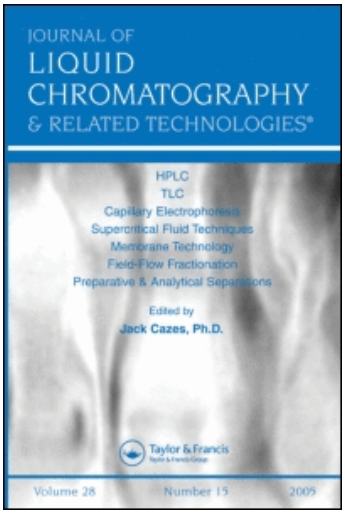

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CARBOHYDRATE ANALYSIS, BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY, OF *CERITHIDEA CALIFORNICA* (GASTROPODA: PROSOBRANCHIA)

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ABSTRACT

Fairbairn has identified glucose and trehalose from a variety of freshwater and marine gastropods. Recent studies on several freshwater snails have identified glucose and maltose, but not trehalose in the freshwater snail *Biomphalaria glabrata*. The purpose of this study was to determine, by HPTLC, the presence of carbohydrates in the economically important marine snail *Cerithidea californica*. In addition to authentic carbohydrate standards, tissue and hemolymph of *B. glabrata* were analyzed along *with C. californica* samples. The HPTLC analysis confirmed the presence of glucose and maltose and the absence of trehalose in the *B. glabrata* samples. None of these carbohydrates were detected in the *C. californica* samples. The only carbohydrate tentatively identified in the *C. californica* samples was raffinose. Possible reasons for not determining the major carbohydrates in *C. californica* are discussed in the paper.

INTRODUCTION

Fairbairn[1] reported the occurrence of trehalose and glucose in the freshwater snails *Biomphalaria glabrata* and *Bulinus africanus* and in the marine snails *Ilyanassa obsoleta*, *Crepidula fornicata*, *C. plana*, *Littorina littorea*, *Urosalpinx cinerea*, *Thais lapillus*, and *Busycon canaliculatum*. He determined glucose by a specific glucose oxidase assay, and trehalose by equating the total carbohydrate in chromatogram eluates with glucose formed by acid hydrolysis of the carbohydrate in both the freshwater and marine gastropods. Cline et al.[2,3] used high performance thin-layer chromatography (HPTLC) to determine carbohydrates in the freshwater snail, *Lymnaea elodes,* and combined HPTLC and gas chromatography-mass spectrometry (GC/MS) to confirm the presence of maltose and glucose and the absence of trehalose in *B. glabrata*.

The purpose of this study was to determine the presence of carbohydrates in the marine snail *Cerithidea californica.* This intertidal snail, abundant on the west coast of the USA, is heavily parasitized with larval trematodes; according to Martin,[4] it is infected with at least 18 species of digeneans. Because parasitism alters the carbohydrate content of infected snails (Perez et al.[5]), we analyzed snails infected with larval trematodes versus those not infected.

EXPERIMENTAL

Preparation of Samples

Uninfected *C. californica* and those infected with the strigeid trematode *Mesostephanus appendiculatus* and the heterophyid trematode *Euhaplorchis californiensis* were obtained from Jones Biological Lab., Inc. (Long Beach, California, USA). The snails were used within several days of receipt, and the digestive gland gonad complex (DGG) and hemolymph were prepared for analysis by HPTLC as described in Cline et al.[2] for studies on *L. elodes*. Snails were grouped by the type of larval infection or by the absence of infection (uninfected controls). Each DGG sample consisted of one DGG of a particular larval trematode type or one DGG of an uninfected snail. Hemolymph samples were pooled from 5 uninfected or 5 infected samples. Four DGG samples were analyzed for each infection type and also for the uninfected DGGs. Three hemolymph samples from each sample type were also analyzed.

HPTLC Analysis

Qualitative and quantitative analyses were carried out as described earlier[2,3] on Merck (EM Separations Technology, Gibbstown, NJ) channeled silica gel HPTLC layers with concentrating zone using triple development with acetonitrile-water (85:15) or ethyl acetate-acetic acid-methanol-water (60:15:15:10)

mobile phase or on Whatman (Clifton, NJ) LK5DF channeled silica gel TLC plates with concentrating zone using single development with ethyl acetate-acetic acid-methanol-water (60:15:15:10). With both systems, sugar zones were detected by spraying with 1-naphthol reagent and heating for 10 min at 110°C. Hemolymph and DGG samples were extracted with 70% aqueous ethanol and reconstitution volumes used to prepare the TLC test solutions were 25-200 μL, resulting in concentrations up to 8x greater than those reported in the studies on freshwater snails by Cline et al.[2,3]

RESULTS AND DISCUSSION

The results (n=3 in each case) of analysis of infected and uninfected DGGs and hemolymph samples failed to show maltose and glucose. Numerous polar 1-naphthol positive zones were detected, but none matched authentic standards with the possible exception of a raffinose zone which migrated at about the same distance as a raffinose standard (see Figure 1). Spiking experiments with

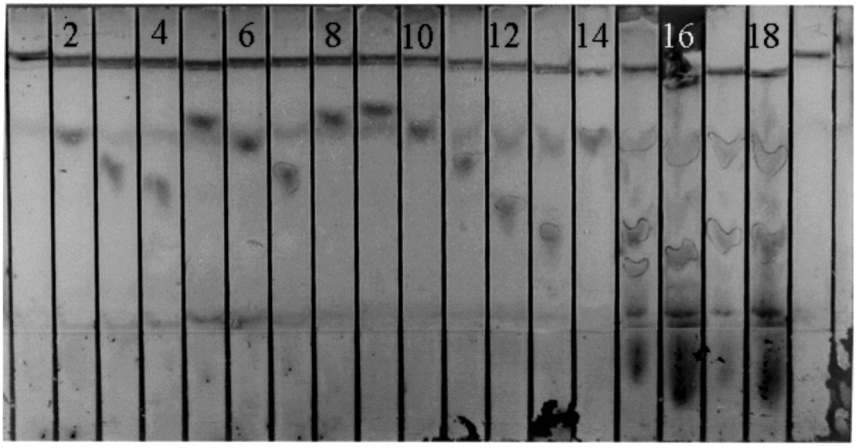

Figure 1. Chromatograms of sugar standards (2 μL aliquots of 1 mg/mL standard) and *Cerithidea californica* samples extracted in 70% ethanol and developed on plates pre-impregnated with sodium bisulfite (0.1 M) and citrate buffer in ethyl acetate-acetic acid-methanol-water (60:15:15:10) and detected with α-naphthol spray reagent. Lanes 2-13 contain the following sugar standards: 2. glucose, 3. maltose, 4. ribose, 5. galactose, 6. trehalose, 7. fucose, 8. xylose, 9. fructose, 10. sucrose, 11. melezitose, 12. raffinose, 13. mannose. Lanes 14-17 contain the snail DGG samples: 14. uninfected *C. californica* (2 μL), 15. uninfected *C. californica* (6 μL), 16. *C. californica* infected with *Mesostephanus appendiculatus* (2 μL), 17. *C californica* infected with *M. appendiculatus* (6 μL). Note the prominent, slow moving zones in the snail samples which do not line up with any of the standard sugars.

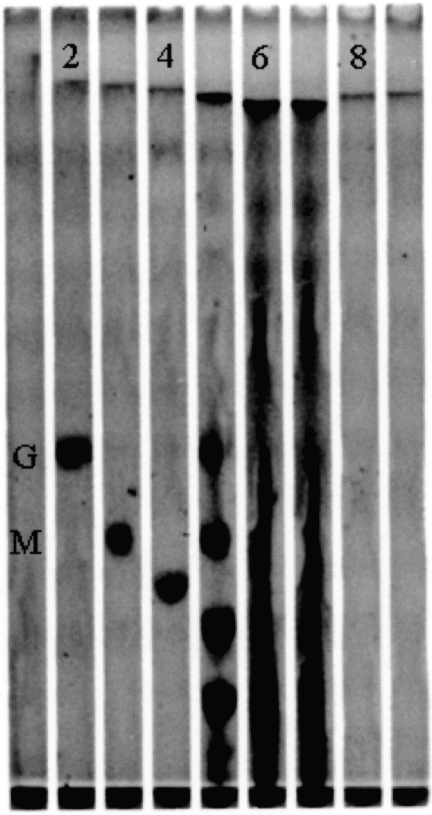

Figure 2. Chromatograms of glucose (G) and maltose (M) standards (lanes 2 and 3, respectively). Lane 4 consists of a lactose standard. Lane 5 contains extract of *Biomphalaria glabrata* DGG. Note the presence of glucose and maltose in this snail. Lanes 6 and 7 contain extracts of *Cerithidea californica* DGG. Note the typical smear patterns in these extracts.

maltose and glucose failed to identify sample zones that preliminarily appeared to co-migrate with maltose and glucose standards. Some plates included *B. glabrata* samples for comparison with the *C. californica* samples. As seen in Figure 2, the chromatograms on these plates clearly showed the maltose and glucose zones in *B. glabrata* samples and the absence of similar zones in *C. californica*.

It is clear from Fairbairn's work,[1] that at least the 8 species of marine gastropods that he studied have glucose in their hemolymph and tissues. We do not

know why we have not been able to determine its presence in either infected or uninfected samples of *C. californica*. Perhaps our extraction method, suitable for previous analysis by HPTLC of sugars in *B. glabrata* and *L. elodes*, was not adequate for a similar assay in *C. californica*. The procedure may have failed to extract the sugars or may have extracted impurities that interfered with the TLC separation. Perhaps the concentration of carbohydrates in *C. californica* (particularly the infected snails, where infection is known to reduce the sugar content) was too low for our HPTLC analysis, which has a detection limit of 0.00075 weight percent for maltose and glucose. In addition, information on carbohydrates in gastropods is very meager, considering that there are more then 40,000 species of living snails occupying various habitats and with diverse feeding styles. Therefore, it is possible that *C. californica* is one among a large array of marine gastropods that may not have the carbohydrates in question.

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