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The ultramicro detection of lipids

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» In the separation of small amounts of lipid on chromatographic columns, the major disadvantage in collecting a large number of small fractions is the lack of a convenient, sensitive assay for the eluted material. Most lipids do not exhibit any appreciable absorption of light, nor is there any derivative common to all

lipids that has a high extinction coefficient. A fairly simple and rapid ester determination can be used with aliquots containing approximately 0.3 μEq of ester (1), but the ferric hydroxamates have a low absorbancy (about one-twentieth of that for P in the very useful phosphorus analysis [2]). Furthermore, neither of these functional groups is common to all lipids (e.g., sphingomyelin, cerebrosides, triglycerides).

In the course of plating small aliquots from chromatographic fractions of lipid that had been labeled with radioactive tracers, it was noted that the relative amounts of lipid in the fractions could be estimated before counting by observing the minute traces of lipid on the smooth stainless steel plates. This fact emphasized that one property all lipids have is the considerable mass of the hydrocarbon chains, a property utilized by Dieckert and Reiser (3) in glass-paper chromatography, in which the lipid is charred with H_2SO_4. Since the smoothest plates gave the most sensitive indication of the amount of lipid, a number of large smooth surfaces were tried and a standard photographic ferrotype plate was found to be the most satisfactory.

By drying 0.05- to 0.10-ml aliquots of the fractions on a ferrotype plate, the relative concentrations of lipid eluted from a column can be quickly estimated. In this way, the tubes containing greater amounts of lipid may be located, and, if necessary, the fractions containing lipid may be concentrated for further analyses. Even with solutions of 0.1 mM egg lecithin, a 5-μl aliquot (5×10^{-10} moles, 0.38 μg) produces, when dried, a readily discernible spot best observed in light reflected at an angle of approximately 60° from the surface of the plate. Aliquots (1, 2, 3, 4, 5, 10 μl) of two solutions of egg lysolecithin (2 μmoles/ml and 20 μmoles/ml) were dried on a plate; Figure 1A shows that amounts ranging from 1 to 100 μg can be readily detected. This method was particularly useful in studying the changes of polyvinyl sponges *in vivo*, where a considerable portion of the extracted material, apparently derived from the sponges themselves, contained little ester, phosphorus, or cholesterol and therefore could not be detected by conventional colorimetric analyses (4).

To demonstrate the application of this method, a sample of total rat heart lipids containing 8.8 μmoles of phosphorus was placed on a 5-g silicic acid column in ether, and the lipids were eluted with a gradient of ethanol and methanol as described earlier (1). Aliquots (30 μl) from each tube were dried on the plate (Fig. 1B). After estimating the relative amounts of lipid in each fraction, the fractions were combined as shown in Table 1 and analyzed for phosphorus (2).

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TABLE 1. ESTIMATION OF RELATIVE LIPID CONTENTS IN CHROMATOGRAPHIC FRACTIONS

Tube No.	Estimated Lipid*					Total P
						μmoles
1–3	++++	+++	++			0
4–7	++	++	++	+		0
8–12	++	+	++	++		0
13–16	++	++	++	++		0
17–19	+	+++	+			0.22
20–22	+	+	++			0.12
23–25	++	+++	++			0.15
26–29	++++	++++	+++	+++		3.84
30–33	±†	±	±	±		0.17
34–36	±	±	±			0.10
37–39	±	±	±			0.04
40–43	+	+	±	+		0.28
44–48	++	++	+++	++	++	2.55
49–52	++	++	++	+		0.83
53–55	+	+	±			0.22
56–58	±	±	±			0.09
59–60	+	±				0
					Sum:	8.61

* The three major lipid fractions in the order of elution are neutral lipids, aminophosphatides, and choline phosphatides.

† The symbol ± indicates uncertainty concerning the presence of anything.

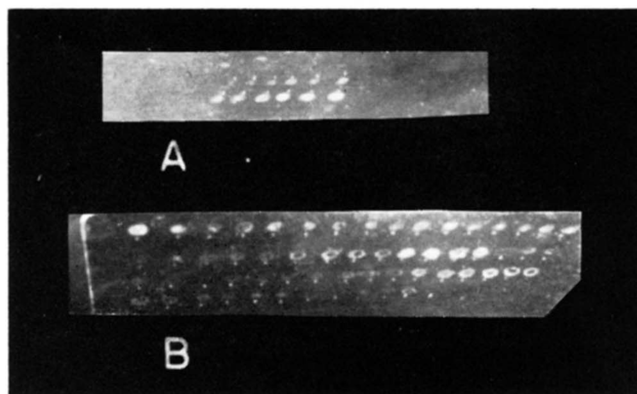

FIG. 1. (A). Appearance of 0.002 to 0.200 μmoles of lysolecithin on a standard ferrotype plate. The material was applied as described in the text.

(B). Appearance of 30-μl aliquots of the chromatographic fractions of total rat heart lipids (60 samples plated in order, to be read left to right, top to bottom).

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