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Note

Quantitation of neutral glycolipids by thin-layer chromatography on pre-coated plates

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Thin-layer chromatography (TLC) is an excellent technique for the qualitative analysis of glycolipids in animal tissues. Van der Eijnden[1] and McCluer and Agranoff[2] increased the sensitivity of the chromatography of gangliosides by using 0.25-mm thick pre-coated silica gel plates instead of the usual plates, which are much thicker. We found that the use of the same pre-coated plates permits an increase in sensitivity in the TLC of neutral glycolipids.

The colorimetric quantitation of neutral glycolipids[3–10] or gangliosides[11–14] after TLC usually requires the elution of the lipids from the plate, or at least the spots have to be scraped off. Sandhoff *et al.*[15], Nutter and Privett[16] and Friedrich and Hauser[17] have shown that the quantitative distribution of gangliosides or neutral glycolipids could be obtained after charring by direct densitometry of the plates. Simultaneously, Smid and Reinisova[18] and Bosch *et al*[19] quantified gangliosides by scanning after detection with a specific orcinol–hydrochloric acid spray. We found that the quantitative analysis of neutral glycolipids could be carried out by densitometry after detection with an orcinol–sulphuric acid spray.

The principal neutral glycolipids in mammalian tissues are sphingoglycolipids derived from ceramide. Galactosyl- and sulphogalactosylceramides are mainly present in nerve tissue, while glucosyl-, lactosyl- and polyhexosylceramides are present in other tissues, such as the kidney and spleen. The separation of galactosyl- and sulpho-galactosylceramides can easily be achieved[3,4,6,8,20–28], and also the separation of glucosyl-, lactosyl- and polyhexosylceramides[29]. However, the separation of mono-glucosyl- and monogalactosylceramides is much more difficult; a good separation can be obtained only if the chromatography is carried out in the presence of sodium tetraborate[28,30,31]. We found that the impregnation of pre-coated plates with tetra-borate is possible and gives both increased sensitivity and fine resolution in the separation of these two types of compounds.

EXPERIMENTAL

Standard lipids

Galactosyl- and sulphogalactosylceramides from human brain and glucosyl-, lactosyl- and polyhexosylceramides from beef spleen were extracted and purified in the Centre de Neurochimie (Strasbourg, France).

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Preparation of glycolipid samples

　　After their extraction from lyophilized tissue by the method of Folch *et al.*[32], the lipids were chromatographed on a silicic acid column according to Vance and Sweeley[33]. The glycolipid fraction was submitted to mild alkaline hydrolysis in order to remove the phospholipid contaminants, before being applied to the plates.

Impregnation of the plates with tetraborate

　　Pre-coated silica gel plates (DC Fertigplatten Kieselgel 60), purchased from Merck (Darmstadt, G.F.R.), were used without additional activation. Before chromatography, the plate was impregnated with sodium tetraborate by immersing the bottom of the plate in a saturated solution of anhydrous sodium tetraborate (Merck) in dry methanol and allowing this solution to migrate up the plate. The migration was stopped when the solvent was 5 cm from the top of the plate, and the plate was then allowed to dry. This impregnation must be carried out at a temperature higher than 30°, otherwise a double front, which interferes in the resolution of the chromatographed lipids, is obtained.

Chromatographic techniques

　　The same procedure was used for normal and impregnated plates. The lipid samples were applied to the part of the plate that was not impregnated with tetraborate, and a reverse migration was carried out. A two-step chromatographic process was then effected. The samples were allowed to migrate first in diethyl ether, in order to move to the top of the plate the fatty acids obtained from the alkaline hydrolysis. After drying, the chromatography of the glycolipids was then carried out in the same direction by using as the mobile phase chloroform–methanol–water (100:42:6)[29]. After drying, the plates were sprayed with the orcinol–sulphuric acid reagent of Svennerholm[34]. Comparison of the migration of the unknown lipids with that of the standards permitted their identification. A quantitative densitometric study was then
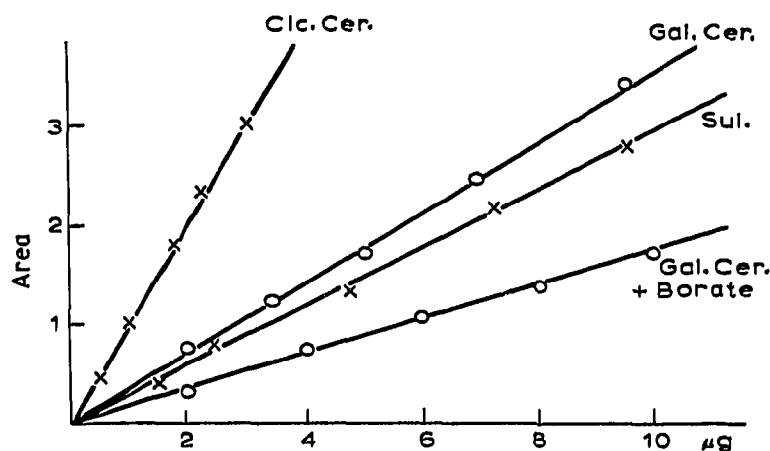

Fig. 1. Calibration graph for monoglucosylceramides (Clc.Cer.), cerebrosides (Gal.Cer.) and sulphatides (Sul.) on normal plates and cerebrosides on impregnated plates (Gal.Cer. + Borate). Areas are expressed in arbitrary units.

possible if several concentrations of standards had been applied on the same plate. The scanning was carried out with a Vernon densitometer.

RESULTS AND DISCUSSION

TLC on pre-coated silica gel plates gives a good separation of glycolipids, as in classical techniques. The main advantage of the use of pre-coated silica gel plates is the increase in sensitivity. While the classical techniques require 20–30 μg of lipid hexose for a quantitative study, in our modification 10–20 times less is needed. The densitometric study of the plates after spraying with orcinol–sulphuric acid reagent shows good proportionality in the range 2–15 μg of lipid. Unfortunately, the slope of each graph varies from one experiment to another. Therefore, a new calibration graph must be made for each measure (Fig. 1). The difference between the results obtained on a sample of glycolipids isolated from rat brain, by densitometry on a pre-coated plate, and by the hexose assay after separation on the usual plate according to Neskovic and co-workers[10,19], is less than 10%.

The impregnation of the plates with a saturated solution of anhydrous sodium tetraborate in methanol gives a good separation of galactosyl- and glucosylceramides (Fig. 2). If the concentration of the tetraborate decreases, the quality of the separation becomes worse. Sulphogalactosyl- and the lactosylceramides are not perfectly separated in this system, but are best separated on non-impregnated plates (Fig. 3). If the purpose is to separate the principal neutral glycolipids of mammalian tissues, two-dimensional chromatography can be used. The lipids are applied on a 20 × 20 cm pre-coated plate, 2 cm from a corner. In the first step, the mobile phase used is chloroform–methanol–water (100:42:6). After drying, the plate is impregnated with sodium
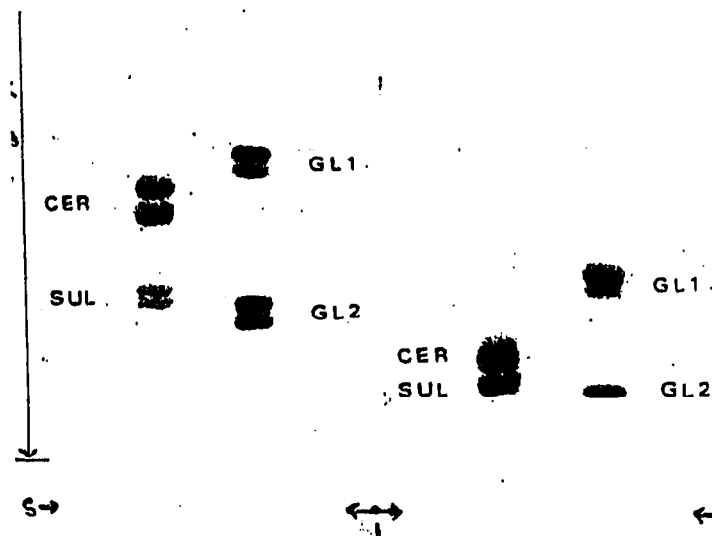

Fig. 2. Migration of glycolipids on borate-impregnated plates (left, 5% tetraborate in methanol; right, saturated tetraborate). Solvent running time: up to the top of the plate. Cer = cerebrosides; Sul = sulphatides; GL1 = monoglycosylceramides; GL2 = lactosylceramides. The arrows show the impregnated areas.
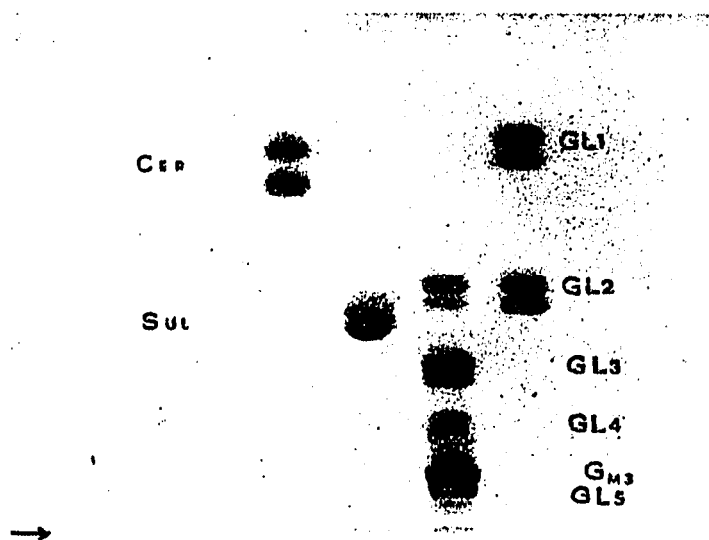
Fig. 3. Migration of glycolipids on normal plates. Solvent running time: up to the top of the plates. Abbreviations as in Fig. 2, plus: GL3, GL4, GL5 = tri-, tetra- and pentahexosylceramides; G_{M3} = monosialolactosylceramides (hematoside).
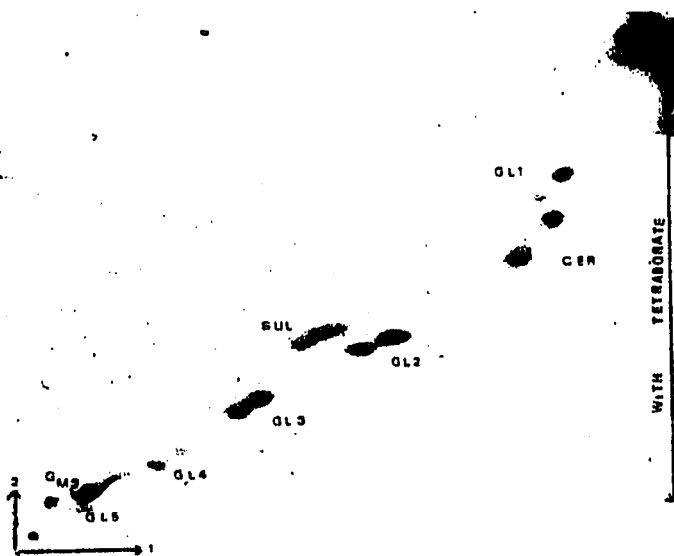

Fig. 4. Migration of glycolipids in two-dimensional chromatography. The second migration is carried out after impregnation of the plate with saturated tetraborate. Solvent running time: first migration, up to the front of the plate + ½ h; second migration, up to the top of the plate + 1 h. Abbreviations as in Figs. 2 and 3.

tetraborate as described above to a distance of 5 cm from the edge where lipids have run. Chromatography is then carried out in the second dimension with chloroform–methanol–water (100:42:6). By this technique, a good separation of all of the principal glycolipids can be obtained (Fig. 4).

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