Comparison of two methods for the separation of polar from nonpolar lipids

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SUMMARY The countercurrent extraction method of Galanos and Kapoulas for the separation of phospholipids from triglycerides was compared with dialysis in petroleum ether in an apparatus modified from that of Eberhagen and Betzing. The efficiencies of the methods are similar, but dialysis, which is slower, can yield triglycerides that are entirely free from phosphorus. The behavior of free fatty acids and monoglycerides is also described.

KEY WORDS countercurrent extraction · dialysis · phospholipids · triglycerides · polar · nonpolar · lipids · free fatty acids · monoglycerides

TWO SIMPLIFIED PROCEDURES for the separation of polar and nonpolar components of lipid mixtures were published in this Journal a few years ago. According to Galanos and Kapoulas (1) polar lipids may be separated from triglycerides by eight countercurrent distributions between light petroleum and ethanol–water 87:13. Eberhagen and Betzing (2) reported a modified dialysis procedure (3) in which the original dialyzer was replaced by a continous liquid–liquid extractor. This report presents a comparison of the efficiencies of the above procedures.

Experimental Details and Results. Materials used in this work included: (*a*) hen egg phospholipids obtained by ethanol–diethyl ether extraction of egg yolks and repeated precipitation of crude phospholipids from acetone [the product contained 3.46% P estimated colorimetrically (4)]; (*b*) pure 1-monolaurin and 1-monoolein; (*c*) pure decanoic and stearic acids; (*d*) refined groundnut oil; (*e*) bleached beef fat. These materials and their binary mixtures were submitted to the two procedures mentioned.

We found that in the apparatus devised by Eberhagen and Betzing the vapors of the light petroleum partly condensed in the dialyzing sector before they reached the water condenser. The resulting rise in the temperature of the solvent in the dialyzer proper caused contamination of the dialysate with phospholipids; furthermore, the rubber membrane containing the retentate was occasionally filled with the solvent to the point of overflowing because of the vapor condensation in the surrounding space. Both drawbacks were eliminated (Fig. 1) by directing the solvent vapors into the condenser, through a detachable vapor tube extension, without allowing them to enter into the dialyzing sector [the stopcock (*G*) may be replaced by a suitable plug or similar blocking device].

Table 1 shows results obtained by the use of Eberhard and Betzing's original apparatus (denoted as EB), its modification (EB Mod), and the procedure of Galanos and Kapoulas (GK). This table includes also the result of acetone dialysis of a phospholipid sample previously dialyzed against light petroleum. All dialysis experiments were carried out for 8 hr.

Dialysis through a rubber or cellulose membrane separates the micelle-forming phospholipids from triglycerides, free fatty acids, and partial glycerides (which do not form high molecular weight aggregates), but the speed of the separation depends greatly on the solubility

TABLE 1 COMPARISON BETWEEN DIALYSIS METHOD AND COUNTERCURRENT EXTRACTION FOR SEPARATING PHOSPHOLIPIDS FROM NONPOLAR LIPIDS

Method	Sample	Weight	Recovery (% of total weight)			
			Dialysate or Light Petroleum Phase	%P	Retentate or Ethanol Phase	%P
		g				
EB*	Phospholipid† (3.46% P)	1.0025	5.38	2.07	95.62	3.71
EB Mod*	"	1.0105	2.74	0.00	97.26	3.70
GK‡	"	1.0101	3.61	0.93	96.39	3.49
EB	Ground-nut oil + phospholipid	1.0744 +1.0722	50.68	0.04	49.32	3.56
EB Mod	Ground nut oil + phospholipid	0.9761 +0.9625	49.23	0.00	50.77	3.50
GK	Ground nut oil + phospholipid	1.0138 +1.0127	50.73	0.04	49.27	3.38
EB Mod	Dialyzed phospholipid (3.70% P) dialyzed against acetone (8 hr)	0.5035	4.47	0.01	95.53	4.01

* EB, dialysis apparatus according to Eberhagen and Betzing (2); EB Mod, the apparatus modified as in Fig. 1.
† Hen egg phospholipids precipitated with acetone.
‡ GK, countercurrent extraction method described by Galanos and Kapoulas (1).

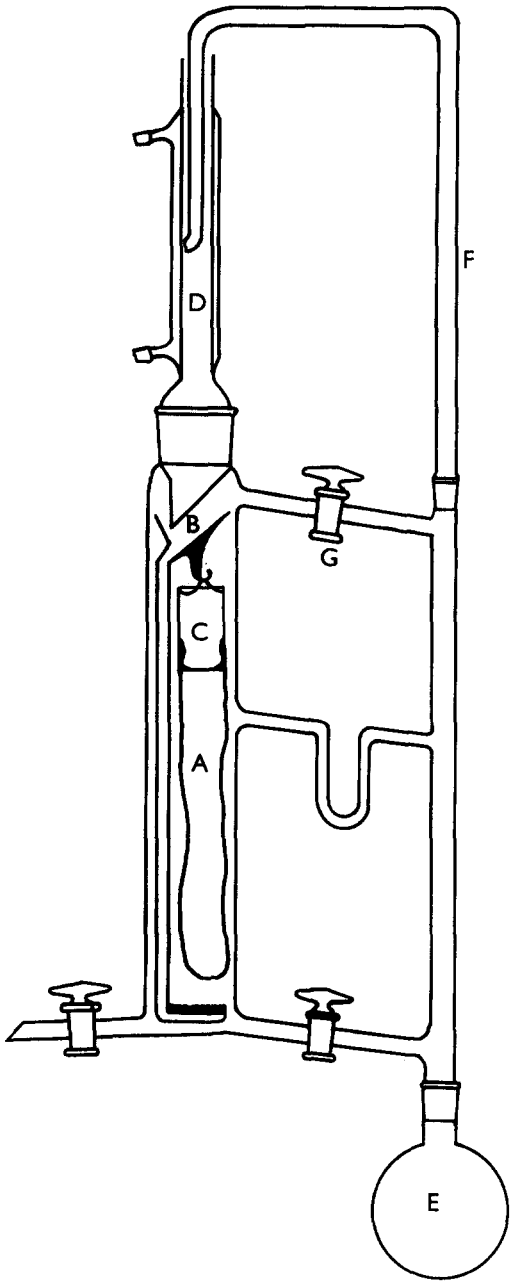

FIG. 1. Diagram of a modified Eberhagen and Betzing dialyzer. The dialysis vessel (*A*), the funnel component (*B*), the device for the suspension of the membrane (*C*), the condenser (*D*), and the evaporating flask (*E*) are as in the original apparatus. A detachable extension of the vapor tube (*F*) and the stopcock (*G*) are the additional elements.

of the dialyzable fraction in light petroleum. In the Galanos and Kapoulas procedure the final result depends, of course, entirely on the partition ratio of the components between the two solvents employed. The different performances of the two methods for the separation of various types of lipids are illustrated in Table 2.

Discussion. Table 1 shows that the countercurrent extraction procedure of Galanos and Kapoulas is almost

TABLE 2 BEHAVIOR OF POLAR LIPIDS AND LIPID MIXTURES SUBJECTED TO COUNTERCURRENT EXTRACTION (GALANOS AND KAPOULAS) OR DIALYSIS

Sample	Weight	Recovery (% of Total Weight)	
		Light Petroleum Phase	Ethanol Phase
	g		
Countercurrent extraction			
Decanoic acid	0.4599	8.07	91.93
Stearic acid	0.5547	59.03	40.97
Phospholipid (3.70% P) + 1-Monolaurin	0.5013 + 0.5124	Nil	100.00
Bleached beef fat (5.3% FFA)	5.0032	97.07	2.93 (98.8% FFA)
Dialysis (EB modified), 8 hr			
		Dialysate	Retentate
Decanoic acid	0.5124	100.00	Nil
Stearic acid	0.5027	67.43	32.57
1-Monoolein	0.2532	100.00	Nil
1-Monolaurin	0.2564	53.56	46.44
Phospholipid (3.70% P) + 1-Monolaurin	0.5105 + 0.5012	11.91	88.09

as efficient in separating phospholipids from triglycerides as the time-consuming dialysis which requires special equipment. The actual extraction, which employs two conventional separatory funnels, may be completed in 30 min, the recovery of triglycerides is rapid, and only the recovery of phospholipids from the ethanol phase requires some time. Dialysis is, however, preferable if it is essential to obtain a nonpolar fraction entirely free from phospholipids. It is also more specific inasmuch as it makes possible the separation of phospholipids from free fatty acids and partial glycerides. However, as already mentioned, solubility in light petroleum determines the rate of the dialysis, which may sometimes be exceedingly slow, particularly in the case of certain monoglycerides. Results obtained after 8 hours' dialysis show that monoglycerides that are appreciably soluble in light petroleum, such as monoolein, pass through the membrane within the above time. The dialysis of monoglycerides such as monolaurin or monostearin may require several days depending on the amounts present. Similarly, the dialysis of stearic acid or tristearin has to be continued for a much longer period than that of low molecular weight or unsaturated fatty acids and glycerides, which detracts from the value of the method.

On the other hand the insolubility of phospholipids in acetone makes possible their purification by dialysis against this solvent beyond the stage attainable by the conventional dialysis against light petroleum. Although the permeability of a rubber membrane in acetone is not

great, certain impurities difficult to solubilize in light petroleum may be removed by acetone dialysis, and phospholipids with an almost theoretical phosphorus content are obtainable (Table 1).

As could be expected, monoglycerides partitioned between light petroleum and 87% aqueous ethanol accumulate in the ethanol phase, whereas the behavior of fatty acids varies according to their molecular weight, and the amount and nature of other components (Table 2). Accordingly, the method of Galanos and Kapoulas cannot be used for the purification of phospholipids in the presence of free fatty acids and monoglycerides.

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