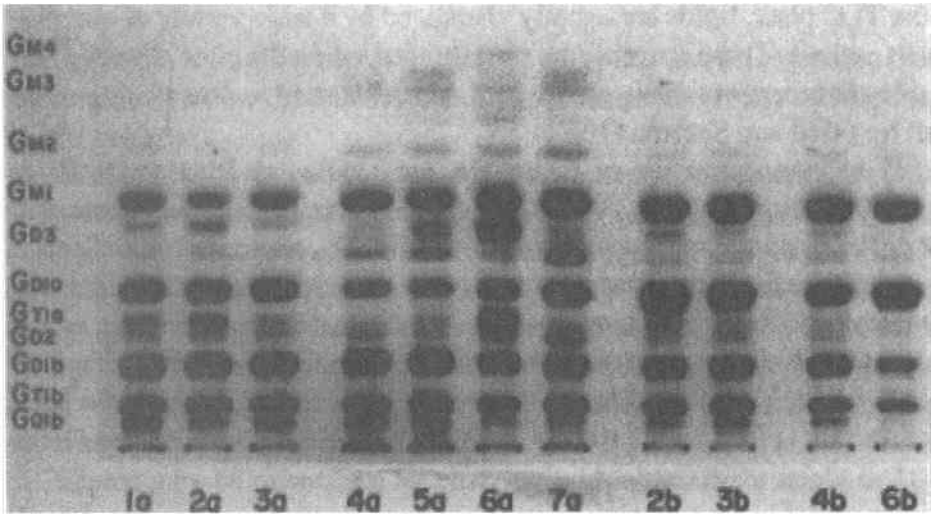

Figure 4 Separation of brain gangliosides on an HPTLC plate developed one-dimensionally with the mobile phase chloroform–methanol–0.22% aqueous $CaCl_2$ (55:45:10). The gangliosides were detected with the resorcinol–HCl reagent. Lanes 1a–3a show brain gangliosides from control cases; lanes 6a, 7a show brain ganglioside patterns from patients with known gangliosidosis diseases. Other lanes are not relevant to this discussion. (Reproduced from Ref. 63 with permission of Elsevier Press, Inc.)

polar lipids such as hydrocarbons, steryl esters, methyl esters, and mixed glycerides that migrate close to each other in one-dimensional TLC. Thompson (97) used a 2-D system to separate the neutral lipids of the digestive gland–gonad (DGG) complex of the medically important snail *Biomphalaria glabrata*. The first development was in hexane–diethyl ether (80:20); after the plate was dried, it was turned 90° and developed in the second direction in hexane–diethyl ether–methanol (70:20:10). Figure 5 shows the results of this separation.

2. Phospholipids

Two-dimensional systems are often used to separate complex phospholipid mixtures in plant and animal tissues. See reviews in Mangold (98) and Rouser et al. (99) for details. The first development is typically in chloroform–methanol–water (65:25:4), and development in the second direction is often in either *n*-butanol–acetic acid–water (60:20:20) or chloroform–acetone–methanol–acetic acid–water (5:2.1:1:0.5). Although 2-D procedures may increase the resolution of some spots, they often result in large spots with tails. Figure 6 shows a 2-D separation of phospholipids from snail tissue, and Fig. 7 shows a 2-D separation of serum lipids. Table 7 lists frequently used 2-D solvent systems for complex lipid mixtures.

3. Glycolipids

Glycolipids are often difficult to separate completely by one-dimensional TLC because of their complex array of oligosaccharides. Therefore, a variety of 2-D TLC procedures have been devised to separate individual glycolipids and phospholipids on the same plate. Glycolipids of plant and bacterial origin are usually separated on silica gel G using the 2-D system first described by Nichols (100).

The solvent system in the first direction is chloroform–methanol–7 N ammonium hydroxide (65:30:4), with chloroform–methanol–acetic acid–water (170:25:25:6) in the second direction. Excellent separation is obtained for neutral lipids, monogalactosyldiacylglycerols, cardiolipin, phosphatidic acid, sterol glycosides, ceramide monohexosides, phosphatidylethanolamine; phosphatidylglycerol, digalactosyldiacylglycerols, sulfoquinovosyldiacylglycerols, phosphatidylcholine, and phosphatidylinositol. An additional 2-D system used for the separation of glycolipids