of *Biomphalaria glabrata* as determined by high performance thin layer chromatography-densitometry and observations on snail fecundity. *J. Liq. Chromatogr. Relat. Technol.* **2010**, *33*, 1005–1012.
12. Vasta, J.D.; Fried, B.; Sherma, J. Determination of estivation-induced changes in the amino acid content of *Biomphalaria glabrata* snails by high performance thin-layer chromatography-densitometry. *J. Liq. Chromatogr. Relat. Technol.* **2010**, *33*, 1028–1037.