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Note

Separation of phosphatidyl inositomannosides of Mycobacteria

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Lipids constitute nearly 40% of the dry weight of certain strains of Mycobacteria[1]. Recent advances in lipid analysis such as effective extraction procedures and separation by thin-layer (TLC) and gas–liquid chromatography greatly facilitated the identification and quantitation of different classes of lipids of mycobacteria. The major phospholipids of the species are cardiolipin[2,3], phosphatidylethanolamine[4] and a family of phosphatidyl inositomannosides[5] that contain 1–6 mannose units. Ballou *et al.*[5] characterized the chemical nature of the mannosides after elaborate procedures of purification, deacylation and paper chromatography of the deacylates or separation of the water-soluble esters by chromatography on DEAE-Sephadex columns[6]. Pangborn and McKinney[7] separated the mannosides after extensive solvent fractionations of the phospholipids and conversion into sodium salts followed by TLC. In view of the importance of the mannosides as immunogens[8], a rapid method for their separation from total lipids and the isolation of the pure mannosides would be of great value in studying the antigen–antibody interactions in greater detail. This paper describes a simple one-dimensional TLC method for the separation of different classes of phosphatidyl inositomannosides from a total lipid extract of the bacilli.

EXPERIMENTAL

The total lipids were extracted from large amounts of *Mycobacterium tuberculosis* $H_{37}R_v$ and M.607, originally obtained from National Collection of Type Cultures (London, Great Britain) grown on liquid media and purified as described elsewhere[9]. The TLC of the total lipids was carried out on silica gel H plates impregnated with either ammonium sulphate or anhydrous sodium carbonate. A 1.806-g amount of the salt was dissolved in 1 l of distilled water, then 30 g of silica gel H were mixed thoroughly with 72 ml of the salt solution and the mixture was spread on to 20 × 20 cm glass plates to a thickness of 0.3–0.5 mm. The plates were pre-run with the solvent used for development. The plates were dried and activated at 110° for 1 h and an aliquot of total lipid in chloroform containing 10–15 μg of lipid-P was spotted and developed for 2 h with chloroform–methanol–water (10:5:1). The solvent reached the top of the plate after 90 min and was allowed to over-run for a further 30 min.

The lipids were identified on the plates by spraying with distilled water or a 1% solution of iodine in chloroform. Phosphorus-containing lipids were located on the

plate by using molybdenum spray reagent[10]. Carbohydrate-containing lipids were detected by spraying with α-naphthol–sulphuric acid reagent[11] and heating the plates at 100° for 3–6 min. For quantitative isolation of mannosides, the spots of the lipids were located on the plates by spraying with water. The spots from ten plates were scraped into tubes containing chloroform–methanol (1:1), mixed thoroughly and the gel was allowed to settle. The supernatant was passed throug ha column plugged with glass-wool. The process was repeated after suspension of the gel in chloroform–methanol (3:2, 3:1 and 4:1). The extracts were evaporated to dryness at 50° under reduced pressure and the residue was dissolved in an aliquot of chloroform. The isolated lipids were analyzed qualitatively for glycerol, mannose and inositol by acid hydrolysis and paper chromatography[12].

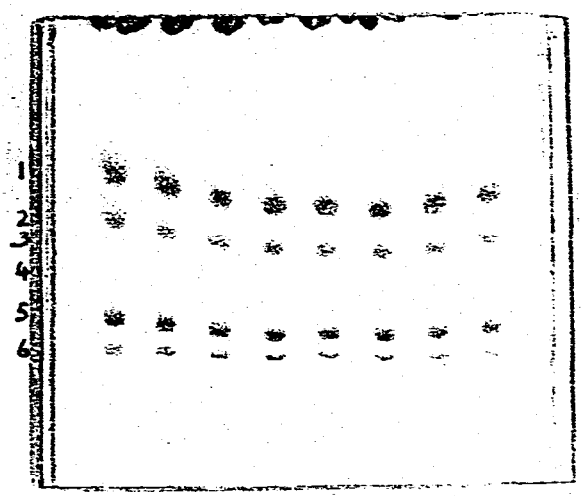

Fig. 1. Separation of phosphatidyl inositomannosides from total lipids on a silica gel H–ammonium sulphate plate. Spots developed with α-naphthol-sulphuric acid spray.

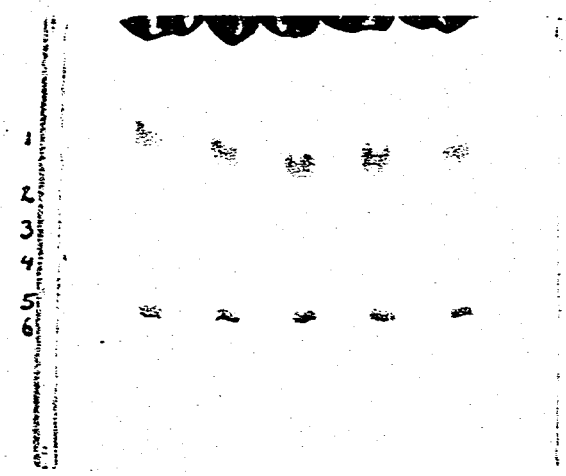

Fig. 2. Separation as in Fig. 1 on a silica gel H–sodium carbonate plate.

RESULTS

Typical separations of the lipids from a total lipid extract isolated from either $H_{37}R_v$ or M.607 on TLC plates impregnated with either ammonium sulphate or sodium carbonate are shown in Figs. 1 and 2, respectively. It can be seen that the separations of the spots on the silica gel H plates impregnated with either of the salts were satisfactory. The six spots in each lane, separated from a total lipid extract, were phosphorus- and carbohydrate-positive. Owing to the over-run of the solvent, cardiolipin and PE, together with neutral lipid, reached the solvent front. The six lipids were eluted separately and, on qualitative analysis, were found to contain glycerol, mannose and inositol in addition to phosphorus.

Further characterization of the lipids with respect to the quantitation of the constituents and their immunological properties will be reported elsewhere.

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