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Note

Elevation of certain polyols in the cerebrospinal fluid of patients with multiple sclerosis

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Multiple sclerosis (MS), a severely disabling disease of the central nervous system (CNS), may be caused by vascular factors, disturbed immune mechanisms, viral infections, or metabolic alterations. While a combination of various factors [1] is believed to be ultimately responsible for the demyelination process in MS, much remains to be learned about the disease etiology. Metabolic alterations of either genetic, dietary, or immune origin could modify certain CNS structures and prepare them for attack by a pathogenic agent (e.g., a virus).

Since lipids are major constituents of the myelin sheath, several studies have been concerned with this class of compounds or related factors [1—7]. Other biochemical studies of this disease are currently rare. This work suggests that an additional biochemical factor, related to the metabolism of carbohydrates, may be implicated. The enhanced concentrations of fructose and sorbitol observed in this study with MS patients suggest the existence of the "sorbitol pathway." This pathway is also believed to be implicated in diabetic neuropathies [8—12].

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EXPERIMENTAL

During the development of multicomponent analytical methodology for polyols [13] and other constituents [14] of human cerebrospinal fluid (CSF), a variety of pathological samples were screened together with normal specimens (i.e., from patients with slipped or cracked disks, but no other known pathological problems). CSF samples (obtained by lumbar puncture) from seven patients diagnosed as having definite symptoms of MS were used to record polyol metabolic profiles [13]; all of these samples were also found positive for oligoclonal bands [15, 16] in the electrophoresis of CSF proteins. Samples of 0.5 ml CSF were first subjected to chromatography on a 4 cm × 0.5 cm DEAE Sephadex column to remove interfering compound classes [14]. The fraction eluted with 5.0 ml deionized water was collected and lyophilized to dryness. Dodecanol was added as an internal standard [13], and 100 μl TSIM (trimethylsilylimidazole, from Pierce, Rockford, IL, U.S.A.) was used to derivatize the samples at 70°C for 5 h. Removal of excess reagent on a Lipidex-5000 column, sample preconcentration and chromatography on a glass capillary column were carried out as described in a previous publication [13].

RESULTS AND DISCUSSION

While analyzing the data obtained from the seven MS patients versus six normal samples, increases in fructose and sorbitol (glucitol) were consistently observed in the former sample type. Fig. 1 shows a typical example of such elevations, while no substantial variations are seen in the remaining identified polyols [13].

A summary of the concentration of polyols, including the standard deviations, is given in Table I. Because of the widely varying concentrations of the different polyols in CSF, normal levels have been scaled to 1.0 to permit inclusion of all data points on the same graph (Fig. 2), indicating also the range of normal values. The points plotted for the MS patients represent average values. A significant difference in the means (normal versus MS) for fructose is placed at better than 99% confidence level, while it is somewhat less for sorbitol (more than 95% confidence).

While there is little doubt about a characteristic elevation of fructose and sorbitol in the MS patients as indicated above, a possible biochemical explanation is also readily offered. The following pathway has been postulated [9] to result in accumulation of fructose and sorbitol:

$$\text{D-glucose} + \text{NADPH} + \text{H}^+ \xrightarrow[\text{reductase}]{\text{aldose}} \text{sorbitol} + \text{NADP}^+$$

$$\text{sorbitol} + \text{NAD} \xrightarrow[\text{dehydrogenase}]{\text{sorbitol}} \text{D-fructose} + \text{NADH} + \text{H}^+$$

It is of interest that this polyol pathway is suspected of having implications in neurological complications of diabetes mellitus [8, 11, 12] in humans and the CNS damage in experimental diabetic animals [9, 10]. Suggestions have been made that accumulation of these polyols may result in osmotic

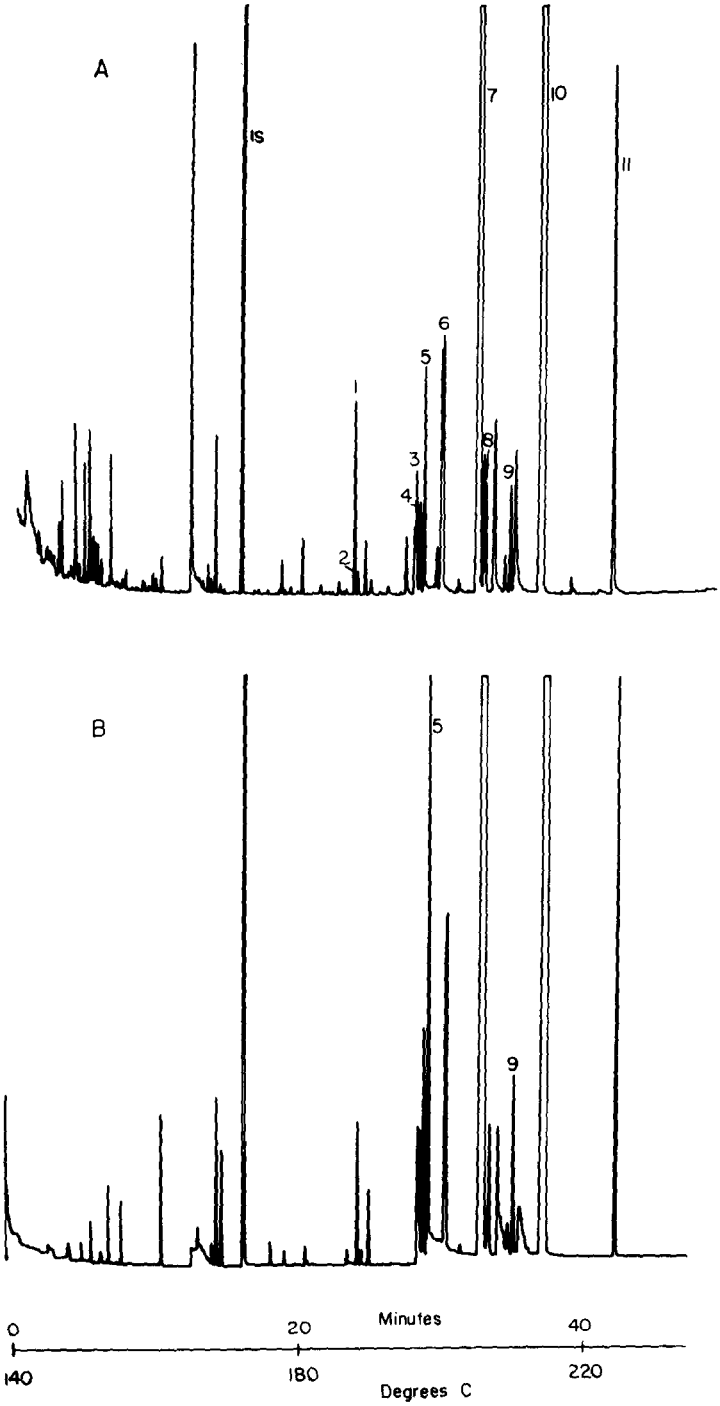

Fig. 1. Chromatograms of the polyols in CSF. (A) Normal sample; (B) sample from patient with multiple sclerosis. *Peaks*: IS = dodecanol (internal standard); 1 = arabinitol; 2 = ribitol; 3 and 5 fructose; 4 and 8 = mannose; 6 = 1,5-anhydroglucitol; 7 and 10 = glucose; 9 = glucitol (sorbitol); 11 = myo-inositol. Conditions: 50 m × 0.25 mm I.D., glass capillary column, temperature programmed from 140°C to 240°C at 2°C/min; flame-ionization detector.

TABLE I

CONCENTRATION OF POLAR NEUTRAL COMPOUNDS IN THE CEREBROSPINAL FLUID OF NORMAL AND MULTIPLE SCLEROSIS PATIENTS

Concentrations (mg/l ± S.D.) are based on six normal and seven multiple sclerosis samples.

Compound	Normal	Multiple sclerosis
Ribitol	3.4 ± 2.0	2.6 ± 3.8
Fructose	7.6 ± 3.6	31.2 ± 11.5
Mannose	8.5 ± 5.5	12 ± 8
1,5-Anhydroglucitol	37.1 ± 16.5	44 ± 12
Glucose	789 ± 275	838 ± 204
Glucitol (sorbitol)	4.8 ± 3.0	9.4 ± 3.2
Inositol	54 ± 12	49 ± 11

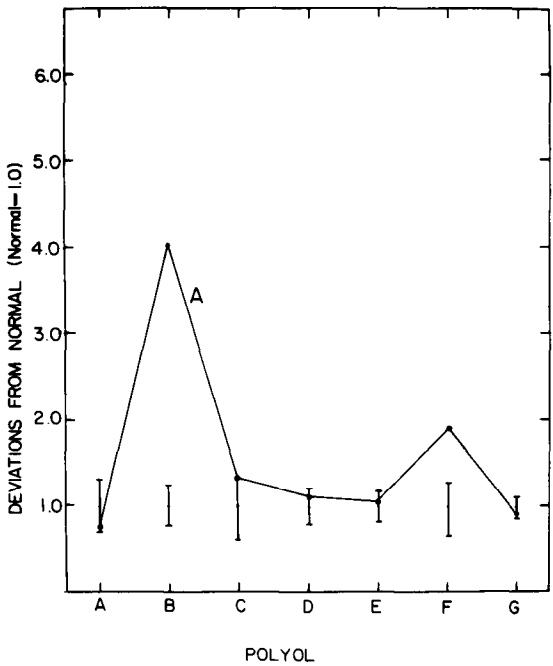

Fig. 2. Normal values for six CSF polar neutral compounds scaled to 1.0 (range of standard deviations indicated). Ordinates: A = ribitol; B = fructose; C = mannose; D = 1,5-anhydroglucitol; E = glucose; F = glucitol (sorbitol); G = myo-inositol. A = plot of average values for polyols in CSF from seven multiple sclerosis patients.

dysequilibrium [12] and subsequent cellular damage, induced abnormalities in lipid synthesis by Schwann cells [17], and the demyelination process itself [18]. If the above were consequences of the polyol accumulation in diabetic neuropathies, the elevated CSF levels of fructose and sorbitol reported in this work would suggest a similar mechanism to be a contributory factor to MS conditions.

While remaining polyols (as seen in Figs. 1 and 2) may have clinical significance in various other CNS diseases [13, 19, 20], we found their levels in MS

within the normal range. This includes inositol in spite of its obvious importance for the cerebral tissue. Only slight elevations of this substance have been found in the CSF of patients with diabetic neuropathies [12].

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