Simultaneous Extraction and Preparative Fractionation of Egg Yolk Lipids Using the Principle of Adsorption

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ABSTRACT

A new approach describing the simultaneous extraction and preparative fractionation of egg yolk lipids is described, a method which can be extended to other tissues. In this method, egg yolk is adsorbed on activated thin layer chromatography grade silicic acid and sequentially extracted with different solvents to get a crude fractionation of its lipids into nonpolar and polar components. These crude concentrates help achieve larger yields of high purity lipids per unit column load during subsequent chromatographic subfractionation.

INTRODUCTION

In the process of preparing high purity lipids for commercial purposes in our recently started institute, we found it necessary initially to prepare crude concentrates of various lipids from naturally occurring complex lipid mixtures. These would yield more high purity lipids per unit column load during subsequent chromatographic fractionation (1). In this communication, we report a new approach which will simultaneously extract and preparatively fractionate egg yolk lipids into crude nonpolar and polar concentrates.

Extraction and preparative fractionation of egg yolk lipids is conventionally achieved by the Folch procedure (2) and acetone precipitation (3), respectively. Alternatively, with the use of chromatography, the same type of fractionation has been achieved from silicic acid columns by either sequential elution with chloroform and methanol (4) or with petroleum ether and ethyl ether (5). In the former silicic acid procedure, the nonpolar lipids are eluted ahead of the polar lipids, while in the latter, the polar lipids (phospholipids) are eluted ahead of the nonpolar lipids (triglycerides) because of the formation of high molecular weight micelles of phospholipids in hydrocarbon solvents. These methods have their limitations in that the Folch procedure followed by acetone precipitation consumes substantial amounts of solvents, while the silicic acid procedures require an extraction step as a prerequisite. The present method, on the other hand, applied the principle of adsorption to obtain simultaneous extraction and preparative fractionation of egg yolk lipids into nonpolar and polar types as mentioned earlier.

EXPERIMENTAL PROCEDURES

Hen eggs were purchased locally. All solvents used were of reagent grade.

Silicic acid of thin layer grade was precipitated in the laboratory. Solution of sodium silicate (1.1 sp gr at room temperature) was treated with concentrated hydrochloric acid under constant stirring until the pH was brought to 2.0. After overnight standing, the precipitate was washed free of acidity and chloride ions and then dried in the sun. The dried silicic acid was sieved, and the material passing through 200 mesh, after heating it at 100 C for 1 hr, was used.

Egg yolk (35 g) was mixed with 25 g of activated thin layer chromatography (TLC) grade silicic acid, and the mixture was ground to a fine powder in a CO_2 atmosphere. A 20-g portion, containing 3.4 g of total lipids was subjected to sequential extraction by petroleum ether (50 ml x 4), chloroform (50 ml x 4), and a 1:1 mixture of chloroform-methanol (50 ml x 4). The pooled fractions from each solvent were monitored by TLC, and their cholesterol (6) and phospholipid (7) contents were determined by standard procedures. A portion of egg yolk was subjected to the extraction procedure of Folch (2) and analyzed for its total and component lipid contents. In all the cases the triglyceride content was determined by difference.

RESULTS AND DISCUSSION

The results given in Table I show the reliability of the present method when compared with the results obtained by the Folch method and also with the results reported in the literature (8).

The advantage of this method over the conventional methods are many-fold: (a) silicic acid serves as a dehy-

TABLE I

Comparison of the Composition of Egg Yolk Lipids[a] as Determined by the Folch Method and the Present Method

Lipid Components of egg yolk	Lipid content (in g) of 12 g of egg yolk				
	Present method				
	Petroleum ether extract	Chloroform extract	Chloroform-methanol extract	Total	Folch method
Triglycerides[b]	1.839 (96.8)[c]	0.490 (88.0)	0.121 (12.8)	2.450 (72.0)	2.218 (69.4)
Cholesterol	0.057 (2.9)	0.067 (12.0)	0.019 (2.1)	0.143 (4.2)	0.140 (4.5)
Phospholipids	0.006 (0.3)	Nil (---)	0.800 (85.1)	0.806 (23.8)	0.820 (26.1)
Total	1.902	0.557	0.940	3.399	3.178

[a]Reported figures in literature (8): triglycerides = 65.0%; cholesterol = 4.0%; phospholipids = 30.0%.
[b]This includes small amounts of carotenoids, vitamins, etc.
[c]Figures in parentheses represent percent of total lipids.

drating agent thus preventing the loss of methanol that results in the Folch procedure due to its dilution with tissue water; (b) at the same time it serves as an absorbent for the chromatographic fractionation; (c) the extraction-cum-fractionation is achieved using ordinary unsophisticated glassware; and (d) its solvent consumption is much less.

The amount of silicic acid required to fractionate a complex lipid mixture successfully will depend not only on the water content but also on the lipid content and composition of the tissue. This has to be decided by experimentation. Thus a sheep brain, which has a higher water content than has egg yolk and also a very different lipid composition, required double its own weight of silicic acid to achieve a proper lipid fractionation. Similarly the amount of solvent required for extraction would also depend on the amount of a particular type of lipid present in the tissue. This also has to be decided by experimentation.

We have herein used two solvent systems—petroleum ether and chloroform—instead of just one (chloroform) for the elution of the nonpolar lipids of egg yolk, as a result of one of our previous observations (unpublished). On adsorption of nonpolar lipids of egg yolk (obtained following acetone precipitation) onto TLC grade silicic acid, we had found we could isolate triglyceride free of cholesterol by petroleum ether extraction. However, an attempt at the same fractionation in the present method, employing two solvent systems, was unsuccessful (Table I). We attribute our failure to achieve the subfractionation of nonpolar lipids in this case to the deactivation of silicic acid by water from egg yolk. This observation has been confirmed experimentally (data not given).

Recently we have successfully extened this method to the fractionation of sheep brain lipids and obtained crude concentrates of cholesterol, sphingoglycolipids, and phospholipids by sequential elution with chloroform, acetone, and methanol. The details of these experiments will form the subject of another communication.

Although this method seems promising in the light of future application, it has certain limitations. Whenever the finely ground biological material is directly absorbed onto the silicic acid, the fractionation one can achieve is limited only to lipid classes differing widely in polarity. This is due to deactivation of silicic acid by water from the tissue. When subfractionation within a class of lipids is desired, one had to absorb a lipid solution (free of moisture) to the silicic acid. Our experiments in progress show that such a possibility exists. We have not only separated chemically synthesized wax esters from a reaction mixture free of the reactants—fatty alcohols and fatty acids (9), but have also achieved a partial preparative isolation of phosphatidylethanolamine (90% pure) from egg yolk phospholipids (unpublished).

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