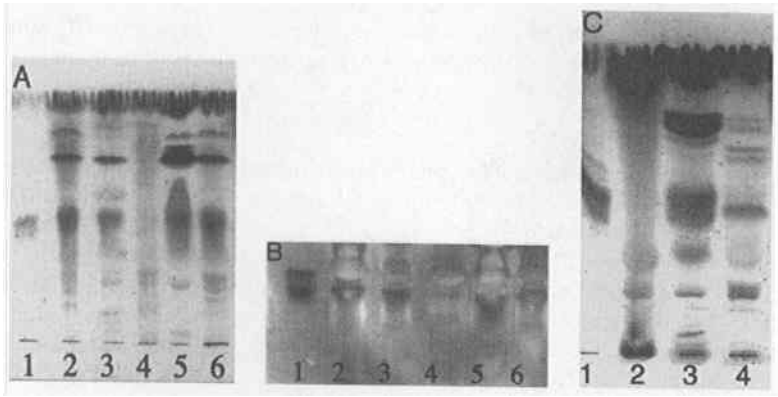

Figure 5 TLC-based analyses of lipid extracts of murine tissue to identify tissues that contain high levels of globotriaosyl ceramide (Gb3), a glycolipid receptor for Shiga toxin (verotoxin). (A) Thin-layer chromatogram of total lipid extracts from mouse (3-week-old) tissues as visualized with an orcinol spray reagent specific for carbohydrate residues. Chromatograms were developed in chloroform–ethanol–water (65:25:4). Lanes contained the following glycolipid standards and tissue extracts: *1*, Gb3 standard; *2*, heart; *3*, testes; *4*, lung; *5*, brain; *6*, kidney. (B) Thin-layer chromatogram overlay assay for identification of mouse tissues containing the Shiga toxin/verotoxin receptor glycolipid Gb3. Thin-layer chromatograms were developed as in (A) and allowed to air dry. Chromatograms were blocked with gelatin and incubated sequentially with Shiga toxin (verotoxin-1), antitoxin monoclonal antibody, anti-mouse antibody conjugated to horseradish peroxidase, and chloronaphthol in TBS solution. Tissues containing Gb3 were identified by lanes containing purple bands comigrating with the Gb3 standard. (C) Thin-layer chromatogram of saponified lipid extracts of some mouse tissues developed and visualized as in (A): *1*, Gb3 standard; *2*, testes; *3* brain; *4*, kidney. Note that orcinol staining of glycolipid bands is much clearer [compare lanes *3* and *4* with lanes *5* and *6* of (A)] when phospholipids have been removed following saponification with sodium hydroxide.

result in artifacts such as false-positive band development in overlays (107). In a similar procedure involving detection of glycosphingolipids blotted from a high-performance thin-layer chromatogram to a polyvinylidene difluoride membrane, the detection limit was confirmed to be more sensitive than detection on an HPTLC plate by typical chemical visualization and immunological staining procedures (85d).

E. TLC Coupled with Other Chromatographic and Spectrometric Analysis

A number of investigations involving carbohydrate or glycoconjugate analysis by gas chromatography or mass spectrometry have used TLC for the initial purification or separation of samples. Initially, such purification or separation from carbohydrate-containing mixtures took the form of preparative TLC for purification of carbohydrate or glycolipid bands. Some recently developed mass spectrometric techniques, however, have the capacity to analyze samples separated by TLC directly from the thin-layer chromatograms. Some examples of such TLC use are given below.

Urashima et al. (108,109) used a combination of gel filtration chromatography, preparative TLC, and ^{1}H-NMR to purify and determine the composition of oligosaccharides from milk. DeJong et al. (110) used TLC with silica gel 60 plates to detect oligosaccharides in urine as a measure of the severity of Gaucher's disease and preparative TLC prior to analysis of the sugar composition of urine by gas chromatography. Kimber et al. (77a) used HPTLC to compare the glycosphingolipid composition of untreated and retinoic acid–treated embryonic stem cells and preparative HPTLC to purify glycolipids prior to fast atom bombardment mass spectrometry. Obermeier et al. (111) analyzed oligosaccharides in milk and urine by a combination of gel filtration chromatography, HPTLC, isotope ratio mass spectrometry (IRMS), and high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Hanisch et al. (2)