Use of tetracyclines as a spray in the thin-layer chromatography of lipids

Fluorescent compounds are widely used for visualizing chromatographic fractions as they mostly have the advantage of great sensitivity. Rhodamine G and dichlorofluorescein are frequently used as general lipid stains. We have made use here of the fluorescence of tetracyclines in the presence of lipids and calcium, as a spray for thin-layer chromatograms of lipids of blood sera, erythrocytes and tissues.

Materials and methods

Human brain and retina were homogenised and extracted with 17 volumes of Folch reagent (chloroform–methanol, 2:1) and evaporated to dryness, dissolved in chloroform and applied to the chromatogram. Erythrocytes were extracted according to REED *et al.*[1], and sera were treated similarly[2]. Glass plates 20 × 20 cm were coated with a 250 μ layer of silica gel (Kieselgel G, Merck), the proportion of gel to water being 1:1.5, and activated for 1 h at 110°.

Solvent systems. The double solvent system described by SACHS AND WOLFMAN[3] was used and followed by a third development in hexane[2].

Sprays. The following solutions were used:

(1) 0.1 % aqueous solution of oxytetracycline;

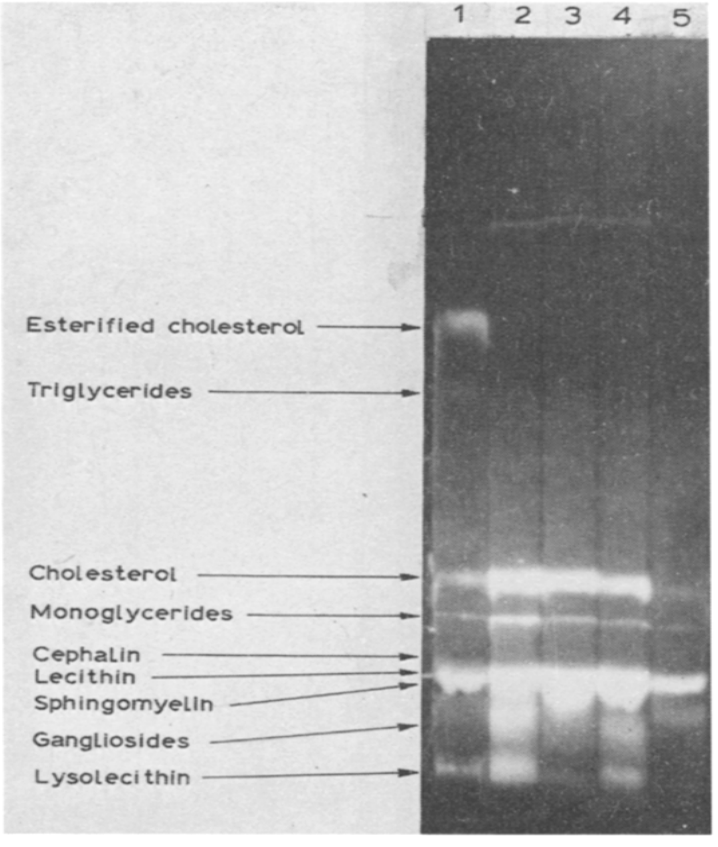

Fig. 1. Fluorescence of lipids on thin-layer chromatograms sprayed with oxytetracycline. 1 = Extract of normal human serum; 2 = extract of erythrocytes (retinitis pigmentosa); 3 = extract of normal human erythrocytes; 4 = extracts of human brain; 5 = extract of human retina. Solvents according to SACHS AND WOLFMAN[3], followed by hexane[2].

(2) 0.1 % oxytetracycline in Tris buffer, *i.e.* 0.36 g Tris [tris(hydroxymethyl)-aminomethane], 0.15 g boric acid, 0.15 g citric acid, 0.10 g sodium chloride, 1000 ml distilled water; pH = 5.5;

(3) 0.1 % chlorotetracycline in Tris buffer (see above).

Results and discussion

The 0.1 % solution of oxytetracycline in Tris buffer (pH = 5.5) proved to be the most satisfactory of the above-mentioned tetracyclines. It was able to detect all the lipid standards applied to thin-layer chromatograms and all the lipid fractions of sera, erythrocytes and tissues (see Fig. 1) which could be detected by other "universal sprays" as *e.g.* bromthymol blue.

No fluorescence occurred, however, when it was applied to paper chromatograms of lipids. The fluorescence of thin-layer chromatograms sprayed with oxytetracycline in Tris buffer proved to be stable in the dark for several weeks. This stabilizing and solubility-promoting effect of Tris buffer has already been proposed by us for other purposes[4]. Other more or less specific sprays could be used after spraying with oxytetracycline, *e.g.* iodine vapour or bromthymol blue for lipids[5], $SbCl_3$ for cholesterol[6], the molybdenum spray of ZINZADZE[7], Dragendorff's reagent for choline phosphatides[5] and the Bial reaction for gangliosides[8].

This feature of tetracyclines giving a strong yellow-greenish fluorescence with lipids in the presence of calcium and in the absence of protein is in agreement with the results of LACZKO *et al.*,[9] who describe the interaction of tetracyclines with lipoproteins. This interaction occurs between the tetracyclines and the lipid moiety of lipoproteins in the presence of calcium. For this reason this interaction does not occur on paper chromatograms devoid of calcium.

The phenomenon of fluorescence of lipid–calcium with the tetracyclines has been used widely in clinical medicine and histochemistry[10–12], but until now it has not been used in chromatography to our knowledge.

Department of Ophthalmology, University of Medicine, Szeged (Hungary) I. L. KAHÁN

1 C. F. REED, S. N. SWISHER, G. V. MARINETTY AND E. G. EDEN, *J. Lab. Clin. Med.*, 56 (1960) 281.
2 I. L. KAHÁN AND A. KAHÁN, *Kisérl. Orvostud.*, in press.
3 B. A. SACHS AND I. WOLFMAN, *Proc. Soc. Exp. Biol. Med.*, 115 (1964) 1138.
4 I. L. KAHÁN AND I. PÁPAI, *Szemészet*, 102 (1965) 157.
5 E. STAHL, *Dünnschichtchromatographie*, Springer, Berlin, 1962, S. 497–515.
6 R. NEHER AND A. WETTSTEIN, *Helv. Chim. Acta*, 34 (1951) 2278.
7 J. C. DITTMER AND R. L. LESTER, *J. Lipid. Res.*, 5 (1964) 126.
8 C. G. HONEGGER, *Helv. Chim. Acta*, 45 (1962) 2020.
9 L. LACZKO, J. KORINEK AND M. BURGER, *Clin. Chim. Acta*, 4 (1959) 800.
10 E. TAPP, K. KOVÁCS AND R. CARROLL, *Stain Technol.*, 40 (1965) 199.
11 E. TAPP, R. CARROLL AND K. KOVÁCS, *Brit. J. Cancer*, 19 (1965) 538.
12 N. STEFENELLI, *Wien. Klin. Wochschr.*, 2 (1964) 33.

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