by using the more absorbent S & S No. 589 Green Ribbon C paper, we successfully extracted 2 ml of plasma from each 14 in. × 41/2 in. strip. Another advantage is economy of technician's time in case of multiple samples. As many as 36 narrow strips can be easily placed in one jar. The inconvenience of paper silication is only real when the procedure is seldom used.

If collection beakers are tared determination of total lipid by weighing may be conveniently done by evaporation of solvent without further transfers. This may be particularly useful clinically in pediatric work.

We were not able to detect any significant lipid degradation but a more detailed analysis might have revealed some alterations such as transesterification. On the other hand drying of samples before extraction and an essentially unhydrous atmosphere of the jar would tend to reduce degradatory process. The lipid extract did not seem to contain non-lipid plasma elements but minute amounts of material extractable from paper itselfa ppeared in the eluate. This was practically eliminated by the prerun.

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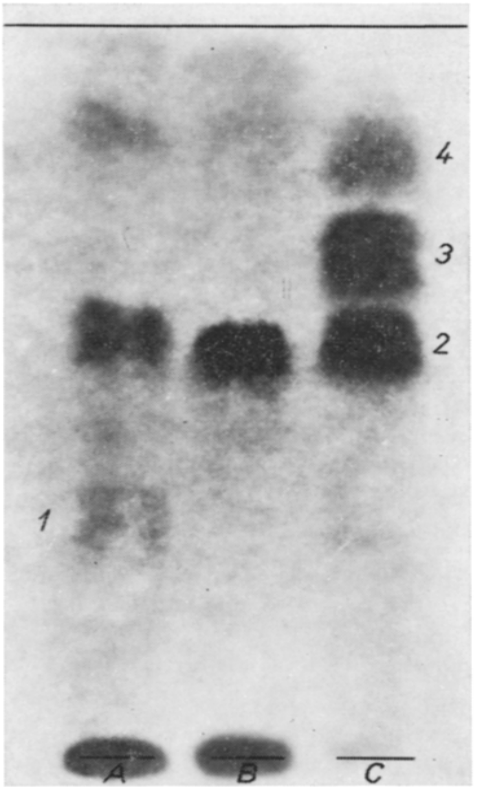
Biochemistry of sphingolipids

XXI. Separation of dinitrophenyl derivatives of long-chain bases by reaction paper chromatography

Thin-layer and paper chromatographic separations of DNP (dinitrophenyl)-derivatives of long-chain bases and their degradation products* originating from different hydrolytic conditions have been reported in our previous papers[1–5].

* The names of the long-chain bases and their degradation products are presented in the form of the semi-systematic nomenclature used up to the present time, and also in the terms proposed by IUPAC-IUB Commission on Biochemical Nomenclature[12].



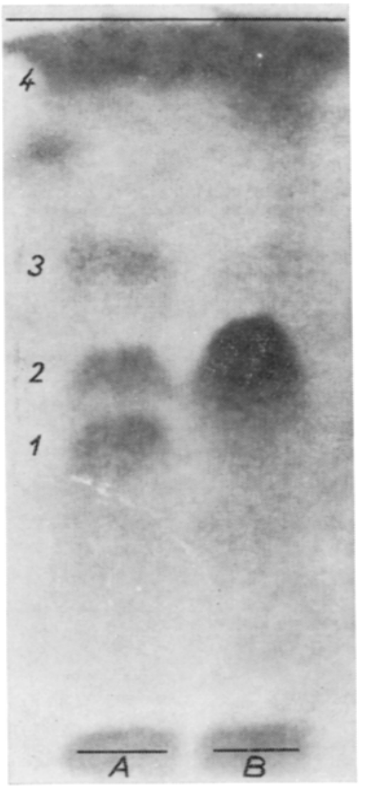


Fig. 1. Separation of DNP-derivatives of long-chain bases by reaction chromatography on Whatman No. 3 silica gel paper in petroleum ether (b.p. 60–90°)–diethyl ether (50:50). A = long-chain bases from normal human brain sphingolipids after hydrolysis with modified aqueous methanolic HCl[11]; B = long-chain bases isolated from human aorta sphingomyelins after enzymatic and alkaline hydrolysis[10]; C = long-chain bases isolated from normal human brain sphingolipids after hydrolysis with methanolic H_2SO_4[11]. A and B are the results of the direct formation of DNP-derivatives on the chromatogram; C are DNP-derivatives formed by the method of KARLSSON[6]. 1 = 1,5-Dihydroxy monoenoic derivatives (5D-hydroxy-3-sphingenine + 5L-hydroxy-3-sphingenine); 2 = sphingosines and dihydrosphingosines(4-sphingenines + sphinganines); 3 = 5-O-methyl ethers (5D-methoxy-3-sphingenine + 5L-methoxy-3-sphingenine); 4 = 3-O-methyl ethers and dienoic derivatives(3D-methoxy-4-sphingenine + 3L-methoxy-4-sphingenine + 3,5-sphingadiene + *cis*-3,5-sphingadiene).

Fig. 2. Separation of DNP-derivatives of long-chain bases by reaction chromatography on Whatman No. 3 paper impregnated with silica gel and 0.05 *M* $Na_2B_4O_7$ in chloroform–methanol (99:1)[10]. A and B are the same samples as in Fig. 1. 1 = threo-sphingosines (3L-4-sphingenines); 2 = erythro-sphingosines(4-sphingenines); 3 = dihydrosphingosines + 1,5-dihydroxy monoenoic derivatives; 4 = 5-O-methyl ethers, 3-O-methyl ethers and dienoic derivatives.

In all our experiments the derivatives were prepared by the reaction of the free bases with an ethanolic solution of 2,4-dinitrofluorobenzene in sodium borate buffer (pH 10) at 60–70° according to KARLSSON[6].

Reaction chromatography (elatography) was introduced by BECKER[7] and has been used in the analysis of DNP-amino acids[8] or lipids[9]. We have used this technique as a simple procedure for the identification of long-chain bases on paper chromatograms.

Experimental

Isolation of sphingolipids. Sphingolipids were isolated from human brain, aorta and other tissues by mild alkaline hydrolysis. The highly purified fractions of glycosphingolipids and sphingomyelins were prepared by column chromatography on florisil and DEAE-cellulose.

Long-chain base extracts free of various degradation products were prepared from sphingomyelins by the action of phospholipase C (phosphatidylcholine phosphohydrolase EC 3.1.4.3) and subsequent hydrolysis with 1 *N* methanolic potassium hydroxide[10].

Reaction paper chromatography. An aliquot of the solution of free bases in diethyl ether was spotted as a narrow band (2 cm) on the starting line of Whatman No. 3 silica gel paper or on the same paper impregnated with 0.05 *M* sodium tetraborate[5].

The area of each sample was wetted with some drops of methanol and then with an 2 % aqueous solution of sodium carbonate and a 1–2 % methanolic solution of 2,4-dinitrofluorobenzene. The chromatograms were incubated for 15 min in a chamber saturated with water vapour at 38° and finally dried for 10 min at room temperature.

Chromatography was performed in petroleum ether (b.p. 60–90°)–diethyl ether (65:35 or 50:50) or in chloroform–methanol (99:1). The homologous bases were separated on Whatman No. 3 silica gel paper impregnated with tetralin in methanol–tetralin–water (90:10:10; upper phase)[5]. The spots were located under U.V. light (Figs. 1 and 2).

The direct formation and chromatography of DNP-derivatives of long-chain bases on paper, represents a reliable method for the identification of their pattern in sphingolipids. The results are identical when compared to the "classical" technique described in our previous papers.

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