Thin-layer chromatography of phospholipids using silica gel without calcium sulfate binder

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» In a previous communication (1), the authors reported that the positions of phosphatidyl serine on chromatoplates prepared from Silica Gel G containing calcium sulfate as a binder depend upon the amount of this phospholipid applied. This "load effect" was eliminated empirically by using "basic" Silica Gel G plates. The cause of this "load effect" has been found to be the calcium sulfate binder present in the silica gel. An improved method for the separation of phospholipids on silica gel chromatoplates free from calcium sulfate is described. Such plates exhibit no "load effect" and also give better resolution of cephalins.

The source of most of the reference compounds and the methods used for preparation of the liver phospholipids was identical to those already described (1). The sodium salt of monophosphatidyl inositol (isolated from wheat sprouts) was a generous gift from Dr. Faure, Pasteur Institute, Paris. The apparatus for preparation of chromatoplates and the purification of solvents were described before (1).

Silica gel without calcium sulfate added as a binder was obtained from Camag, Muttenz, Switzerland.[1] Three types of plates were prepared from this silica gel: (*a*) "neutral" plates, 20 g silica gel slurried with 45 ml distilled water; (*b*) "neutral" plates prepared from the same silica gel but to which was added 5 or 15% calcium sulfate (w/w), the usual commercial range of this binder (2); and (*c*) "basic" plates, 20 g silica gel made into a slurry in 45 ml of 0.001 M Na_2CO_3. The technique for preparation of chromatoplates was essentially as described before (1) except that air drying of the silica gel plates was increased to ½ hr. All chromatoplates were activated at 110° for 30 min just prior to application of samples.

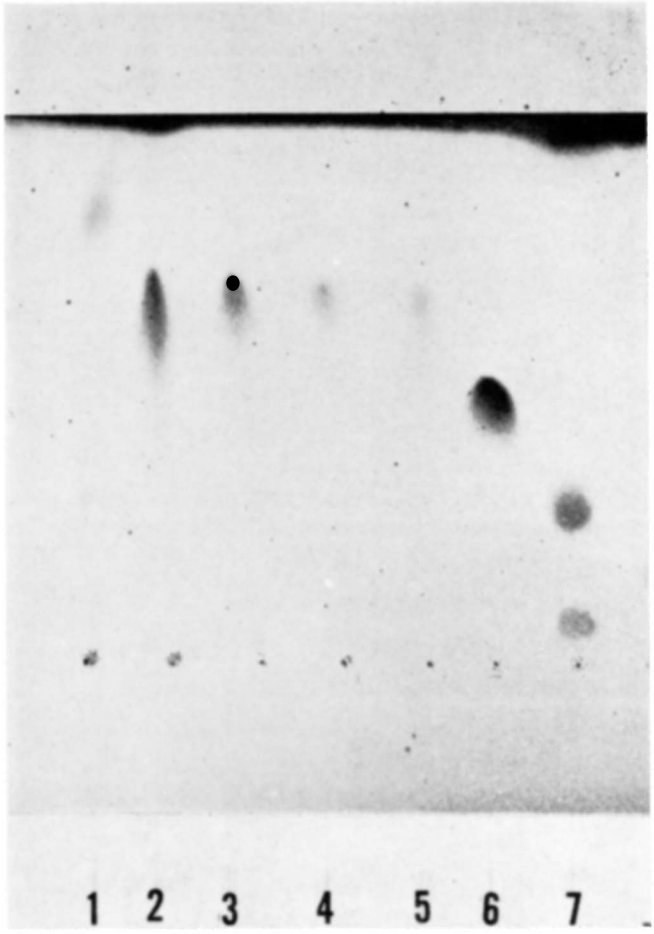

FIG. 1. Thin-layer chromatogram of phosphatidyl serine on silica gel without calcium sulfate added, "neutral plate". Developing solvent: chloroform–methanol–glacial acetic acid–water 50:25:8:4 (v/v). Detection method: ammonium molybdate–perchloric acid spray. Compounds: (1) phosphatidyl ethanolamine, 25 μg (upper spot), and lysophosphatidyl ethanolamine, 10 μg (lower spot); (2) phosphatidyl serine, 50 μg; (3) phosphatidyl serine, 25 μg; (4) phosphatidyl serine, 13 μg; (5) phosphatidyl serine, 6 μg; (6) phosphatidyl inositol, 20 μg; (7) lecithin, 20 μg (upper spot), and lysolecithin, 20 μg (lower spot).

The developing solvents were mixtures of chloroform–methanol–glacial acetic acid–water, in the volume proportions cited in the figures. Spots were detected by iodine vapor (3), ninhydrin, ammonium molybdate–perchloric acid sprays (1, 4), and 40% sulfuric acid spray (2). The first two methods of detection were found to be most useful in the routine work. However, when a permanent record of a chromatoplate was desired, either ammonium molybdate–perchloric acid spray or sulfuric acid spray was used.

Figure 1 shows a thin-layer chromatogram of different

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[1] The authors wish to thank Dr. H. K. Mangold, Hormel Institute, for calling our attention to this silica gel.

amounts (6–50 μg) of phosphatidyl serine on silica gel "neutral plate" containing no calcium sulfate. The positions of all phosphatidyl serine spots are identical; there is no "load effect." However, thin-layer chromatograms performed on the same silica gel, but with addition of calcium sulfate, showed a definite "load effect" for phosphatidyl serine. Similar results were obtained on commercial Silica Gel G containing calcium sulfate as a binder.

The use of silica gel without calcium sulfate for thin-layer chromatography gives better separation of some of the phospholipids, especially in the cephalin group. "Basic" plates also facilitate separations. Chromatography of reference phospholipids, their mixtures, and phospholipids isolated from rat liver on a "basic" plate is shown in Fig. 2. This chromatogram demonstrates the separation of phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, lecithin, sphingomyelin, and lysolecithin. To our knowledge, this is the first one-dimensional thin-layer chromatography system reported that permits separation of phosphatidyl inositol along with other phospholipids.

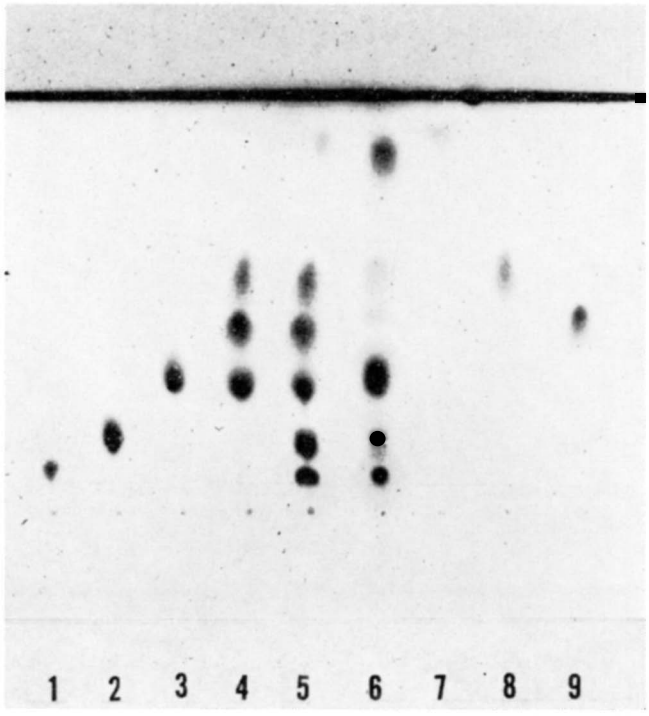

FIG. 2. Thin-layer chromatogram of different reference phospholipids and phospholipids extracted from rat liver. "Basic plate" prepared from silica gel without calcium sulfate. Developing solvent: chloroform–methanol–glacial acetic acid–water 50:25:7:3 (v/v). Detection method: 40% sulfuric acid spray. Compounds: (1) lysolecithin, 10 μg; (2) sphingomyelin, 15 μg; (3) lecithin, 15 μg; (4) mixture of (3), (8), and (9); (5) mixture of (1) to (3) and (7) to (9); (6) phospholipids extracted from rat liver, 120 μg; spots identified as (from bottom to the top): lysolecithin, sphingomyelin, lecithin, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, and polyglycerophosphatides fused with solvent front band; (7) phosphatidyl ethanolamine (synthetic), 5 μg; (8) phosphatidyl serine 12 μg; (9) phosphatidyl inositol, 10 μg.

The phospholipid fraction from liver is shown to contain the following components: lysolecithin, sphingomyelin, lecithin, phosphatidyl inositol, phosphatidyl serine, and phosphatidyl ethanolamine. Phosphatidic acid and polyglycerolphosphatides move with the solvent front.

Similar results were obtained with the reference compounds and with liver phospholipids when chromatography was performed on "neutral" plates free from calcium sulfate. However, phosphatidyl inositol and phosphatidyl serine were usually more clearly separated on "basic" plates.

When lysophosphatidyl ethanolamine was chromatographed on either "neutral" or "basic" plates in the solvent system cited in Fig. 2, its R_f was close to that of lecithin. A slight change in the developing solvent, chloroform–methanol–glacial acetic acid–water 50:25:6:2 (v/v), gave good separation of these compounds. However, phosphatidyl serine and phosphatidyl inositol have a tendency to fuse in this system.

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