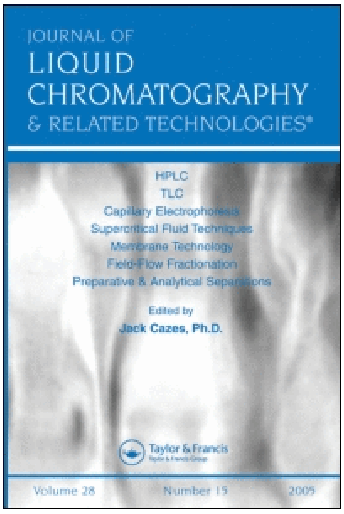

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Thin Layer Chromatographic Analysis of Neutral Lipids in the Digestive Gland-Gonad Complex of *Biomphalaria Glabrata* Snails Maintained on Various Diets

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THIN LAYER CHROMATOGRAPHIC ANALYSIS OF NEUTRAL LIPIDS IN THE DIGESTIVE GLAND-GONAD COMPLEX OF *BIOMPHALARIA GLABRATA* SNAILS MAINTAINED ON VARIOUS DIETS

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ABSTRACT

Thin layer chromatography on channeled, high performance preadsorbent silica gel plates was used to study the neutral lipid composition of six diets, i.e., hen's egg albumen, hen's egg yolk, leaf lettuce, Tetramin fish food, lettuce/Tetramin (1:1), and Spirulina, and the digestive gland-gonad complex of Biomphalaria glabrata snails maintained on these diets. Differences in the neutral lipid profiles between the diet and the DGG suggest that factors other than dietary uptake, presumably metabolic, play a role in determining the lipid composition of the snail.

INTRODUCTION

The digestive gland-gonad complex (DGG) of the medically important snail Biomphalaria glabrata is the major site for the deposition of neutral lipids (1). Thin layer chromatography (TLC) has been used to study the effects of starvation on the DGG of B. glabrata (1), and TLC and GLC have been applied to the examination of the effects of a leaf lettuce versus hen's egg yolk diet on the DGG of this snail (2, 3). These studies showed that B. glabrata snails fed hen's egg yolk had elevated levels of triacylglycerols (TG) and cholesterol in the DGG. The increase in TG resulting from the egg diet could be reversed by returning the snails to the lettuce diet (4).

In addition to yolk and lettuce diets, there are other diets that have been used to maintain B. glabrata snails in the laboratory, including the blue-green algae Spirulina (5) and "Tetramin Baby Food E" (6). The purpose of this study was to determine the effects of six specific diets that have been reported in the literature, i.e., hens' egg yolk, hen's egg albumen, leaf lettuce, Tetramin, lettuce/Tetramin (1:1), and Spirulina, on snail growth and fecundity and the lipid composition of the DGG of B. glabrata. TLC was used to analyze the lipid contents of the six diets and the DGG of snails maintained on each diet for two weeks.

EXPERIMENTAL

Sample preparation

Snails with 5+/-2 mm shell diameter were maintained in glass vessels (7 snails per vessel) with 1000 ml of spring water (1) and fed the respective diets. Each experimental group of 7 snails was allowed to feed ad libitum for two weeks. After two weeks of feeding, snails from each group were weghed and pooled, their shells removed, and their DGGs dissected in saline. The DGG sample was then adjusted to give a wet weight of about 100 mg per pool. Each DGG pool was extracted with two 2 ml portions of chloroform-methanol (2:1), the combined extract was filtered through glass wool, and non-lipid components were removed by Folch washing as previously described (3). The DGG extract was evaporated just to dryness under a nitrogen stream, and the residue was reconstituted with 10 ul of chloroform-methanol (2:1) per milligram (wet weight) of tissue. Analysis of 100 mg portions of each snail diet was performed in the same manner as the DGG samples.

TLC of lipids

TLC analysis was performed using Whatman LHP-KDF channeled, preadsorbent silica gel plates, petroleum ether-diethyl ether-glacial acetic acid (80:20:2) mobile phase, and PMA detection reagent as described earlier (7). Using a Drummond digital microdispenser, plates were spotted with 5 and 10 ul of lipid standard 18-5A (Nu-Check Prep, Elysian, MN), containing 0.25 ug/ul each of phosphatidylcholine (phospholipid), oleic acid (free fatty acid), cholesterol (sterol), triolein (triacylglycerol), and cholesteryl oleate (cholesterol ester), and 5 and 10 ul of reconstituted DGG or diet extract. Qualitative identification of lipids was based on comparison of R_f values of samples and standards, and relative concentrations of lipid fractions were estimated by comparing the intensity of the zones detected in the sample chromatograms. TLC analyses were replicated by analyzing two samples of each diet and DGGs from three pools of snails. In each case, essentially identical results were obtained for the repeated analyses.

RESULTS

The results of the chromatographic analysis of neutral lipids in the various diets (except for Spirulina) are illustrated in Figure 1. Neutral lipids were not detected in Spirulina, and only trace amounts of sterols were detected in the albumen (not visible in Figure 1, lane 3). TG were very abundent in yolk (lane 11), moderately abundent in Tetramin (lane 9), and less abundent in combined lettuce/Tetramin (lane 7). TG were not detected in lettuce (lane 5). Sparse to moderate amounts of cholesterol esters were present in the yolk, Tetramin, lettuce/Tetramin, and lettuce diets. An additional zone that migrated just below cholesterol esters (presumably methyl esters) was seen in the Tetramin and lettuce/Tetramin diet chromatograms. Moderate to heavy amounts of both free fatty acids and free sterols were present in the yolk, Tetramin, lettuce/Tetramin, and lettuce diets. Some additional zones that migrated between the free sterol and free fatty acid fractions were seen clearly in the lettuce chromatogram and less clearly in the

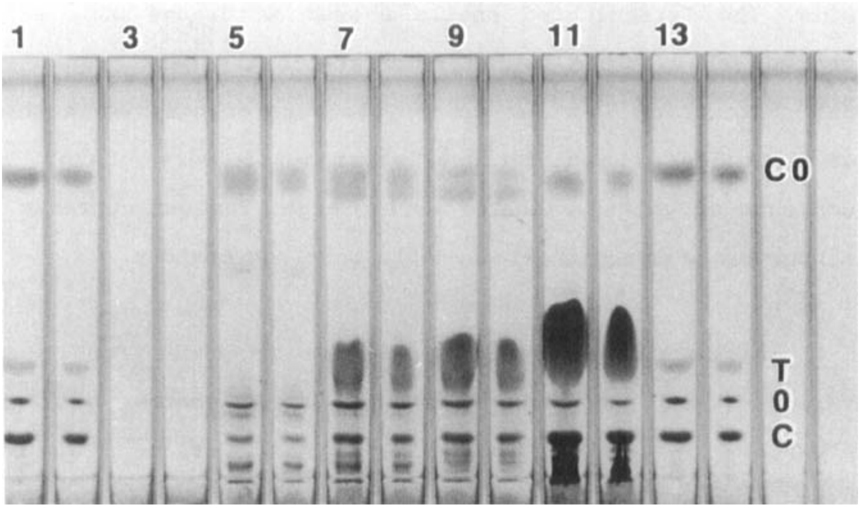

FIGURE 1. Photograph of the chromatograms of extracts of various diets developed with petroleum ether-diethyl ether-acetic acid (80:20:2) on a laned, high performance preadsorbent silica gel plate. Lanes 1 and 13 contain 10 ul of the mixed lipid standard, and lanes 2 and 14 contain 5 ul of the same standard. Lanes 3, 5, 7, 9, and 11 contain 10 ul of the extracts of albumen, lettuce, lettuce/Tetramin, Tetramin, and yolk, respectively. Lanes 4, 6, 8, 10, and 12 contain 5 ul of the respective diets. Abbreviations: CO=cholesteryl oleate, T=triolein, O=oleic acid, C=cholesterol.

lettuce/Tetramin chromatogram. These bands were not identified. Additional uncharacterized zones, probably phospholipids, were observed below the free sterol fraction in all of the chromatograms except albumen.

The yolk-fed snails showed a yellow-white DGG, while that of lettuce-fed snails was green-brown. Both Spirulina-and albumin-fed snails showed small, light-colored, fragile DGGs. The Tetramin-fed snails showed an orange DGG, while that of the lettuce/Tetramin-fed snails was dark brown. The gut contents of Tetramin-fed snails were orange, while those of lettuce-fed and lettuce/Tetramin-fed were green. Gut contents of yolk-fed snails were orange. The gut contents of albumen- and Spirulina-fed snails were not obvious.

The results of the DGG analysis of snails fed on the various diets are shown in Figure 2. TG content was very abundent in the DGG from yolk-fed snails (lane 11), less abundent in the lettuce/Tetramin DGG (lane 7), moderate in the lettuce (lane 5) and Tetramin (lane 9) DGG, and sparse in the DGG of snails maintained on albumen (lane 3) and Spirulina (lane 13). The cholesterol ester fraction was abundent in the DGG of yolk-fed snails (lane 11) but was absent or sparse in the other populations. A presumed methyl ester band (between TG and cholesterol esters) was present in the DGG of yolk-fed snails (lane 11) but was absent or sparse in the other populations. The free fatty acid fraction was moderate in the DGG of yolk-, lettuce-, and lettuce/Tetramin-fed snails, and was sparse in the other pools. The free sterol fractions were moderate to abundant in the DGG of snails maintained on all diets. Additional zones seen beolw the free sterol, particularly in the yolk, lettuce, and lettuce/Tetramin chromatograms, were not characterized.

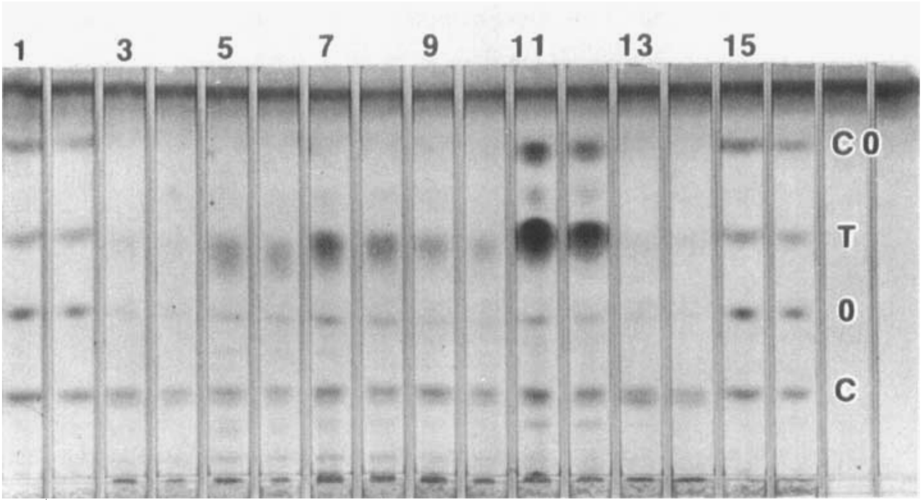

FIGURE 2. Photograph of the chromatograms of DGG extracts of B. glabrata snails maintained on various diets. Mixed lipid standard was applied to lanes 1 and 15 (10 ul) and 2 and 16 (5 ul). DGG extract from snails fed albumen, lettuce, lettuce/Tetramin, yolk, and Spirulina was applied to lanes 3, 5, 7, 9, 11, and 13 (10 ul) and 4, 6, 8, 10, 12, and 14 (5 ul), respectively. Conditions and abbreviations are the same as in the caption of Figure 1.

Average starting weights of B. glabrata were 125 mg per snail. Snails on the lettuce/Tetramin diet had the greatest increase in body weight, reaching an average of 235 mg at 14 days post-feeding, compared with increases to 169 mg for Tetramin and 176 mg for lettuce. Snails on the yolk diet had essentially no change in body weight, while those on Spirulina and albumen decreased to 100 and 115 mg, respectively.

Fecundity, i.e., the number of egg masses per snail, was also observed for 5-7 snails on each diet as a function of time. By day 14 post-feeding, B. glabrata reared on lettuce/Tetramin had the highest fecundity rate, averaging 3.5 egg masses per snail. This is considerably higher than the number of egg masses per snail for lettuce (2.1) or Tetramin (1.5) alone. Snails fed yolk, albumen, and Spirulina diets had average fecundity rates of 0.2, 0.4, and 0 during the 14 days of the experiment.

DISCUSSION

Optimal snail growth and fecundity occurred with the lettuce/Tetramin diet, and in contrast to the findings of Thompson and Mejia-Scales (5), the Spirulina diet was not effective in promoting growth or egg laying in our B. glabrata cultures. Although neutral lipids were not detected in the ambumen diet, with the exception of a trace of free sterol, lipid fractions were present in the DGG extract of snails maintained on this diet. Cholesterol esters were found in all of the diets except albumen and Spirulina, but they were sparse or not detected in all of the DGGs except those from yolk-fed snails. Therefore, although dietary intake influences the lipid composition of the DGG of B. glabrata, other factors, presumably metabolic, also play a role. Snails on the yolk diet did not gain weight nor lay eggs at a rate equal to those on the lettuce/Tetramin diet, suggesting that a high TG/cholesterol diet is not conducive to promoting optimal development in B. glabrata.

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