

Gas-Liquid Chromatographic Profile of Neutral and Acidic Metabolites in Cerebrospinal Fluid from Newborns and Infants

Richard D. Malcolm and Richard Leonards

A profile (chromatographic pattern) of the neutral and acidic metabolites present in cerebrospinal fluid from newborns and infants was obtained by gas-liquid chromatography. The metabolites are those extracted with ethyl acetate and diethyl ether. They are converted to trimethylsilyl ether derivatives, and chromatographed on a column containing 5% silicone OV-101 (methyl) packed on Chromosorb W. Several substances were tentatively identified from their methylene unit values. We established a control profile for infants, and compared profiles for infants and adults. Noteworthy qualitative and quantitative differences from the control were seen for cerebrospinal fluid from subjects with neurological disorders or infections. The technique may be of use in neurological diagnosis.

Additional Keyphrases: *newborns • pediatric chemistry • diagnostic aid • neurological disorders • screening*

CSF¹ from adults has been examined by GLC for neutral and acidic metabolites (1), fatty acid composition (2-4), and metabolic products of cerebral metabolism that are associated with neurological disorders (5-7). There is no information about the GLC profiles of CSF from newborns and infants younger than three months. However, the composition of their CSF is known to differ in some respects from that of adults (8-10); therefore the GLC patterns may also differ.

Department of Pediatrics, Children's Hospital of San Francisco, San Francisco, Calif. 94119.

¹ Nonstandard abbreviations used: CSF, cerebrospinal fluid; GLC, gas-liquid chromatography (-ic); and MU, methylene unit (a measure of retention time).

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Here, we report the metabolic profile of neutral and acidic components of CSF from newborns and infants. Sub-milliliter amounts of the CSF are extracted, derivatives are formed, and the product is chromatographed. We show that the resulting profiles may be of value in the diagnosis of neurological disorders and infections.

Materials and Methods

Subjects

A total of 37 samples were obtained from 33 babies, 26 of whom were males, admitted to the Neonatal Intensive Care Unit at Children's Hospital of San Francisco. Their ages ranged from 1 to 84 days, with 28 younger than 30 days. Their various diagnoses included suspected sepsis, ventriculitis, meningitis, hydrocephalus, and intracranial hemorrhage.

Reagents

Reagents and chemicals were: ethyl acetate, diethyl ether, and methanol (all "Nanograde"; Mallinckrodt Chemical Works, St. Louis, Mo. 63160); and bis(trimethylsilyl)-trifluoroacetamide in glass ampules, with 10 ml of trimethylchlorosilane added per liter; and glass-distilled pyridine (both from Regis Chemical Co., Morton Grove, Ill. 60053).

Standards

Hydrocarbon standards and fatty acid standards were obtained from Applied Science Laboratories, State College, Pa. 16802. Homovanillic acid, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindoleacetic acid, 3-methoxy-4-hydroxyphenylethylene glycol, and 3-methoxy-

4-hydroxyphenylethanol were obtained from Calbiochem, San Diego, Calif. 92112. Lactic acid, succinic acid, maleic acid, and citric acid were obtained from Arthur Thomas Co., Philadelphia, Pa. 19105.

Sample Treatment

CSF, obtained by lumbar puncture from newborns and infants with suspected sepsis or neurological disorder, was centrifuged at $1000 \times g$ for 5 min and the supernate was stored at -40°C until use. We saw no changes in the chromatographic profile after three months of storage at -40°C .

The CSF was extracted with ethyl acetate and diethyl ether (11). Half-milliliter samples of CSF were diluted to 20 ml with de-ionized water, saturated with sodium chloride, acidified to pH 1.0 with a few drops of dilute hydrochloric acid (6 mol/liter), and the mixture was placed in a separatory funnel. The diluted sample was extracted three times with 30-ml portions of ethyl acetate and three times with 30-ml portions of diethyl ether. The extracts were pooled, dehydrated over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure and the residue transferred in about 3 ml of methanol to a Teflon-lined mini-reaction vial with a screw cap (Regis Chemical Co.). The methanol was evaporated in a stream of dry nitrogen at room temperature.

The residue containing the extracted substances was redissolved in $25 \mu\text{l}$ of pyridine. Trimethylsilyl ether derivatives were prepared by adding $25 \mu\text{l}$ of bis(trimethylsilyl)-trifluoroacetamide and allowing the silylation reaction to proceed overnight at room temperature before GLC analysis.

Gas Chromatography

We used a Model 1440 chromatograph (Varian Aerograph, Walnut Creek, Calif. 94598), equipped with a hydrogen-flame ionization detector and a 25.5-cm potentiometric recorder (Beckman Instruments, Fullerton, Calif. 92634). Glass columns, 1.83 m long, 2 mm i.d., and 6.35 mm o.d. were silanized with dimethyldichlorosilane and packed with 5% silicone OV-101 (methyl) coated on Chromosorb W, 80/100 mesh, AW, DMCS (Applied Science Laboratories). A small amount of silanized glass wool held the packing in place. The injection-port temperature was $210 \pm 15^\circ\text{C}$ ($190\text{--}240^\circ\text{C}$), and we used an injection-port extender.

Detector temperature was $280 \pm 15^\circ\text{C}$ ($270\text{--}320^\circ\text{C}$), and nitrogen ("zero" grade; Liquid Carbonic Corp., Chicago, Ill. 60603) was used as the carrier gas. Gas-flow rates (ml/min) were: hydrogen 30, air 300, and nitrogen 30.

A total of $6.0 \mu\text{l}$ was injected (12% of total sample volume). The column was immediately temperature-programmed at a rate of $2^\circ\text{C}/\text{min}$ from 65 to 260°C , and then held at 260°C for 20 min. The range setting was 10^{-11} A/mV. Attenuation was reduced from 128 to 16 after succinic acid emerged. All samples were analyzed in duplicate.

A de-ionized water blank was extracted and analyzed

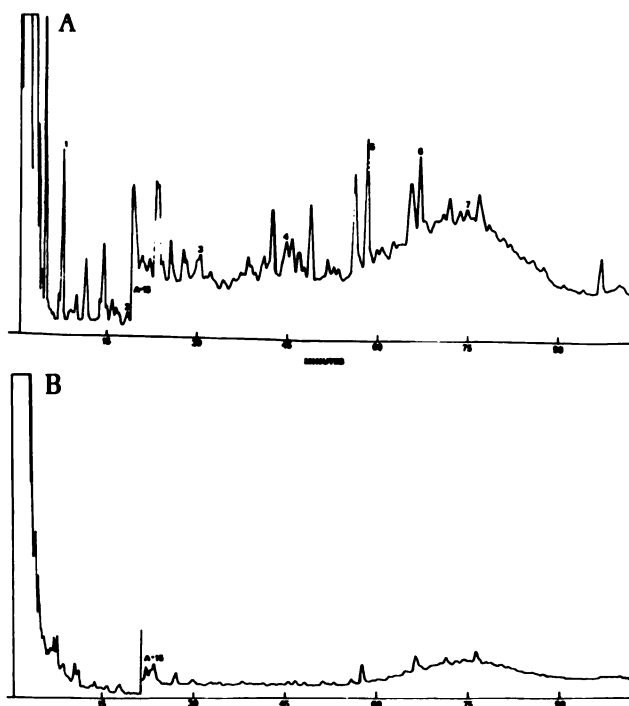


Fig. 1. A. Control profile for cerebrospinal fluid from a 6-day-old boy with hepatic and renal failure, but no nervous system disorder

1, lactic acid; 2, succinic acid; 3, maleic acid; 4, homovanillic acid; 5, palmitic acid; 6, stearic acid; 7, arachidic acid

B. Blank chromatogram for de-ionized water that was extracted, derivatized, and analyzed identically to the CSF samples

as a control for each batch of CSF samples. Trace amounts of organic compounds, which we believe to be from the de-ionized water, gave some very small peaks (Figure 1).

Results

If the infants without metabolic or neurological disorders had normal CSF values (8) as shown in Table 1, and the results of the Gram stain and bacteriological culture were negative, their profiles were considered controls. Twenty such control profiles were examined. Figure 1 shows a typical one.

Despite the variety of the clinical disorders, all not directly affecting the central nervous system, their profiles demonstrated a consistent pattern. Two large peaks and three small peaks were present in all profiles. They were tentatively identified as palmitic, stearic, lauric, myristic, and arachidic acids, respectively. Peaks consistent with the other fatty acids were seen in small amounts in some profiles. The peaks corresponding to lactic, succinic, and homovanillic acids varied in size but appeared consistently for all samples. A peak consistent with 3-methoxy-4-hydroxyphenylethanol was seen in 25 of the 37 samples we analyzed. Other peaks in the profiles corresponded in their chromatographic behavior to 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylethyleneglycol and 5-hydroxyindoleacetic acid.

Table 1. Values for Leukocyte Count, and Glucose and Protein Concentration in CSF from Control Infants

	Range	Mean	SD
Leukocytes per μ l	0-25	6.76	\pm 6.35
Protein, g/liter	0.38-1.82	0.934	\pm 0.345
Glucose, g/liter	0.27-0.82	0.548	\pm 0.135

Table 2. MU Values on Three Chromatographic Stationary Phases for Compounds in CSF from Newborns and Infants^a

Compounds	OV-101	OV-17	OV-25
	MU values		
Lactic acid	10.63	11.20	12.08
Succinic acid	13.03	14.10	14.50
Maleic acid	14.88	16.69	17.14
3-Methoxy-4-hydroxy-phenylethanol	17.04	18.21	19.90
Homovanillic acid	17.58	19.31	18.80
3,4-Dihydroxyphenyl-acetic acid	18.32	19.31	20.00
Citric acid	18.33	19.02	19.44
Myristic acid	18.46	19.02	19.46
3-Methoxy-4-hydroxy-phenylethylene glycol	18.52	18.21	19.80
Palmitic acid	20.44	20.99	21.21
5-Hydroxyindoleacetic acid	22.03	24.23	25.26
Stearic acid	22.42	22.97	23.24
Arachidic acid	24.45	24.91	25.15

^a Compounds were chromatographed as trimethylsilyl ether derivatives. All stationary phases are 5% liquid phase on Chromosorb W 80/100 mesh, AW, DMCS: OV-101 (liquid methyl silicone), OV-17 (methyl phenyl silicone), and OV-25 (phenyl silicone).

Metabolites present in the extracts of the CSF were tentatively identified on the basis of their MU values (11). MU values for standards and substances in the profile were systematically determined by using three columns with different liquid phases (5% OV-101, 5% OV-17, 5% OV-25). Table 2 shows MU values for metabolites in the CSF of newborns and infants.

Profiles of CSF from newborns with neurological disorders had patterns that differed from those for the controls. Figure 2 demonstrates the gross alterations seen in a case of intracranial hemorrhage. Several additional peaks are present in the later portion of the profile. In another example of neurological disorder, a baby with hydrocephalus secondary to toxoplasmosis, the profile had a high 5-hydroxyindoleacetic acid peak and two additional peaks in the later portion of the chromatogram.

Certain peaks in the profiles for newborns and infants with neurological infections rather than neurological

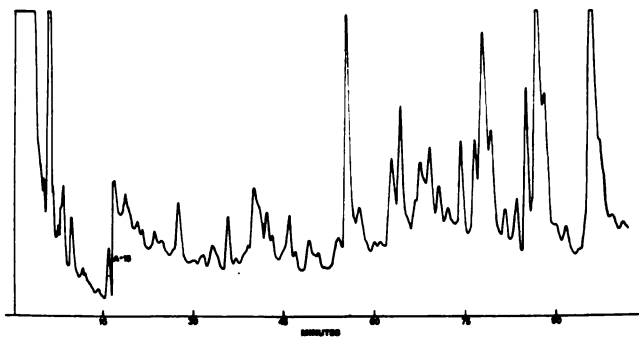


Fig. 2. Profile for cerebrospinal fluid from a 1-day-old premature female infant with an intracranial hemorrhage

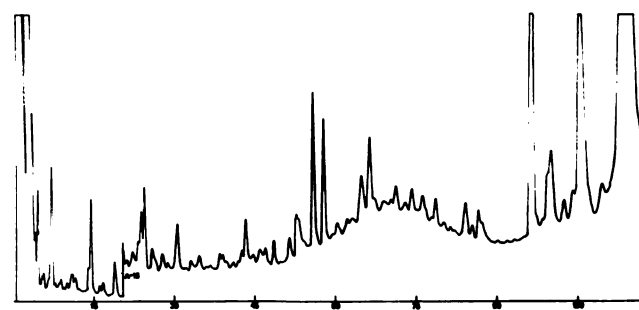


Fig. 3. Profile for cerebrospinal fluid from a 72-day-old premature male infant with ventriculitis

disorders, also differed qualitatively from the controls. The pattern for an infant with ventriculitis (Figure 3) had additional peaks at the end of the profile. All of the meningitis samples examined showed a series of six peaks that were absent in all other profiles. The peaks were regularly spaced, suggesting that they may represent homologs.

Discussion

A profile of the neutral and acidic metabolites in the CSF from newborns and infants has been obtained by a method that allows measurement to be made on very small samples. A number of substances in the profiles have been provisionally identified. Although many of these were previously reported as components of CSF from adults (1-3, 5-7, 12, 13), no quantitative or qualitative studies have been made on infants.

3-Methoxy-4-hydroxyphenylethanol is inconsistently present in infants' CSF, as was the case for adults (1). However, stearic acid, a relatively minor component of CSF from adults (1), is a major component in CSF from infants.

We found that the profile for newborns was reproducible enough that we could establish a control profile, which can be compared with profiles from adults (1) and from infants with neurological disorders and infections.

Quantitative changes in the existing peaks or the appearance of new peaks may reflect neurological or

metabolic disorder. Further work is necessary to determine whether the alterations seen are sufficiently specific to be used as major diagnostic criteria. Moreover, the components should be identified by other analytical methods, especially the new peaks seen in pathological conditions. These findings do suggest, however, that such GLC profiles may be of diagnostic value in suspected neurological infections and disorders in the newborn.

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