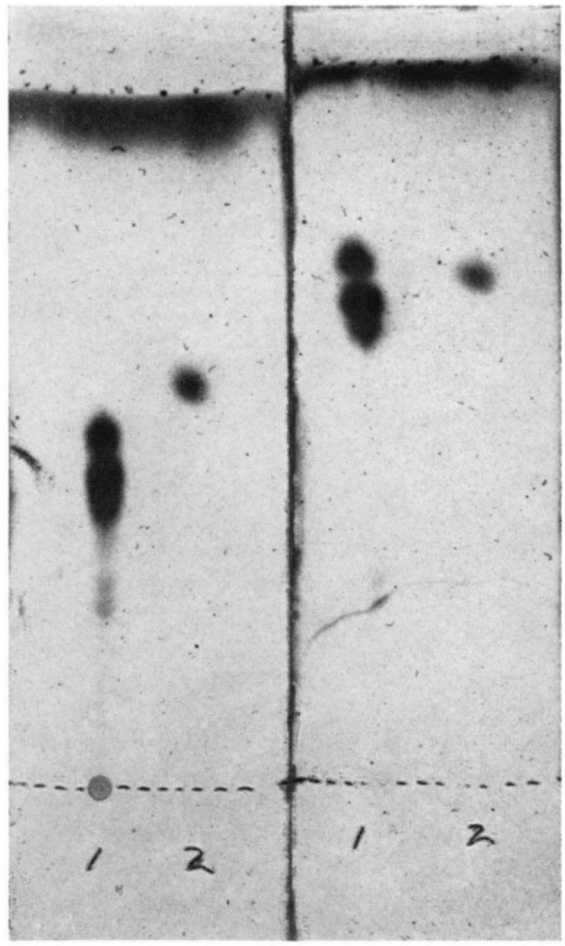

FIG. 1. Thin-layer plates prepared in water (right) and sodium borate (left). *1*, galactocerebroside; *2*, glucocerebroside. Solvent system 1: chloroform–methanol–water 65:25:4 (10). Development time: 45 min.

Separation of gluco- and galactocerebrosides by means of borate thin-layer chromatography

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SUMMARY Gluco- and galactocerebrosides can be separated by thin-layer chromatography on Silica Gel G prepared with sodium borate solution instead of water. The most successful developing system was chloroform–methanol–water–15 M NH_4OH 280:70:6:1.

KEY WORDS thin-layer chromatography · borate-impregnated silica gel · gluco- and galactocerebrosides · ceramide monosaccharides

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CEREBROSIDES containing glucose or galactose have been isolated from a variety of tissues (1), yet thin-layer chromatographic techniques relying solely on adsorption and partition differences between these similar classes of ceramide monosaccharides are at best only moderately successful in distinguishing between them. The ability of sugars to form borate complexes offers a technique that utilizes the major distinguishing factor between these two species, the configuration about carbon atom 4 of the hexose. By taking advantage of the differences in the ability of the *cis*-glycols in glucose and galactose to form borate complexes, excellent separation of the glucose- and galactose-containing cerebrosides has now been achieved on thin-layer plates prepared in a borate medium.[1]

Methods. Glass plates, 20 cm long, were coated with a 250 μ layer of Silica Gel G (Brinkmann Instruments Inc., Westbury, Long Island, N.Y.) prepared as a

[1] After this work had been completed, similar findings were reported by Young and Kanfer (2).

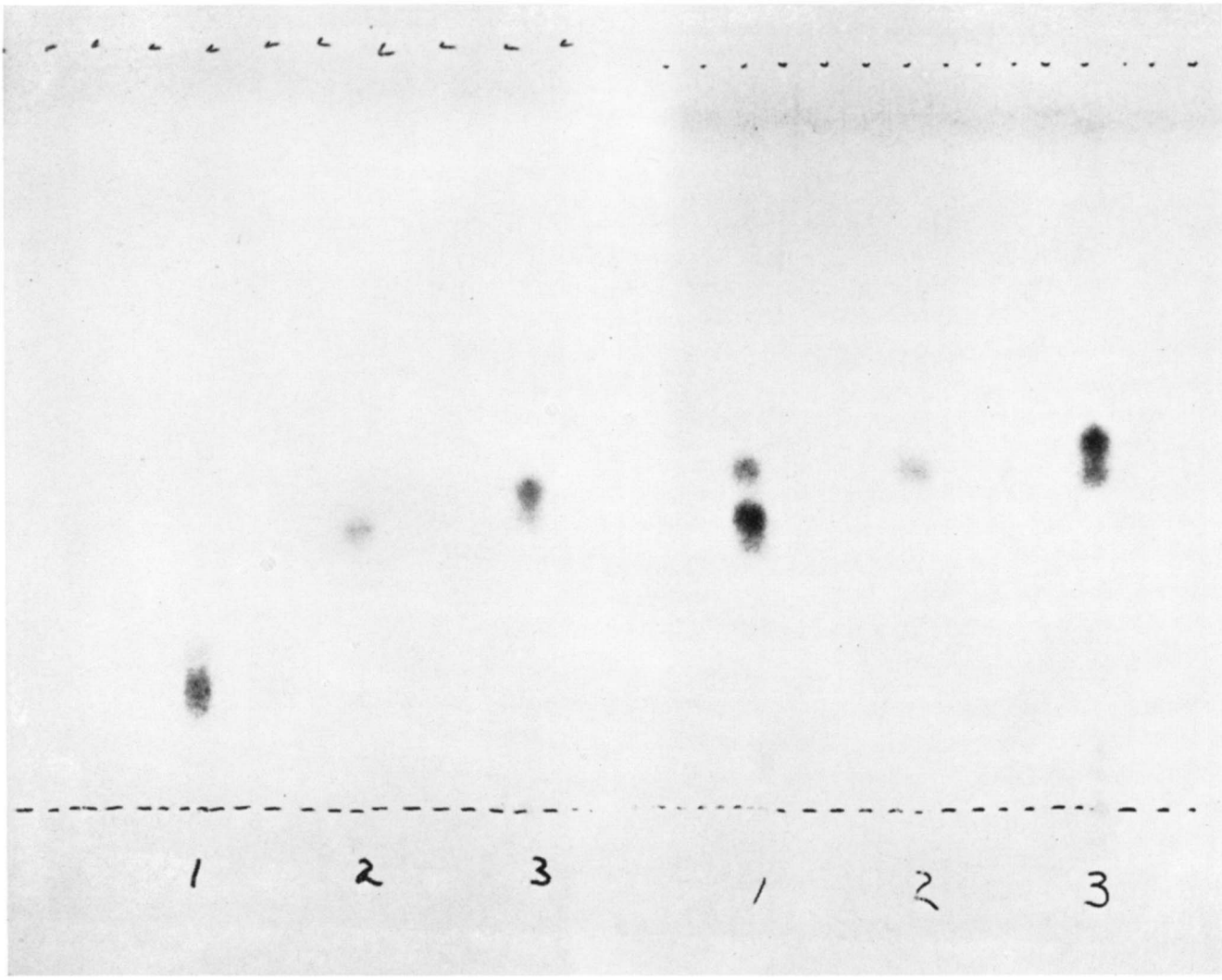

FIG. 2. Thin-layer plates prepared in water (right) and sodium borate (left). *1*, galactocerebroside (beef brain); *2*, glucocerebroside (from degradation of gangliosides); *3*, cerebroside from Gaucher spleen. Solvent system 2: chloroform–methanol–water 24:7:1 (11). Development time: 45 min.

slurry of 30 g/65 ml of either water or 1% $Na_2B_4O_7 \cdot 10H_2O$(pH 9.20). The plates, prepared using a Desaga/Brinkmann adjustable applicator, Model S-11, were allowed to dry overnight at 30°C, activated by heating for 1 hr at 125°C, and stored in a desiccator until use. After chromatography of about 5 μg of glycolipid, the plates were dried and the spots were made visible by charring according to the procedure of Privett and Blank (3). In addition, a positive reaction for carbohydrate-containing materials was obtained with the α-naphthol spray reagent (4).

Materials. Standard cerebrosides were obtained from Drs. B. Kaufman, S. Basu, and S. Roseman of Johns Hopkins University (galactocerebrosides prepared from beef brain[2] and glucocerebrosides prepared by the selective hydrolysis of gangliosides). A preparation containing glucocerebrosides isolated from Gaucher spleen was obtained from Dr. N. S. Radin of the University of Michigan. Ceramide lactoside (cytolipin H) was received from Dr. M. M. Rapport and digalactosyl cerebroside, from Dr. C. C. Sweeley. The author gratefully acknowledges these generous gifts. Sphingosine was prepared according to the method of Tipton (5).

Analytical Procedures. All chromatographic and analytical studies to be described were performed on the glucocerebroside prepared by the selective degradation of gangliosides and the galactocerebroside from beef brain, unless indicated otherwise. The purity of these materials was established by measuring their sugar and base components after methanolysis of the cerebrosides with a solution of 5% HCl in methanol (6). Sphingosine was analyzed by the Sweeley modification (6) of the

[2] This preparation also contained about 5% cerebroside sulfate.

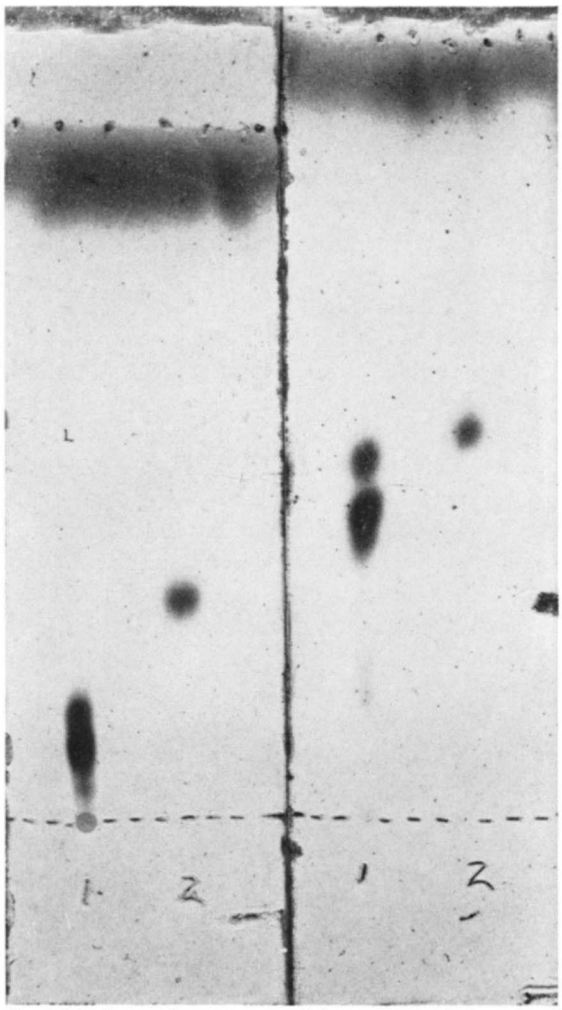

FIG. 3. Thin-layer plates prepared in water (right) and sodium borate (left). *1*, galactocerebroside; *2*, glucocerebroside. Solvent system 3: chloroform–methanol–water–15.0 M NH_4OH 280:70:6:1 (12). Development time: 40 min.

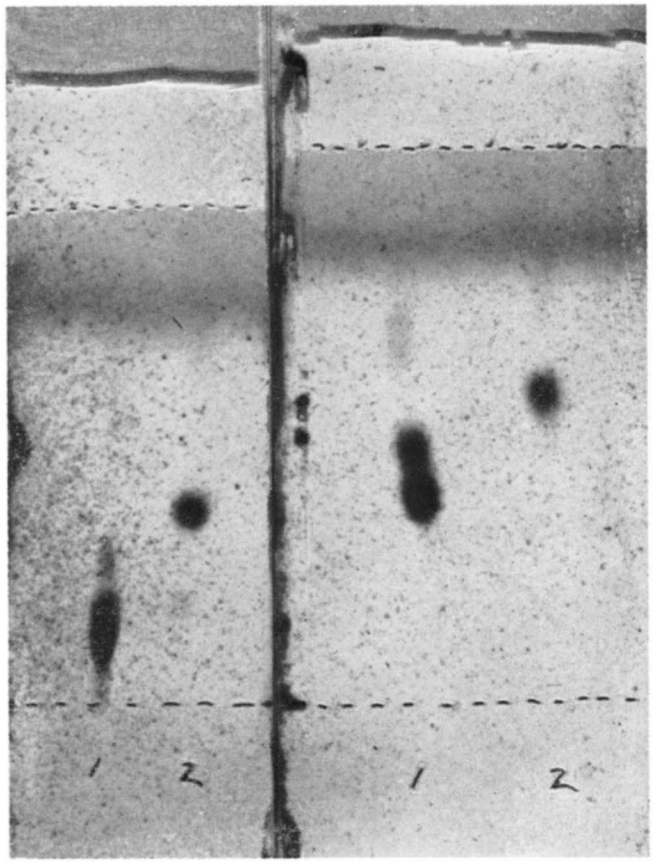

FIG. 4. Thin-layer plates prepared in water (right) and sodium borate (left). *1*, galactocerebroside; *2*, glucocerebroside. Solvent system 4: *n*-propanol–15.0 M NH_4OH–water 160:25:15 (13). Development time: 140 min.

method of Lauter and Trams (7). After further hydrolysis of the methyl glycosides in N HCl in a boiling water bath for two hours, glucose was determined by measuring the reduction of NADP fluorimetrically (8) by means of a Farrand Fluorimeter (Farrand Optical Co., Inc., Mt. Vernon, N. Y.) after reaction with hexokinase and glucose-6-phosphate dehydrogenase (Sigma Chemical Co., St. Louis, Mo.). Galactose was determined by means of the galactose oxidase assay (9) utilizing the "galactostat" reagent prepared by Worthington Biochemical Corp., Freehold, N.J.

Hexose and Sphingosine Analyses. The analytical data for the cerebrosides showed essentially equimolar amounts of hexose and sphingosine. The molar ratios for glucose, sphingosine, and galactose were 1.0, 1.2, 0.03 and 0.02, 1.0, 1.0 for the gluco- and galactocerebrosides respectively. The galactocerebroside had a galactose content of 21.5% (theoretical 22.2%) when calculated on the basis of lignoceric acid as the fatty acid constituent. Insufficient glucocerebroside was available to obtain an accurate weighing.

Thin-Layer Chromatography. Little or no separation between the gluco- and galactocerebrosides was obtained on thin-layer plates prepared in water (Figs. 1–4) yet marked differences in migration between these two species of cerebrosides were seen with each of four solvent systems when the plates employed were prepared with borate-impregnated Silica Gel G. The galactocerebrosides migrated as two of three major spots, all of which were affected in a similar manner on borate-treated plates, while the glucocerebroside migrated as a single spot.

The galactocerebroside is completely separated from the double spot given by Gaucher cerebroside on the borate plate (Fig. 2), while only slight separation is evident in the plate prepared in water. Similar results were obtained using solvent system 3 (Fig. 3). The slight separations that were noted in systems 3 and 4 when silica gel was slurried in water were enhanced on borate-impregnated gel. Varying solvent system 2 by the use of 1% borate instead of water as a component of the solvent system was ineffective by itself in producing separation.

Similarly, increasing the ammonia concentration up to fourfold in system 3 did not change the migration pattern. In terms of the degree of separation and speed of development, solvent system 3 is preferred among those tested for separating gluco- and galactocerebrosides.

None of the above systems, however, was effective in separating the digalactosyl and lactosyl cerebrosides, whether the plates were prepared in the presence of borate or not.

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