CHROM. 4158

A simple two-stage, one-directional thin-layer chromatography procedure for separating the products of fat digestion

For the study of fat digestion it is desirable to have a TLC procedure which separates from one another the main eight classes of lipids present in gut contents, namely mono-, di- and triglycerides, free fatty acids, cholesterol, cholesterol esters and phospholipids. No one-stage procedure has yet been published for this purpose. With the more polar solvent mixtures, cholesterol esters and triglycerides run together at the solvent front, while with the less polar solvents, one or other of the diglyceride isomers runs either with the cholesterol or with the free fatty acid, while the monoglyceride may not be separated from the phospholipid. For this reason, several two-stage one-direction procedure have been published. Of these, the method of FREEMAN AND WEST[1] gives complete separation of the eight classes of lipid if the extra long plates (34 cm) recommended by the authors are used, necessitating specially made equipment. Using the methods of KELLEY[2] and SKIPSKI *et al.*[3], we were able to separate the eight lipid classes but did not get a sufficient distance between the 1,2-diglycerides and the cholesterol to permit removal of the zones without risk of contamination.

We have developed a method using the same principle as that of FREEMAN AND WEST and SKIPSKI *et al.*, namely development by a relatively polar solvent mixture which is allowed to run two-thirds of the way up the plate, followed by a less polar solvent mixture which is allowed to run to the top. The second solvent mixture does not affect the more polar lipids, but separates the less polar lipids which ran at the solvent front in the first solvent mixture.

Method

Preparation of plates. Plates were spread with 0.25 mm layers of silica gel (Whatman SG 41, and when this was no longer available, Merck Silica Gel G). They were air-dried, dried in an oven at 60° for 45 min and washed by running twice to the top with diethyl ether, after which the plates were again dried and put into a desiccator until required. Immediately before use, individual plates were re-dried at 60° for 60 min.

Application of samples and treatment of plates. Samples were applied as spots or streaks, 2 cm from the bottom edge of the silica gel layer. The first solvent mixture was allowed to run to 6 cm from the top of the plate which was then dried for 15 min at 60°. The second solvent mixture was run to the top of the plate. After drying again at 60°, the plate was sprayed with 5 % aqueous sulphuric acid containing 0.1 % potassium dichromate. The separated bands of lipid were made visible by heating the plate to 250° in a ventilated furnace.

When individual lipid fractions were required for further analysis, 0.4 g of 'Ultraphor WT High Conc.' (Badische Anilin und Sodafabrik) was added to each 100 g of silica gel before spreading the plates. This fluorescent agent enabled the lipid bands to be delineated when viewed under U.V. light.

Solvent mixtures. The components of the mixtures mentioned in Table I were redistilled before use.

TABLE I

	Mixture 1 (*parts by volume*)	*Mixture 2*
Diethyl ether	65	6
Petroleum spirit (b.p. 40–60°)	35	94
Acetic acid	0.5	—

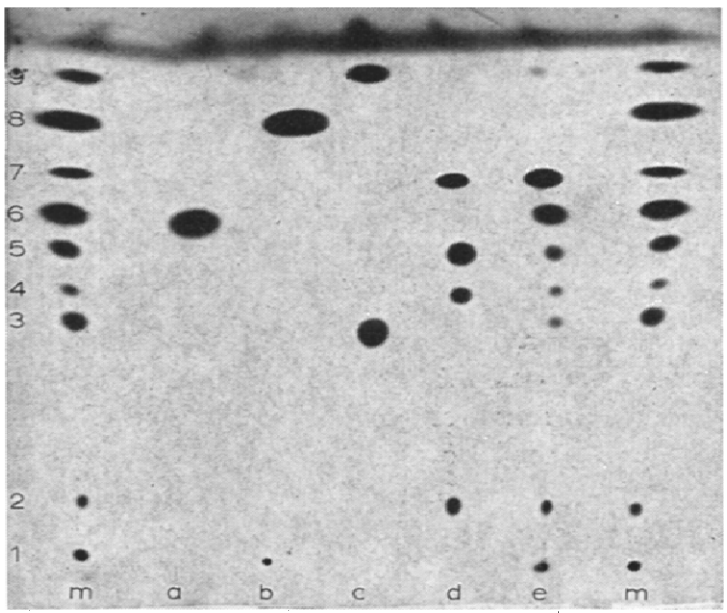

Fig. 1. Thin-layer chromatogram produced using the method described.
a = palmitic acid, b = methyl stearate + lecithin, c = cholesterol oleate + cholesterol, d = crude dipalmitin, e = lipids from piglet gut contents, and m = mixture of a, b, c and d.
1 = phospholipid, 2 = monoglyceride, 3 = cholesterol, 4 = 1,2-diglyceride, 5 = 1,3-diglyceride, 6 = fatty acid, 7 = triglyceride, 8 = methyl ester of fatty acid, and 9 = cholesterol ester.

Results and discussion

The method described requires only three common laboratory solvents and gives clear separation of the main eight lipid classes present in gut contents. The method also separates methyl esters of long chain fatty acids (Fig. 1). The latter could therefore be used as markers or standards in analytical procedures. The sequence of components found on the plate is as follows: solvent front, followed by cholesterol ester, methyl ester, triglyceride, fatty acid, 1,3-diglyceride, 1,2-diglyceride, cholesterol, monoglyceride, with phospholipid remaining at the point of application.

School of Veterinary Science,
University of Bristol, Bristol
(Great Britain)

M. J. MANNERS
D. E. KIDDER
PATRICIA M. PARSONS

1 C. P. FREEMAN AND D. WEST, *J. Lipid Res.*, 7 (1966) 324.
2 T. F. KELLEY, *J. Chromatog.*, 22 (1966) 456.
3 V. P. SKIPSKI, J. J. GOOD, M. BARCLAY AND R. B. REGGIO, *Biochim. Biophys. Acta*, 152 (1968) 10.

Received May 7th, 1969