0.160 mm). The rate of flow of helium was 60 ml/min, and the thermostat temperature 210°C. The alkaline hydrolysis of the combined HLs, TAGs, 2-MAGs, DAGs, and MAGs was carried out as in [2].

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PREPARATIVE FRACTIONATION OF CEREBRAL GANGLIOSIDES WITH THE AID OF ION-EXCHANGE CHROMATOGRAPHY ON SPHERON CH 300

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A new preparative method is proposed for the separation of natural mixtures of gangliosides into fractions of mono-, di-, and trisialogangliosides which is based on ion-exchange chromatography on a weak anion-exchange resin — Spheron CH 300. The latter ensures a considerably faster separation than the ion-exchange resins usually employed.

The demand for effective methods for the preparative separation of complex mixtures of nerve-tissue gangliosides is due to the modern development of investigations of the physiological functions and biochemical properties of this class of glycolipids [1, 2]. According to the existing methodology, the isolation of individual gangliosides is carried out in several stages [2]. In the first stage, fractions consisting of components with the same number of sialic acid residues are usually used. For this purpose, ion-exchange chromatography on anion-exchange resins containing amino or diethylamino groups is frequently employed [2-4]. With the use of the ion-exchange materials proposed in the cited papers in high-performance liquid chromatography, a rapid and satisfactory separation of small amounts (up to 15 mg) of combined gangliosides is ensured. However, in large-scale (1 g and more) fractionation the process lasts several days and a risk of the modification of the native compounds appears.

The authors of the present paper have used for this purpose Spheron CH 300 — an anion-exchange resin containing hydrazine groups produced in Czechoslovakia (Lachema) and have found that preparative chromatography on this sorbent requires a considerably shorter time and permits the isolation of mono-, di-, and trisialogangliosides in satisfactory yield. Thanks to the relatively low basicity of Spheron CH 300, the probability of the appearance of artefacts in the chromatographic

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TABLE 1. Results of the Fractionation of 1.0 g of the Total Gangliosides from Harp Seal Brain on Spheron CH 300

Lipids eluted	Fraction no.*	Volume of the eluent, ml	Proportion of MeOH in the eluent (MeOH:1 M NaCl) in vol. %	Weight of the eluted lipids, mg	Yields of pure subfractions, %**
Sphingomyelin and neutral glycolipids	5–15	330	100	53	
	16–19	120	95		
G_M	20–26	210	85	217	92
	27–28	60	65		
$G_M + G_D$	29–32	120	65	93	
G_D	33	30	65	486	80
$G_D + G_T$	34–39	180	40		
	40–41	60	0	54	
G_T	42–45	120	0	51	72
$G_T + G_Q$	46–49	120	0	12	

*Fractions with a volume of 30 ml were collected.

**In relation to their amount in the total ganglioside fraction.

process is excluded, and the high mechanical stability of the gel formed by them makes it possible to perform chromatography under a pressure of up to 3 MPa. The column filled with the ion-exchange resin is washed with 0.05 M ammonium acetate buffer solution (AABS), pH 5.1, until ionic equilibrium has been established. Since the detection of the gangliosides in the eluate was carried out from the optical density of the latter at 206 nm while the AcO^- ion possesses pronounced absorption in this region of the spectrum, this counterion was replaced by Cl^- by washing the anion-exchange resin with a solution of NaCl.

For the final preparation of the column it was washed with distilled water and with methanol. The mixture of gangliosides was deposited on the column in the form of a solution in methanol. Elution was carried out with methanol to eliminate sphingomyelin and neutral glycolipids, after which the ganglioside fractions were eluted with mixtures of ethanol and 1 M aqueous NaCl with gradually increasing proportions of the latter. Table 1 gives the results of the separation of the proposed method of the total gangliosides of the harp seal. The components of the fractions of the gangliosides obtained were identified, in the first place, from their retention volumes as found for the mono-, di-, and trisialogangliosides in the chromatography of a standard mixture and, in the second place, by the use of TLC with markers. Preparations of the gangliosides were obtained by the desalting of the corresponding fractions of the eluate by dialysis and the evaporation of the dialyzates.

The fractionation of 1.0-1.5 g of the total gangliosides of harp seal brain took about 17 h. The performance of the same process under a pressure of 0.2 MPa enabled the time of chromatography to be brought to less than half without an appreciable deterioration of the quality of the separation. The fractionation of 1.0 g of the same mixture of gangliosides on a column (3 × 35 cm) of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals, AB, Sweden) led to similar results, but this required about 120 h.

The column of Spheron CH 300 can be used for the above-described separation not less than 30 times, its regeneration being performed by the same operations as in its initial preparation, i.e., washing with AABS until ionic equilibrium was achieved and then with a solution of NaCl, with distilled water, and with methanol.

EXPERIMENTAL

The total fraction of gangliosides from the brain of the harp seal *Phoca groenlandica* was isolated by the usual procedures [7], but for the complete elimination of glycerophospholipids the fraction was subjected to alkaline methanolysis and then to dialysis [4]. From 1.0 kg of natural brain 1.8-2.0 g of total gangliosides were obtained. These were analyzed with the aid of high-performance liquid chromatography on a column of silica gel (4.6 × 250 mm, μ-Porasil, Du Pont), elution being performed in the solvent system isopropanol–hexane–water with a linear change in their volume proportions from (55:43:2) to (55:25:20) over 1.5 h at a rate of flow of 1.2 ml/min. The substances were detected from the optical density of the eluate at 206 nm. For the identification and quantitative determination of the gangliosides, known amounts of standard samples were chromatographed under the same conditions. The standard samples of gangliosides were supplied by N. V. Prokazova (Institute of Experimental Cardiology of the All-Union Cardiological Scientific Center of the USSR Academy of Medical Sciences, Moscow).

It was found that 1.0 g of total gangliosides contained 235 mg of G_M (19% of G_{M1} and 81% of G_{M3}), 612 mg of G_D (57% of G_{D1a} and 43% of G_{1b}), and 71% mg of G_T.

Preparation of Chromatographic Column. Commercially available Spheron CH 300 (22 g; 0.3 mmole/g of covalently bound NH_2NH groups; particle size 63-100 μm) was mixed with 400 ml of distilled water and was left for 24 h, after which the sorbent was resuspended and was left for sedimentation for 10 min, and the liquid phase was decanted off. The deposit was suspended in 1 liter of distilled water, the suspension was left for sedimentation for 10 min, and the liquid phase was decanted off. This operation was repeated twice more. The deposit was degassed for 5 h at a residual pressure of 20 mm Hg at 20°C and was then suspended in 400 ml of 0.05 M AABS (pH 5.10), and the suspension was transferred to a column 30 mm in diameter. The sorbent was washed with the same AABS (~700 ml; here and below at a rate of 1.5 ml/min) until a pH of 5.1 had been established in the eluate. Then the column was washed with 800 ml each of 1 M aqueous NaCl, distilled water, and methanol, and was used for chromatography.

Chromatography of the Total Seal Brain Gangliosides. A solution of 1.0 g of the gangliosides in 40 ml of methanol was added to the prepared column. Elution was performed first with methanol, then with mixtures of MeOH and 1 M aqueous NaCl as shown in Table 1. The elution process was monitored continuously from the optical density of the eluate at 206 nm using a Uvicord SD Model 2510 with a flow-through cell having a volume of 8 μl (l 2.5 mm). The eluate was collected in 30-ml fractions. The fractions were combined in accordance with the chromatogram and were subjected to dialysis against distilled water (3 liters) in Servapor fine-pored dialysis bags (Serva, FRG) until Cl^- ions had been eliminated completely (usually 45-50 h with three changes of water), and were evaporated in a rotary evaporator with the addition of isopropanol as foam suppressant. The ganglioside fractions were analyzed with the aid of TLC on high-resolution plates with silica gel 60 (Merck, FRG) in the $CHCl_3$—MeOH—0.2% aqueous $CaCl_2$ (55:45:10 by volume) system. The substances were detected on the chromatograms by a nonspecific method — carbonization at 80-200°C after the plates had been sprayed with 50% H_2SO_4 — and also by the resorcinol reagent [6], which is specific for sialic acid derivatives, and the anthrone reagent [7], which is specific for glycosides.

Regeneration of the Column. After the end of chromatography, the column was washed with 0.05 M AABS having pH 5.1 until a pH of 5.1 had been established in the eluate (about 600 ml of AABS) and then with 1 M aqueous NaCl, distilled water, and methanol (800 ml each).

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