and 10 ml samples. Initially, for both sample sizes, there was an increase in sensitivity. However, during a period of 2 h, the oxygen sensitivity fell back to its original level. It would appear, therefore, that either "conditioning" with pure oxygen had not been extensive enough, or that the column requires "conditioning" before each sample injection.

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Biochemistry of sphingolipids

XXIII. Paper chromatography of human brain gangliosides

Gangliosides represent a complex group of glycosphingolipids characterized by the presence of N-acetylneuraminic acid in the carbohydrate chain. They occur primarily in brain tissue, but are now known to exist outside the central nervous system as well (*e.g.* spleen, erythrocytes etc.) Alterations of their pattern in certain neurological diseases has provided additional impetus to the study of their chemistry and metabolism.

The separation of gangliosides has recently been achieved through the use of thin-layer chromatography. The heterogeneity of these substances in chromatographic systems has been well documented; however, the number of individual types and their chemical composition reported by various authors varies[1–7]. An excellent comparative thin-layer chromatographic study has been published recently by PENICK *et al.*[8]

Only a few authors have reported the separation of gangliosides on paper, so far. SVENNERHOLM[5,9] describes some solvent systems including tetrahydrofuran–diisobutyl ketone–water, diisobutyl ketone–acetic acid–water and *n*-butanol–pyridine–water mixtures for the separation of these compounds. MICHALEC AND KOLMAN[10] used Schleicher and Schüll No. 289 silica gel paper and several solvent systems for the one- or two-dimensional chromatography of gangliosides.

During our study on the brain ganglioside spectrum, we have applied paper

chromatographic techniques successfully for the qualitative and semiquantitative identification (characterization) of these substances.

Experimental

Isolation of gangliosides. A relatively pure ganglioside fraction was isolated from human brain according to the method described by SUZUKI[12].

Chromatography. An aliquot of the ganglioside solution was spotted on Whatman No. 2 or Schleicher and Schüll No. 2043b glatt paper (18 × 18) in a straight line (1.5 cm) and developed in isoamyl alcohol–pyridine–water (1:1:0.8, v/v/v) by an ascending technique at laboratory temperature. When the solvent front was 1 cm from the upper end of the paper the chromatogram was removed and dried at 110–120°.

Detection. The chromatogram was immersed in a 0.002 % solution of cresyl violet in 1 % acetic acid for 12 h and then washed well in 1 % acetic acid for several hours.

Semiquantitative estimation. The direct densitometry of the coloured spots in reflected light was performed using an ERI 10 apparatus (VEB Zeiss, Jena). The areas corresponding to the individual spots were estimated by planimetry. The molecular percentages of all ganglioside fractions was calculated from these values.

Results and discussion

The chromatography of gangliosides on unimpregnated paper results in the separation of these substances into three main groups (MSG, DSG, TSG*). It appears very likely that this separation depends on the different NANA contents of the individual fractions (Fig. 1). These preliminary speculations could be verified by the estimation of NANA in each fraction.[3]

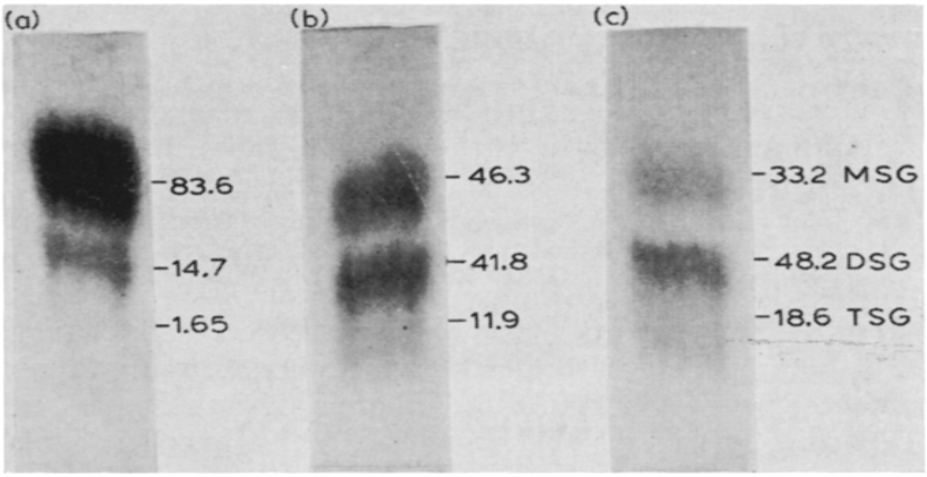

Fig. 1. Paper chromatography of human brain gangliosides on Whatman No. 2 paper in isoamyl alcohol–pyridine–water (1:1:0.8). Detected with cresyl violet. (a) Tay-Sachs' brain; (b) gargoylism; (c) normal human brain. The values are expressed as the mole % of each fraction.

A similar picture of the separation was observed by POWNING AND IRZYKIEWICZ[14] on paper chromatography of oligosaccharides in the same solvent system.

The amount of other carbohydrate units in the molecule of various major

* Abbreviations used: MSG = monosialogangliosides; DSG = disialogangliosides; TSG = trisialogangliosides; NANA = N-acetylneuraminic acid.

ganglioside types seems to have no significant effect on the mobility in our system. These observations are in apparent contrast to the results obtained by SVENNERHOLM[5]. In his system it was possible to distinguish *e.g.* G_{M3}, G_{M2} and G_{M1} fractions, although the mobilities are very similar. A possible explanation might be sought in the different technique (descending chromatography) and the duration of chromatography (16 hours).

The visualisation of the ganglioside spots on paper chromatograms gives considerable difficulties since many of the reagents frequently used in thin-layer chromatography are very drastic and attack the cellulose (carrier). The only one which could be used is cresyl violet.

This compound belongs to the group of metachromatic basic dyes, and is frequently used in histological and histochemical work.

According to KELLY[15] various organic compounds with definite functional groups, so-called chromotropes, in the molecule could interact with the dye and cause shifting of the original absorption maximum to the range of short wave lenghts, resulting in a change of color. Among the functional groups which could produce these changes are the $-SO_3^-$, $-OPO_3H^-$, $-OPO_3^{2-}$, and COO^- groups. It seems likely that gangliosides, or the presence of NANA in these substances has a similar effect.

In one of our previous papers[10] we found that of all the sphingolipids analysed, only sulfatides and gangliosides give a metachromatic reaction.

ŠMÍD[13] applied this reaction to the semiquantitative estimation of gangliosides on paper (Fig. 2). Comparison of his and SUZUKI's[7,16] method showed a good correlation. For this reason we believe that both methods are based on the determination of the NANA content in gangliosides (Table I).

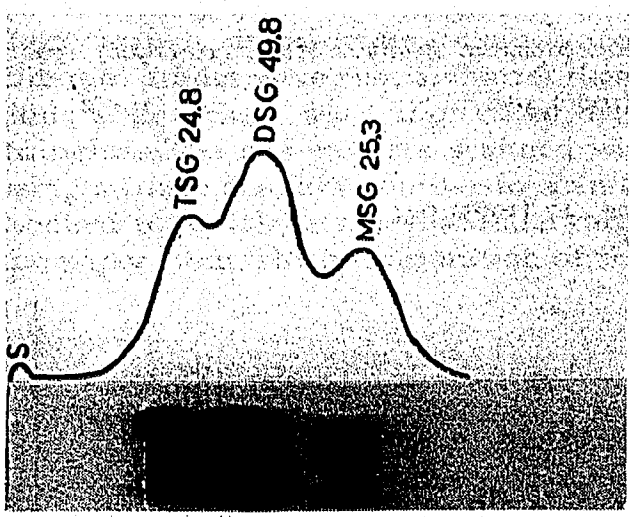

Fig. 2. Densitometric estimation of human brain gangliosides after paper chromatography on Whatman No. 2 paper in isoamyl alcohol–pyridine–water (1:1:0.8) and detection with cresyl violet. The values are expressed as the mole % of each fraction.

Although the differences in the concentration of MSG and DSG as determined by the two methods, are remarkable, we think that it would be possible to use paper chromatography for the semiquantitative determination of gangliosides in biological

TABLE I

COMPARISON OF PAPER AND THIN-LAYER CHROMATOGRAPHIC ESTIMATION OF GANGLIOSIDES IN NORMAL HUMAN ADULT BRAIN (30–40 YEARS)

Fraction	*Paper chromatography* (1)	*Thin-layer chromatography* (2)
MSG	25.1	16.4
DSG	46.6	55.0
TSG	28.2	28.2

(1) Densitometry (this paper); (2) colorimetric estimation of NANA after the reaction with modified resorcinol–HCl[7].

The values are expressed as mole % of NANA.

material or, better still as a "screening test", because the procedure is less complicated than thin-layer chromatography.

More details of the application of this technique will be given in another paper[17].

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