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# Rapid Diagnosis of Infection by Gas-Liquid Chromatography: Analysis of Sugars in Normal and Infected Cerebrospinal Fluid

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A highly reproducible procedure was developed for gas-liquid chromatographic analysis of trimethylsilyl derivatives of normal human cerebrospinal fluid. Fourteen normal human cerebrospinal fluid samples tested with this procedure contained $\alpha$- and $\beta$-glucose as well as isomers of two unidentified sugars. Chromatographic changes in three cases of meningeal inflammation (two cryptococcosis and one thalamic astrocytoma) were limited to decreased concentrations of all sugars. In one case of early meningitis, the concentrations of the unknown sugars decreased before glucose. Now that a reproducible chromatogram of the trimethylsilyl derivatives of normal human cerebrospinal fluid has been established, more samples of abnormal cerebrospinal fluid should be prepared by these methods and examined by gas-liquid chromatography. It may be possible to identify unique products of infectious agents which will permit rapid diagnosis of central nervous system infection.

Gas-liquid chromatography (GLC) is a sensitive technique for the detection and identification of small amounts of volatile derivatives of organic material. Methods have been described for the evaluation of normal constituents of animal tissues (1, 8), the determination of abnormal metabolites in human genetic diseases (7), the detection of drugs and drug metabolites in human serum and urine (8), and the analysis of bacterial cell components and fermentation products in vitro (4, 5). Recently two groups (9; G. Miller, A. I. Braude, and C. E. Davis, unpublished data) have attempted to identify the etiological agents of experimental and natural human bacteremias and fungemias by GLC analysis of serum. Although the results are promising, the method is still experimental.

This study was undertaken to determine a reproducible gas chromatogram under specified chemical and chromatographic conditions for normal human cerebrospinal fluid (CSF) and to characterize the changes which might occur during meningeal inflammation. If CSF alterations during infection of the central nervous system are specific for the causative agent, GLC would be an invaluable clinical tool for rapid diagnosis of infections caused by organisms which are difficult to recover and identify (cysticercosis and borreliosis) or grow slowly (tuberculosis and cryptococcosis).

## MATERIALS AND METHODS

**Samples.** Portions of normal CSF were collected from clinical samples which had a normal cell count (zero erythrocytes per high-power field, less than two leukocytes per high-power field), negative cultures (fungal, mycobacterial, and routine bacterial), and normal protein and glucose. The samples were stored at 4 C until use. CSFs from two patients with culturally proven relapsing cryptococcal meningitis and from one patient with a fatal thalamic astrocytoma were centrifuged at 3,000 rpm for 15 min, and the supernatants were passed through a 0.45-$\mu$m membrane filter (Millipore Corp.). The cell-free filtrates were stored and processed exactly as the normal CSFs.

**Preparation of derivatives.** Portions (1 ml) of CSF were deproteinated by adding 1 ml each of 4.5% $Ba(OH)_2$ and 5% $ZnSO_4$ followed by dilution to a 5-ml total volume with distilled water. These solutions were allowed to stand for 15 min and centrifuged at 3,000 rpm for 15 min; the supernatants were removed and dried at 40 C under vacuum (Rotovap, Buchler Instruments). The residue was redissolved in 2 ml of 0.5 N methanolic HCl and incubated at 75 to 80 C for 12 h. HCl was subsequently removed by passing the sample over a 3-ml column of Amerlite CG-4B in the hydroxyl phase. The sample was eluted with 15 ml of ethanol. The eluants were taken to dryness under nitrogen and converted to *O*-trimethylsilyl derivatives (*O*-TMSi) by the method of Sweely et al. (10) as modified by Sweely and Walker (11). Briefly, 200 $\mu$liters of a mixture of pyridine, hexamethyldisilazane, and trimethylchlorosilane (10:4:1,

vol/vol) was added to the residue at least 30 min before chromatography. A 2-μliter portion of the resultant solution was chromatographed directly.

**Chromatographic conditions.** All studies were performed on a model F&M 402 gas chromatograph equipped with a hydrogen flame ionization detector and a computerized peak integrator (Hewlett-Packard Instruments). The column employed was a U-shaped glass structure (6 feet by ¼-inch internal diameter, ca. 183 by 0.635 cm) packed with 3% SE-30 on acid-washed, silanized 60- to 80-mesh Chromosorb W (Applied Science). The carrier gas was nitrogen at a flow rate of 40 ml/min. The injector and detector temperatures were maintained at 210 C; the column temperature was maintained at 160 C. After 30 min the column temperature was increased to 200 C for 20 min to insure the elution of late peaks.

**Identification procedures.** Compounds were first classified as either fatty acids or water soluble by extraction of the methanolic solution with 3 equal volumes of hexane just prior to silanization. Both fractions were then dried, silylated, and chromatographed. All compounds present in the methanolic solution after hydrolysis, deproteination, and ion exchange were found to be water soluble.

Peaks were compared to *O*-TMSi derivatives of authentic standards of sugars, sugar alcohols, and a purified *E. coli* 0:111 endotoxin, well characterized on 3% SE-30 by GLC and by mass spectroscopy (4). Samples were chromatographed with authentic glucose, mannitol, and *E. coli* 0:111 as internal or external standards, and all peaks were assigned retention times relative to α-glucoside for comparison to published data (4, 10, 11).

Combined GLC-mass spectroscopy was performed on all CSF peaks and authentic standards of *O*-TMSi derivatives of α-glucose, β-glucose, 3-*O*-methylglucose, mannose, 2-deoxygalactose, and fructose (provided by J. Wright) with an LKB mass spectrophotometer gas chromatograph unit (Departments of Biology and Medicine, University of California, San Diego). C. C. Sweeley, Department of Biochemistry, Michigan State University, examined mass spectrograms of all unknown peaks.

## RESULTS

Six major peaks were noted in each of 14 normal CSFs (Fig. 1A). Peak 7 (Fig. 1B) was found in five "normals" but was too small and inconsistent for further analysis. Peaks 5 and 6 were identified by mass spectroscopy as α- and β-glucoside, respectively, on the basis of characteristic fragmentation products and identity to authentic standards. Mass spectra from peaks 2 and 4 were identical to those of peaks 1 and 3, respectively, indicating that these four peaks represent isomers of two compounds. Their retention times relative to α-glucoside (0.49 to 0.69) were greater than TMSi derivatives of pentoses such as rhamnose and ribose (about 0.25) and less than galactose (γ-galactose = 0.76). The retention times of peaks 1 through 4 relative to α-glucoside were completely reproducible in all chromatograms. Small differences in absolute retention times were due to minor variations in column temperature or flow rate of the carrier gas. When co-chromatographed with *E. coli* 0:111 endotoxin, peaks 1

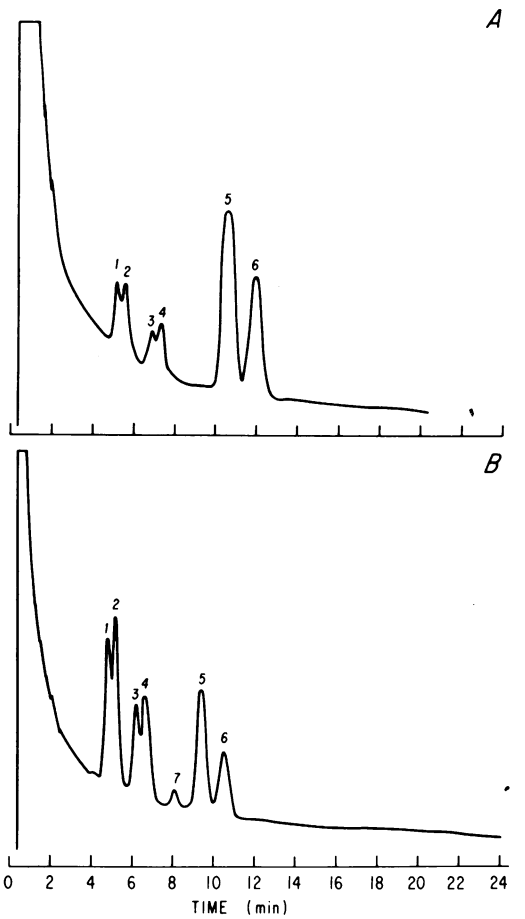


FIG. 1. *Gas chromatograms of normal CSF on a 6-foot glass column packed with 3% SE-30 on Chromosorb W. Carrier gas was nitrogen at 40 ml/min. Injector and detector temperatures were 210 C; column oven temperature was 160 C. A 2-μliter portion of the trimethylsilyl ether derivatives was injected onto the column. (A) Typical chromatogram showing peaks 1 through 6. Peaks 5 and 6 are the TMSi ethers of α- and β-methyl-D-glucopyranoside. Relative retention times of peaks 1 through 6 with α-glucopyranoside as the standard are 0.49, 0.52, 0.65, 0.69, 1.0, and 1.12, respectively. (B) Gas chromatogram showing peak 7 which appeared inconsistently in normals and in all three abnormal CSF. Relative retention times of peaks 1 through 7 are 0.49, 0.52, 0.65, 0.69, 1.0, 1.12, and 0.85, respectively.*

through 4 eluted between TMSi derivatives of ribose and galactose, and peaks 5 and 6 co-chromatograph with $\alpha$- and $\beta$-glucoside. Mass spectroscopy of peaks 1 through 4 showed some similarities to authentic standards of deoxy or partially methylated sugars. The following compounds have been ruled out by co-chromatography or mass spectroscopy: mannose, 6-deoxymannose, 2-deoxygalactose, fructose, glucuronic acid, fructose, sorbitol, and mannitol. Peaks 1 and 2 and 3-*O*-methyl-glucoside co-chromatographed and were similar but not identical on mass spectroscopy. It is possible that these peaks represent partial methylation or silanization of one of these compounds, but it seems unlikely that the pattern of partial derivatization would be so consistent. The mean areas of peaks 1 through 6 in the 14 normals, as calculated by the computerized peak integrator in arbitrary units, were found to be 70 (standard deviation [SD], 58), 82 (SD, 61), 57 (SD, 53), 65 (SD, 53), 136 (SD, 76), and 107 (SD, 68), respectively.

Three abnormal CSFs were studied. Figure 2A represents the chromatogram of a patient with severe, relapsing cryptococcal meningitis. Areas of peaks 1 through 6 were found to be 11, 9, 1, 2, 33, and 25, respectively, denoting a significant depression of more than one SD in each case.

Figure 2B represents the chromatogram of a patient who was being observed in the outpatient clinic after numerous previous admissions for cryptococcal meningitis. There had been eight negative CSF cultures during the previous 4 months. On the day that this CSF was obtained, the patient was subjectively unchanged, and CSF chemistries and cell counts were stable. However, culture of the spinal fluid was positive for cryptococci. Peak areas values in Fig. 2B were 3, 11, 1, 12, 65, and 54, respectively, indicating that peaks 1 through 4 are decreased by more than 1 SD, whereas the glucose peaks are within normal limits both chromatographically (less than 1 SD from the mean) and quantitatively (50 mg/100 ml in our clinical lab).

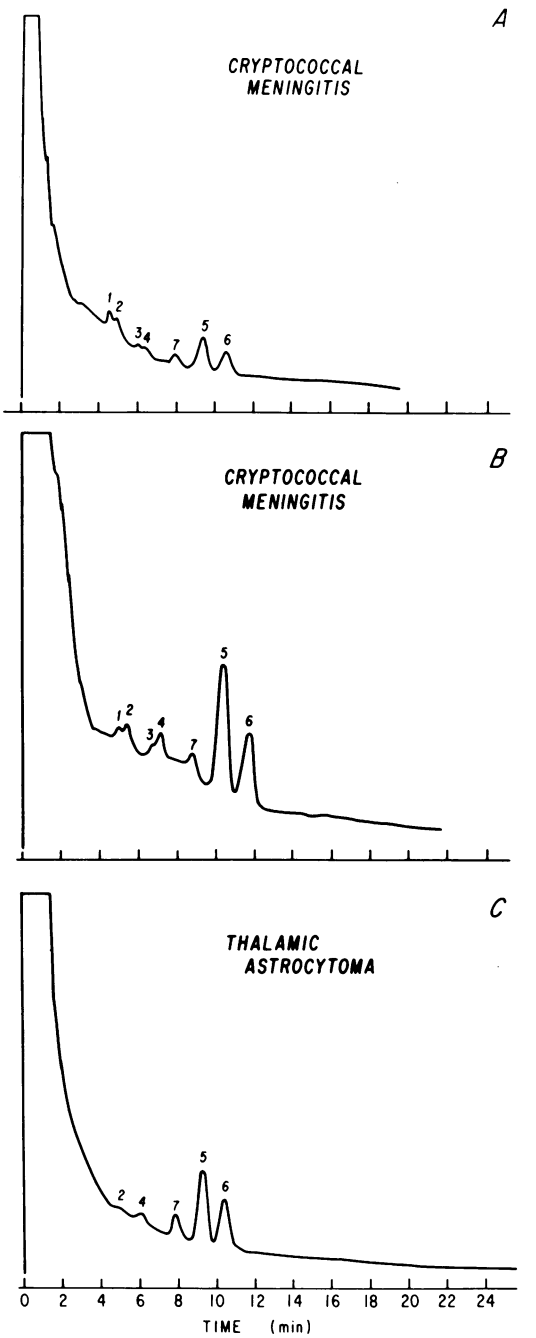


FIG. 2. *Gas chromatograms of abnormal CSF. (A) Patient with severe, relapsing cryptococcal meningitis. Note reduction in size of peaks 1 through 6 correlating with low CSF glucose concentration. Relative retention times of peaks 1 through 7 are 0.49, 0.52, 0.65, 0.69, 1.0, 1.12, and 0.85, respectively. (B) Patient with early, relapsing cryptococcal meningitis. Peaks 5 and 6 (TMS ethers of $\alpha$- and $\beta$-methyl-D-glucopyranoside) are normal in size, correlating with CSF glucose concentration of 50 mg/100 ml. Peaks 1 through 4 are depressed more than one standard deviation from the mean of 14 normals (see text). Relative retention times of peaks 1 through 7 are identical to the other chromatograms. (C) Patient with fatal thalamic astrocytoma. Note depression of all peaks. Relative retention times of peaks 2 through 7 are unchanged.*

The CSF chromatogram of one patient with a fatal thalamic astrocytoma is shown in Fig. 2C. Again there is a depression of more than 1 SD for all peaks. Integrated values for peaks 1 through 6 are 0, 5, 0, 8, 55, and 43, respectively.

## DISCUSSION

These results demonstrate that there is a highly reproducible chromatogram for TMSi derivatives of normal human CSF under the conditions specified. The peaks obtained did not vary significantly with age (subjects ranged from 15 weeks to 67 years old), diet (diets including breast feeding, tube feeding, adult regular, and intravenous fluids only), associated disease not involving the meninges (acute lymphocytic leukemia, pneumococcal pneumonia, trauma, seizure disorders, and benign prostatic hypertrophy), or certain drugs (phenobarbital, valium, ASA, prednisone, digitalis, 6-mercaptopurine, and methotrexate). This finding in normal human CSF of a reproducible chromatogram (Fig. 1) has not been achieved by investigators working with GLC analysis of other body fluids such as urine (8) and serum (9). Thus, the blood-brain barrier serves to decrease or eliminate those changes due to diet, drugs, and other nonpathological factors which make the analysis of serum and urine difficult.

Our results also reveal two unknown components of human CSF. Other investigators, using GLC (with different derivative techniques) and other methods, have described in CSF glucose, citrate, homovanillate, 3-methoxy-4-hydroxyphenethylene glycol, 5-hydroxyindoleacetate, 3,4-dihydroxyphenylacetate, homovanillyl alcohol, acid mucopolysaccharide, and numerous fatty acids (2, 3, 6, 12–15). Mass spectrograms of peaks 1 and 2 were similar but not identical to the TMSi derivative of 3-*O*-methyl-glucose. Peaks 1 through 4 may represent previously undescribed components of human CSF since their spectrogram is not compatible with the compounds described by other investigators. However, it is possible that they represent in vitro hydrolysis breakdown products of the acid mucopolysaccharide described by Constantopoulos and Dekaban (3). This question merits further investigation.

Significant changes of the CSF chromatogram were demonstrated in all three abnormal spinal fluids (Fig. 2). These aberrations consisted of diffuse peak depression in every case, whether the underlying meningeal inflammation was due to cryptococcosis or metastasizing thalamic astrocytoma. The selective depression of peaks 1 through 4 very early in the course of one patient's cryptococcal relapse (Fig. 2B) might indicate that these sugars are better indicators of early meningeal inflammation than glucose and CSF protein. The appearance and persistence of peak 7 in all three abnormal spinal fluids may be a significant lead, and it will be characterized by mass spectroscopy.

Although GLC of CSF from two patients with cryptococcal meningitis showed only a decreased concentration of CSF sugars, the rapid diagnosis of meningeal infection by detecting chromatographic changes in spinal fluid is still an exciting possibility. Many microorganisms probably release unique metabolic intermediates and cell wall breakdown products into the surrounding media, and distinctive host response products may be induced by some invasive agents. In either case, now that a reproducible chromatogram of normal CSF has been established, more samples of abnormal CSFs should be screened for diagnostic changes.

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