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Recovery of lipids from thin-layer chromatography for radioassay

A popular method currently in use for recovery of lipids from thin-layer chromatography (TLC) plates involves the suspension of the silica gel scraped from the TLC plate in a scintillation solution containing a dioxane–water system[1]. However, the efficiency of this system for ^{14}C is only about 70–75% (refs. 1 and 2). The radioassay of lipids in a more conventional counting system containing 1 ml of added methanol is 96 ± 2% for neutral lipids and 78 ± 2% for phospholipids[3]. We have investigated this problem in the interest of finding a system which will give quantitative recovery of both neutral and phospholipids. Our observations with several different elution and counting systems are the basis of this report.

The radioactive compounds used, with one exception, were obtained from commercial sources (New England Nuclear Corp., Boston, Mass., U.S.A. and Applied Science Labs., Inc., State College, Pa., U.S.A.) and were chromatographically purified before use. [U-^{14}C]Sphingomyelin was prepared by the separation of phospholipids obtained from the liver and spleen of two rats injected with [3-^{14}C]serine and [1-^{14}C]-acetate. The sphingomyelin was separated by the TLC method of SKIPSKI *et al.*[4].

Liquid scintillation solvents which are capable of dissolving relatively large amounts of water have recently become available. We tested one of these (Aquasol, New England Nuclear Corp.) as our primary scintillator and solvent and compared the results with those obtained by elution of the lipids from silica gel and by suspension of the silica gel in the solvent mixture of SNYDER AND SMITH[2].

Neutral lipids are readily recoverable in several systems. Plates were developed in hexane–ether–acetic acid (90:10:1), and the unsaturated lipids visualized by exposure to iodine vapor[5]. In the case of saturated lipids two parallel spots were developed and one was identified by spraying with sulfuric acid. After sublimation of the iodine, the silica gel was either suspended directly in Aquasol or the solvent of SNYDER AND SMITH or the neutral lipid was eluted with three successive 5-ml portions of chloroform–methanol (3:2). The results are summarized in Table I. In all experiments the results were within 2–3% of the average value.

It was ascertained that up to 300 mg of silica gel could be added to Aquasol before the counting efficiency of a solution of [4-^{14}C]cholesterol was impaired.

TABLE I

RECOVERY OF NEUTRAL LIPIDS FROM TLC FOR RADIOASSAY

Eluent: chloroform–methanol (3:2) (3 × 5 ml).

Compound	*% Recovery*[a,b]		
	Aquasol	*Elution*	*Ref. 2*
[1-^{14}C]Cholesteryl stearate	98	97	90
[1-^{14}C]Tripalmitin	98	94	92
[16-^{14}C]Palmitic acid	95	90	84
[4-^{14}C]Cholesterol	100	100	93

[a] Average of three to five experiments.
[b] Similar results have been obtained with ^{3}H-labeled lipids.

The phospholipids presented the real problem since recovery of radioactivity by direct counting has been generally poor (70–80%). We found that phosphatidylcholine (PC) gave the poorest recovery in Aquasol and elution with the solvents used in developing the plates was little better. However, an elution system containing ammonia gave close to quantitative recovery of PC. Thus, we now have methods in our hands which will permit quantitative recovery of phospholipids for radioassay. The results with phospholipids are given in Table II. The plates were developed in

TABLE II

RECOVERY OF PHOSPHOLIPIDS FROM TLC FOR RADIOASSAY

Eluents: (A) chloroform–methanol–acetic acid–water (55:45:4:2); (B) chloroform–methanol–14 *M* ammonia (56:42:2).

Compound	*% Recovery*[a]			
	Aquasol	*Ref. 2*	*Elution A*	*Elution B*
[U-^{14}C]Phosphatidylglycerol	97	66	81	75
[U-^{14}C]Phosphatidylinositol	100	78	72	85
[U-^{14}C]Phosphatidylethanolamine	99	76	60	51
[U-^{14}C]Spingomyelin	98	—	90	85
[U-^{14}C]Phosphatidylcholine	70	83	74	96[b]

[a] Average of four to six experiments.
[b] Cumulative result of four elutions.

chloroform–methanol–7 *M* ammonia (230:90:15), and spots were visualized by exposure to iodine vapor or sulfuric acid spray. All results were within 3–5% of the average values.

The elution of PC from silica gel required at least three extractions with chloroform–methanol–14 *M* ammonia, (56:42:2). The percentages of counts eluted were 52, 38, and 6, respectively, in three successive extractions, so that even two elutions will give about 90% recovery of radioactivity.

Our results indicate that combination of a multipurpose scintillator, such as Aquasol, and elution can give complete radiorecovery of all classes of lipids from TLC plates.

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The Wistar Institute of Anatomy and Biology, Philadelphia, Pa. 19104 (U.S.A.)

DAVID KRITCHEVSKY
SAROJ MALHOTRA

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