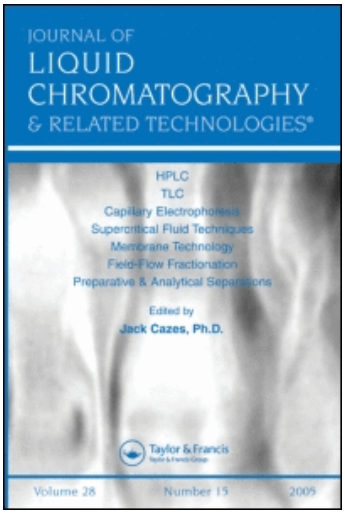

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THIN LAYER CHROMATOGRAPHIC DETERMINATION OF NEUTRAL LIPIDS AND PHOSPHOLIPIDS IN BIOMPHALARIA GLABRATA SNAILS FED LETTUCE VERSUS HEN'S EGG YOLK AS A FUNCTION OF TIME

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ABSTRACT

TLC on HP preadsorbent silica gel plates was used to determine, at various times over a 21-day period, neutral lipids and phospholipids in the plasma and digestive gland-gonad complex (DGG) of Biomphalaria glabrata snails maintained on diets of leaf lettuce and hen's egg yolk. The DGG of yolk-fed snails showed considerable elevation of triacylglycerols by day 3, and free fatty acids and sterols by day 5. Hemolymph from two pools of yolk-fed snails was analyzed on day 7, and the respective results for free fatty acids, sterols, and triacylglycerols were 0.036, 0.0023, and 0.028 g/dL. Comparable values in lettuce-fed snails were 0.0061 for free fatty acids, 0.0016 for sterols, and triacylglycerols were not detected. Semiquantitative TLC showed that compared to lettuce-fed snails, the plasma of yolk-fed snails had elevations in sterols, free fatty acids, triacylglycerols, and phospholipids from days 5 to 21. The DGG of snails fed both diets showed approximately equal amounts of phosphatidylcholine and phosphatidylserine throughout the 21 days, but phosphatidylethanolamine was elevated in the DGG of yolk-fed snails by day 5. All phospholipid fractions were elevated in the plasma of yolk-fed snails during the 21-day period, with plasma from lettuce-fed snails showing only negligible amounts of phospholipids.

INTRODUCTION

Biomphalaria glabrata, a freshwater planorbid snail, is a vector of the medically important blood fluke Schistosoma mansoni. Because this snail is easily maintained on simple diets in the laboratory, it has been used for numerous immunological, anatomical, physiological, and biochemical studies. Recent research has revealed that B. glabrata fed hen's egg yolk show a significant increase in hemolymph and tissue lipids compared to controls maintained on a diet of leaf lettuce (1, 2). Therefore, this snail may serve as a convenient invertebrate model to study dietary-induced hyperlipidemia in humans.

In the first quantitative TLC study (3) of the neutral lipids in snails fed lettuce or yolk diets, triacylglycerols and sterols were found to be the major neutral lipid fractions in the digestive gland-gonad (DGG) complex, while free fatty acids were a minor component. The amount of free fatty acids in the DGG of yolk-fed snails increased by a factor of almost three compared to lettuce-fed snails for a period of seven days, and triacylglycerols increased by a factor of approximately six.

Whereas the previous study (3) examined only the neutral lipids in the DGG on day 7 after the lettuce and yolk diets were initiated, this study used qualitative and quantitative TLC to determine the neutral lipid and phospholipid content of both the DGG and plasma of the two snail groups at various time intervals throughout the 21-day feeding protocol, and

the neutral lipid content of hemolymph after 7 days on the yolk diet.

EXPERIMENTAL

Sample preparation

Groups of five snails were maintained and fed on lettuce or yolk diets for 3, 5, 7, 10, 14, and 21 days as previously described (1, 3, 4). Procedures for isolation of DGG (3), collection of hemolymph (2), removal of hemolymph by cardiac puncture and separation of plasma (5), and extraction of lipids from DGG (3), hemolymph (2), and plasma (5) were also reported earlier. Extracts were evaporated just to dryness with a stream of nitrogen. DGG samples were reconstituted with 4.0 ul of chloroform-methanol (2:1) per mg (wet weight) of tissue. Hemolymph extracts were reconstituted with 1.0 ul of solvent per ul of sample for lettuce-fed snails and 1.5 ul/ul sample for yolk-fed. Plasma extracts were reconstituted with 0.5 ul solvent/ul sample.

TLC of lipids

Whatman LHP-KDF silica gel plates and PMA detection reagent were used in all experiments. Qualitative identification of lipids was based on comparison of R_f values of samples and standards, and semiquantitative analysis was performed by visual comparison of the intensity of sample and standard zones. Quantitative TLC of neutral lipids in DGG was performed as described previously (3), except that the

Mangold solvent system, petroleum ether-diethyl ether-glacial acetic acid (80:20:1), was used, the reconstitution volume was 4 ul/mg, as described above, and 4.0, 8.0, and 16.0 ul of reconstituted extract were spotted. Quantitative analysis of neutral lipids in hemolymph was carried out as described in Reference 3. The concentration of lipid in g/dL was calculated as follows: g/dL = 1/10 x (ug interpolated from calibration curve/ul spotted) x (ul reconstituted extract/ul hemolymph sample). For semiquantitative analysis of plasma for neutral lipids, 10.0, 15.0, and 20.0 ul of reconstituted extract were applied on the same plate with standards, and the Mangold system was used for development.

TLC of phospholipids

For the semiquantitative determination of phospholipids, 2.0, 8.0, and 16.0 ul of a 1.0 ug/ul solution of Supelco Polar Lipid Mix B [containing equal amounts of phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), and cholesterol] and 10.0 ul of a 1.0 mg/ml solution of an individual phosphatidylserine (PS) standard (also obtained from Supelco) were spotted with the same volumes of reconstituted DGG and plasma extracts as specified above for lipid analysis. Plates were developed with chloroform-methanol-deionized water (65:25:4) (6).

RESULTS

Snails maintained on lettuce or yolk for three weeks survived equally well. During this period, snails fed lettuce

laid more than three times the number of egg masses than those fed yolk. Beyond three weeks, snails fed yolk had increased mortality compared to those fed lettuce. Gross examination of yolk-fed snails revealed an enlarged, fatty, yellow-orange DGG in contrast to a firm, brown-green organ in snails fed lettuce (1). Plasma obtained from the two groups showed color differences as early as day 3; plasma from lettuce-fed snails was red-pink, while that from yolk-fed snails was orange-brown.

Neutral lipids in DGG

Triacylglycerols, sterols, and free fatty acids were identified as the major neutral lipid fractions present in DGG extracts, and these zones were quantified against triolein (R_f 0.81), cholesterol (0.29), and oleic acid (0.44), respectively, in the lipid standard 18-4A after development of plates with the Mangold solvent. DGG of yolk-fed snails showed elevated levels of sterols (Fig. 1) and free fatty acids (Fig. 2) by day 5, and triacylglycerols (Fig. 3) as early as day 3. Triacylglycerols were present in the greatest quantity in the DGG. The absolute values found for the percentage composition of these lipids at day 7, as read from Figs. 1-3, are similar to those reported earlier by Higgs *et al*. (3).

Neutral lipids in hemolymph and plasma

Triacylglycerols, sterols, and free fatty acids were also the major neutral lipids found in the hemolymph and

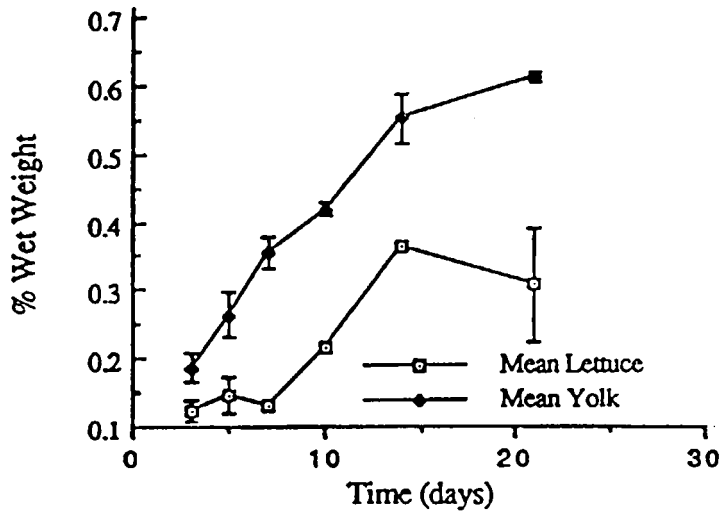

Figure 1. Percentage wet weight of free sterols in the DGG of B. glabrata snails on a diet of leaf lettuce (n=3) or hen's egg yolk (n=3) plotted against the number of days on the diet. Symbol and bar indicate mean +/- standard error.

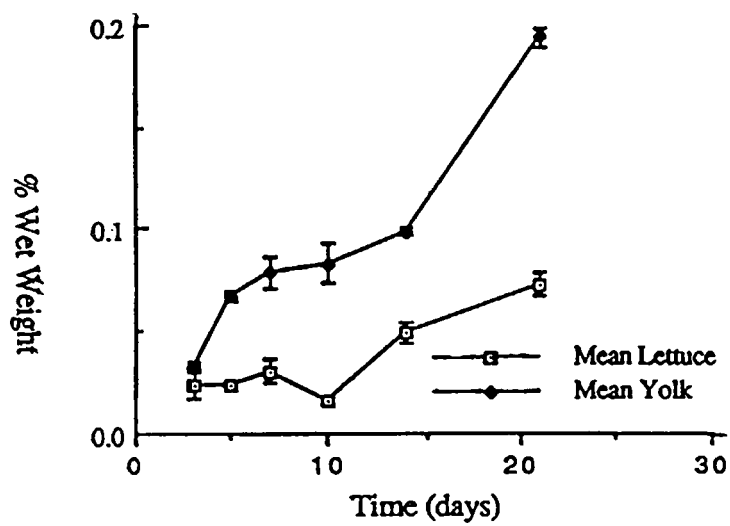

Figure 2. Percentage wet weight of free fatty acids as a function of diet and time, as described in Figure 1.

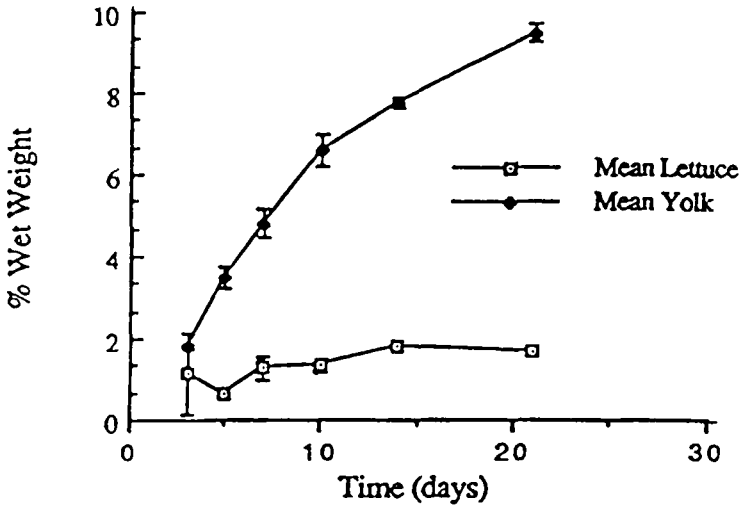

Figure 3. Percentage wet weight of triacylglycerols as a function of diet and time, as described in Figure 1.

plasma of yolk-fed snails, and sterols and free fatty acids in lettuce-fed snails. Quantitative TLC of hemolymph extracts from two 5-snail pools each of lettuce- and yolk-fed snails at day 7 gave the following concentrations (g/dL): lettuce-fed: fatty acids- 0.0048, 0.0074; sterols- 0.0023, 0.0010; triacylglycerols- not detected (<0.001); yolk-fed: fatty acids- 0.041, 0.032; sterols- 0.0024, 0.0033; triacylglycerols- 0.035, 0.022. These data show that sterols increased by a factor of about two, fatty acids by about six, and triacylglycerols fron nil to a value almost as high as fatty acids in yolk-fed snails. A time study of plasma neutral lipids made using semiquantitative TLC showed that

fatty acids were the predominant lipid fraction throughout the 5-21 day period, and that lettuce-fed snails had slightly increasing lipid concentations and yolk-fed snails much greater increases over this period. No triacylglycerol fraction was observed in plasma from lettuce-fed snails throughout the 21-day period.

Phospholipid determinations

Phospholipid semiquantitative analysis using the Wagner et al. solvent system revealed that the DGG of snails fed both diets contained approximately equal amounts of PS (R_f 0.36) and PC (0.44) throughout the 21 days, but PE (0.69) was elevated in the DGG of yolk-fed snails by day 5. Semi-quantitative analysis of phospholipids in plasma showed that PC, PE, and PS were present in both populations. Concentrations of all three compounds were low in lettuce-fed snails and increased upon yolk feeding from day 5-21.

DISCUSSION

This research verifies earlier findings (3) that triacylglycerols, sterols, and free fatty acids are the major lipid fractions in B. glabrata DGG extracts and that free fatty acids increase by a factor of about three and triacylglycerols by about six at day 7 for yolk-fed snails compared to lettuce-fed. In addition, Figures 1-3 show that differences for these two lipid classes in lettuce- and yolk-fed snails are significant (Student's t-test, $P < 0.05$) as

early as day 5, and maximum divergences occur at day 21. Our earlier study (3) found no statistically valid difference in sterol content at day 7, whereas in the present study yolk-fed snails had a significantly higher concentration of sterols by day 5, and as much as a 2-fold higher amount by day 21 (Figure 1). This increase in sterols is consistent with recent GC results (5). The considerable intrinsic variability of lipid composition in B. glabrata snails (7) is undoubtedly reflected in the anomalous results obtained for sterols in the two TLC studies.

The results showing significant increases in free fatty acid and triacylglycerol fractions in the hemolymph of yolk-fed snails compared to lettuce-fed snails confirm a previous study (2). Although most of the lipid in the hemolymph is probably in the plasma fraction, undoubtedly some is associated with the amoebocytes (hemocytes). Analysis of lipids in the amoebocytes of B. glabrata remains to be accomplished. The significance of elevated levels of both neutral and phospholipid fractions in yolk-fed snails is not clear at present.

A lipid zone at the top of the chromatograms (R_f ca. 0.95), which contained cholesteryl esters, was significantly darker for yolk-fed snails than for those that were lettuce-fed. Because this zone undoubtedly contained nonpolar compounds other than cholesteryl esters, such as wax esters and hydrocarbons, the conclusion that cholesteryl esters are elevated in yolk-fed snails must be considered a preliminary result. TLC and HPLC studies aimed at reliably separating and

quantifying steryl esters in snail DGG are underway in our laboratory and will be reported later.

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