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Note

Screening of plasma lipids by thin-layer chromatography with flame ionization detection on chromarods

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Flame ionization detectors (FIDs) are widely used in gas—liquid chromatography (GLC) and are now applied to other types of chromatography. Column chromatographic elution can be monitored continuously by an FID on a moving wire system [1]. Cotgreave and Lynes [2] fed a vapourised sample from a narrow chromatoplate to the FID. Further approaches in this area were conceived by Padley [3] and Szakasits et al. [4]. The idea of Padley to use a rod as a support instead of a plate was further developed by Okumura and Kadono [5]. The latter developed a sintered thin-layer chromatographic (TLC) rod (chromarod) to which silica gel powder or alumina is fused using fine glass powder as a binding agent.

In Japan several analytical procedures using the TLC—FID combination have been developed. Kawai et al. [6], Nakano et al. [7] and Ueda et al. [8] have analysed serum lipids by this technique. Phospholipids have been analysed by Tokunaga et al. [9], Ishii and Yoshioka [10] analysed phospholipids with respect to the sphingomyelin—lecithin ratio in amniotic fluid. Tanaka et al. [11] described the technique for triglycerid analysis in view of the degree of unsaturation.

This paper describes a method for the separation and identification of lipids by a TLC—FID combination in view of the development of a screening method for plasma lipids.

EXPERIMENTAL

The equipment used is the Iatroscan TH-10 (Iatron Lab). A differential and integral curve is recorded by the Omniscribe TM-recorder (Houston Instruments).

Lipids extracted from plasma [12] or standard mixtures are separated on

silicagel—chromarods in a 20 min run in light petroleum—diethyl ether (85:15, v/v). In a more polar solvent system, chloroform—methanol—water (80:35:5), the plasma phospholipids can be fractionated within 30 min.

The chromarods are activated in a preliminary step by passing them through the FID just prior to use. For nonpolar lipid separation the lipid extract of 0.25 ml plasma is redissolved in 0.1 ml chloroform and 1 μl is applied to the chromarod. For phospholipids 0.5 ml of plasma is redissolved in 0.1 ml chloroform and 1 μl is applied. After the run, scanning is performed under the following conditions: hydrogen pressure, 0.7 kg/cm^2; air flow-rate, 1400 ml/min; scanning speed, 32 sec/rod.

RESULTS AND DISCUSSION

Two non-polar plasma lipid profiles obtained are shown in Fig. 1. Similar separations of plasma neutral lipids by TLC—FID were already described [6—8]. The relative distribution of the peak areas is obtained either by triangulation or by measurement of the appropriate step height of the integral trace. The reproducibility of the method on a single sample is given in Table I. Both results are in good agreement. In some cases of hyperlipidemia, however, the integral curve may show an inflection in the trace, due to incomplete separation of the components, so that it becomes difficult to separate the steps but qualitative screening of the hyperlipidemia by the TLC—FID method is always possible (Fig.2). When the separation is performed in a more polar solvent the plasma sphingomyelin—phosphatidylcholine (S—PC) ratio can be calculated as demonstrated in Fig.3.

In general the most important requirement of this method is the selectivity

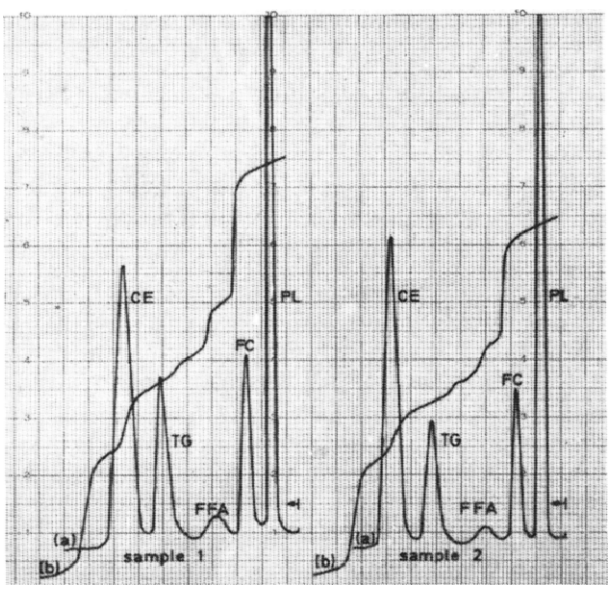

Fig.1. TLC—FID of plasma lipids. CE = Cholesterol esters; TG = triglycerides; FFA = free fatty acids; FC = free cholesterol; PL = phospholipids. (a), Differential curve; (b), integral curve.

TABLE I

REPRODUCIBILITY OF A PLASMA LIPID SCREENING BY TLC—FID ON CHROMARODS, MEASURED BY TRIANGULATION AND INTEGRATION

Compound	Triangulation	Integration
Cholesterol esters	33.3 ± 1.95	35.0 ± 1.02
Triglycerides	11.6 ± 1.08	11.6 ± 2.51
Free fatty acids	2.8 ± 1.24	2.4 ± 0.64
Free cholesterol	14.9 ± 1.22	14.8 ± 0.64
Phospholipids	37.4 ± 1.76	36.2 ± 2.05

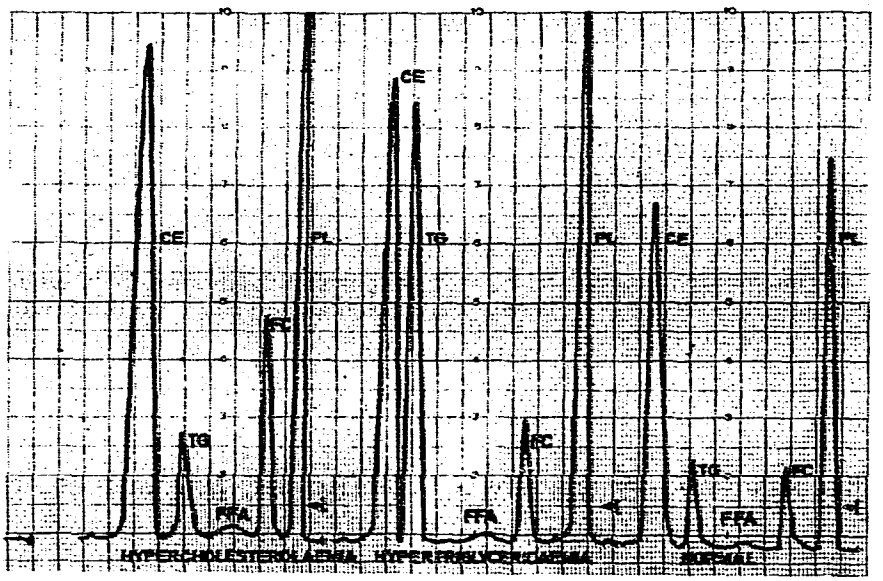

Fig.2. Screening of plasma hyperlipidemia by TLC—FID.

and the purity of the extraction since the FID is not a specific but a general detector. As a first step towards a quantitative evaluation, a mixture of lipid standards has been analysed in a concentration of 4 mg/ml for each lipid. The purity and homogeneity of the standards applied were checked by classical TLC methods. The results obtained by the FID indicate that the relative responses of the individual lipids are different. Especially the triglycerides give a lower response so that a quantitative analysis requires the introduction of correction factors. At any rate the relationship of peak area response to sample concentration remains linear within the examined concentration range of 2—16mg/ml of the lipid standards. A comparison of lipid profiles obtained by the TLC—FID technique and those obtained by classical chemical analysis is underway in order to establish practical correction factors.

In conclusion the TLC—FID technique is a rapid and simple screening procedure applicable to epidemiological studies such as the screening of hyperlipidemia in newborn or to prevention studies of atherosclerosis in adults.

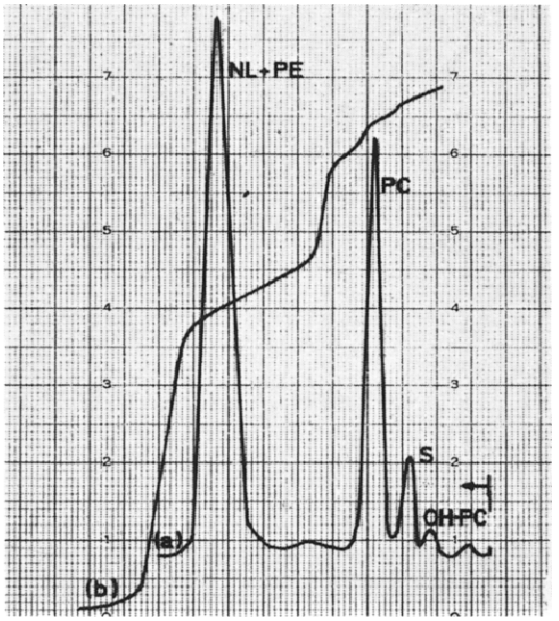

Fig.3. TLC-FID of plasma phospholipids. OH-PC = lysophosphatidylcholine; S = sphingomyelin; PC = phosphatidylcholine; PE = phosphatidylethanolamine; NL = neutral lipids. (a), Differential curve; (b), integral curve.

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