Triphenyltetrazoliumchlorid. (a) TTC, 1%ig in Wasser; (b) NaOH, 10%ig in 60%igem Methanol; Spray mit Mischung von (a) und (b) (1:3).

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The separation of gangliosides by glass fiber chromatography

Owing to the close similarity of the various gangliosides which in the past few years have been identified and isolated from various types of brain tissue, most investigators in the field have leaned heavily on chromatography and allied techniques.

Column and ion exchange chromatography have been used but have not been very successful except in the preliminary fractionation[1]. Counter current distribution methods[2], while adequate, require elaborate equipment; hence, most workers have used thin-layer chromatography (TLC), which has been employed both for analytical and preparative purposes[1].

This method has some disadvantages and we have accordingly utilized instant thin-layer chromatography (ITLC) on glass fiber. Though this technique has been applied extensively to both the carbohydrate and lipid field in recent years[3], it has not, thus far, been used in the ganglioside field. Advantages consist of increased speed of separation and increased sensitivity as compared to conventional TLC.

Experimental

Materials. "Gelman chromatographic media ITLC" type SA was used for most of the separations described here. The much faster type SG was found to be less satisfactory for general purposes, but excellent for the separation and identification of monosialogangliosides.

Before use, the sheets were activated by heating to 110° for about 90 min and then used immediately. Activation for a shorter period increased the speed with which the solvent rose and the R_F values, but decreased the separation of the bands. Solvents used were *n*-propanol–water (75:25) and various combinations of water (or 2.5 *N* ammonia), chloroform and methanol with SA and *n*-butanol saturated with water with SG.

Procedure. The gangliosides were spotted on the paper with 2 μl, 5 μl or 10 μl micropipettes. If necessary, the spots were dried with a hot air gun and another spot or spots superimposed. The use of chloroform–methanol (2:1 or 1:1) as a solvent gave smaller spots than did aqueous solutions and thus smaller amounts could be detected.

Development was carried out in a Gelman developing chamber. Time required for development was about 1½–3 h for SA paper (depending on time of activation) and ¾–1½ h for SG. The developed sheets were air or oven dried, sprayed with 50% sulfuric acid and charred on a heated silica plate. Other methods used for visualization were SVENNERHOLM's resorcinol spray[4] reagent or *p*-dimethylaminobenzaldehyde[5] to confirm the presence of acetylneuraminic acid in the spots, and iodine vapor. In addition, at least the major spots were found to have a very faint bluish fluorescence in 350 mμ light and could thus be observed directly.

For small scale preparative ITLC up to 3,000 μg ganglioside mixture was streaked onto the central region of an ITLC sheet, leaving sufficient space on the sides for the next step and developed as before. After air drying the major bands were identified under 350 mμ light and the sheet cut into strips. The topmost region (*i.e.* the original right side) was cut so as to come to a point. These were then placed vertically in the developing tank and developed 2–3 times when substantially all the adsorbed substance was concentrated in the uppermost narrow region of the strips. This technique leads to more satisfactory elution than attempts to elute the bands directly.

The terminology used is that of SVENNERHOLM[6], that of KOREY AND GONATAS[7] being given in parentheses in Table I.

Results and discussion

An ITLC chromatogram of mixed beef gangliosides (Pierce) is shown in Fig. 1. The total amount used for spotting was 4.5 and 9 μg ganglioside dissolved in 2 μl and 4 μl respectively of chloroform–methanol (1:1). For comparison, the purified gangliosides G_{D1a}, G_{M1} and G_{M2} are also shown. The solvent system used was *n*-propanol–water (75:25). This system is fairly tolerant of small variations in experimental conditions.

For convenience, the R_F values of the spots from Figs. 1 and 2, together with those for the same components using TLC are summarized in Table I. The TLC data are abstracted from SVENNERHOLM[6].

The use of chloroform–methanol–ammonia or chloroform–methanol–water was

TABLE I

R_F VALUES OF SOME GANGLIOSIDES

Ganglioside	*TLC n-propanol–water (70:30)*[6]	*ITLC-SA n-propanol–water (75:25)*	*ITLC-SG n-butanol–water (saturated, 23°)*
G_{M2} (G_5)	0.42	0.55	0.72
G_{M1} (G_4)	0.37	0.50	0.56
G_{D1a} (G_3)	0.27	0.44	0.27
G_{D1b} (G_2)	0.22	0.38	<0.25
G_{T1} (G_1)	0.19	0.33	<0.25

less satisfactory. The composition of the best mixture used was chloroform–methanol–water (65:35:4), and under these conditions the various R_F values varied widely from experiment to experiment for no apparent reason. The other system studied consisted of Gelman ITLC-SG, and as can be seen in Fig. 2, this produced very satisfactory separation of the monosialogangliosides in a short time. Below the level of G_{M1} this system is unsatisfactory, though adequate separation could probably be achieved by redeveloping the sheets one or more times.

In all the systems mentioned, the R_F values of the individual components depend somewhat on the amount of substance present, increased concentrations

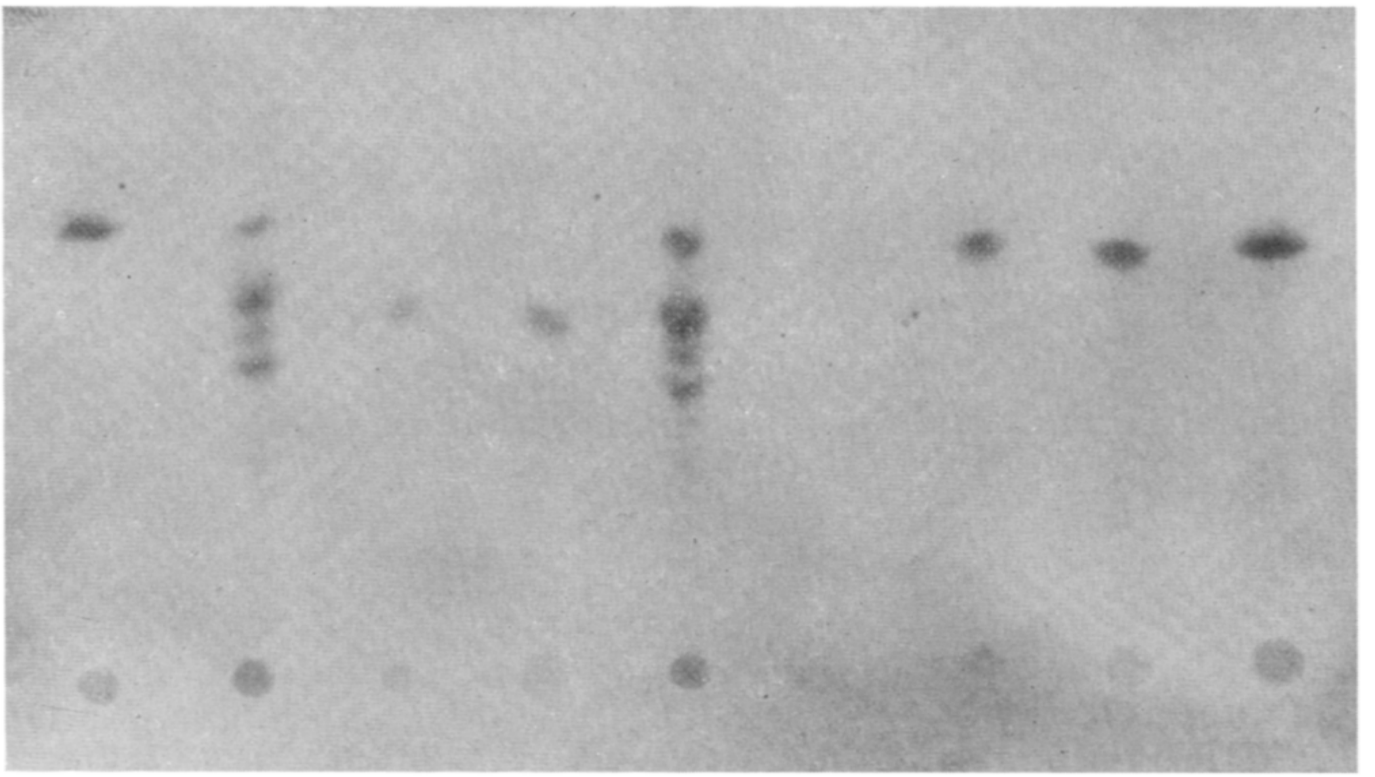

Fig. 1. ITLC chromatogram of gangliosides on SA paper. The spots from left to right are; (1) G_{M2}; (2) mixed beef brain gangliosides (4.5 μg); (3), (4) G_{D1a}; (5) mixed beef brain gangliosides (9.0 μg); (6), (7) G_{M1}; (8) G_{M2}. Solvent system, *n*-propanol–water (75:25); running time, 3 h.

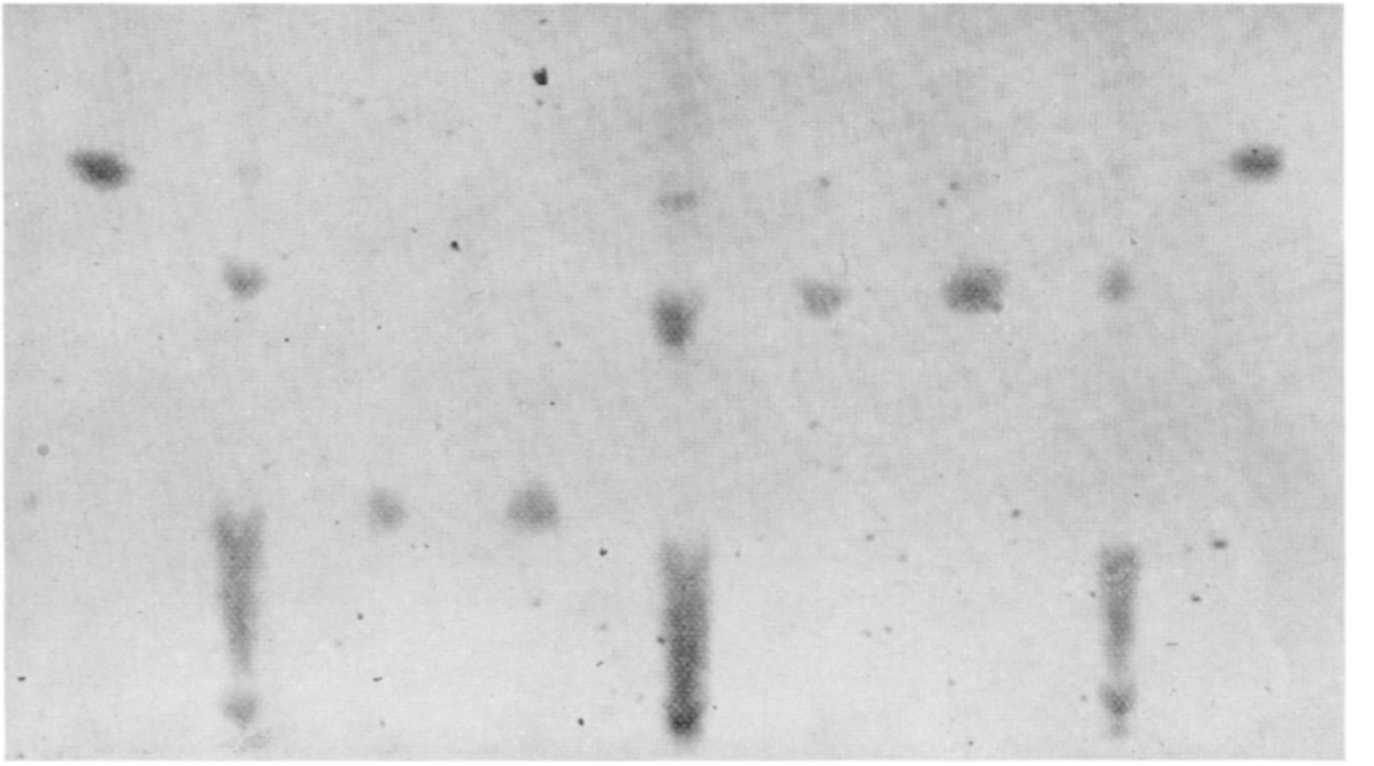

Fig. 2. ITLC chromatogram of gangliosides on SG paper. The spots from left to right are: (1) G_{M_2}; (2) mixed beef brain gangliosides (4.5 μg); (3), (4) G_{D1a}; (5) mixed beef brain gangliosides (9.0 μg); (6), (7) G_{M1}; (8) mixed beef brain gangliosides (4.5 μg); (9) G_{M_2}. Solvent system, *n*-butanol saturated with water (23°); running time, 75 min.

leading to smaller values. A similar phenomenon has also been described for amino acids[8]. The larger separations produced about 1 mg of the monosialoganglioside G_{M1} in a fairly pure state, but the fractions of lower R_F were always contaminated with faster moving material and required re-chromatography for purification.

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