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Note

Two isotachophoretically separated components in human cerebrospinal fluid identified as folic acid and uric acid

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The technique of isotachophoresis was first used in 1975 to separate and analyze human cerebrospinal fluid (CSF) [1, 2]. Since then a number of workers have examined the CSF from patients with a variety of different neurological disorders (e.g., multiple sclerosis, acute lymphocytic leukaemia, subacute sclerosing panencephalitis, viral and bacterial meningitis) and have identified some of the separated components using the spiking method and the Tachofrac (LKB, Bromma, Sweden) [3—9].

There has, however, been variable reporting of UV-absorbing substances migrating ahead of the albumin component [1, 4, 5]. These front running peaks (FRPs) are consistently found in the CSF and this study was undertaken to identify some of these components. Two of the FRPs are identified as folic acid and uric acid.

EXPERIMENTAL

The following reagents were used: morpholinoethanesulphonic acid (MES, Sigma); 2-amino-2-methyl-1,3-propanediol (Ammediol, Sigma); hydroxypropyl methylcellulose (HPMC, Sigma); ϵ-aminocaproic acid (EACA, Sigma); barium hydroxide (Sigma); Ampholine carrier ampholytes pH 6—8, pH 7—9, pH 9—11 (LKB); chromatographic-quality glycine, valine, leucine and β-alanine (BDH); folic acid (pteroylglutamic acid, Sigma); uric acid (Merck); uricase (Boehringer Mannheim).

CSF samples

Two CSF samples were used in this study. The control CSF, for spiking ex-

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periments, was obtained from a patient undergoing myelography for leg pain, probably associated with disc problems. The second CSF sample, for the uricase experiments, was from a patient with meningitis of unknown cause. Both CSF samples had been previously stored at −20°C.

Isotachophoresis

The LKB 2127 Tachophor (LKB) was used in this investigation. Separation was carried out in a PTFE capillary tube (23 cm × 0.5 mm I.D.), kept at a constant temperature of 15°C. A UV detector equipped with a 280-nm filter was used. The Tachophor was connected to a Hewlett-Packard 3390 A reporting integrator (Hewlett-Packard). Chart speed set at 6 cm/min. The operational electrolyte system is given in Table I.

TABLE I

ELECTROLYTE SYSTEM FOR THE ISOTACHOPHORESIS OF HUMAN CSF

	Electrolyte	
	Leading	Terminating
Anion	MES (0.005 *M*)	EACA (0.005 *M*)
Counterion	Ammediol (0.010 *M*)	None
pH	9.1	10.6*
Additive	HPMC (0.5%, mass/volume)	None

*Terminating electrolyte adjusted to pH 10.6 with a saturated aqueous solution of barium hydroxide.

The initial current setting was 200 μA and this was reduced to 50 μA when a voltage of 10 kV had been reached. During detection at 50 μA the voltage rose from 4 to 8 kV. The sample was 9 μl of a CSF—spacer mixture. This mixture was prepared in the following manner: to 30 μl CSF were added 4 μl of an amino acid spacer solution (6 mg each of glycine, valine, leucine and β-alanine dissolved together in 10 ml distilled water) and 0.6 μl of the ampholyte spacer solution (equal volumes of 1%, v/v, aqueous solution of ampholytes with pH ranges 6—8, 7—9 and 9—11).

RESULTS

Fig. 1a shows the control CSF separation pattern with the positions of the FRPs, albumin and gammaglobulins indicated. The addition of 1 μl saturated aqueous solution of folic acid (concentration 3.6 μ*M*) and 1 μl saturated aqueous solution of uric acid (concentration 0.4 m*M*) separately to the control CSF sample shows an increase in height and area of FRP 1 and FRP 3, respectively (Fig. 1b and c).

Further evidence confirming the identity of FRP 3 as uric acid was obtained when a CSF sample from a patient with meningitis was found to have an enlarged FRP 3 when analyzed by isotachophoresis (Fig. 2a). When this CSF was incubated with uricase (60 μl CSF + 1.5 μl of 2 mg/ml uricase at 37°C for 30 min) and re-examined, the FRP 3 was considerably reduced (Fig. 2b).

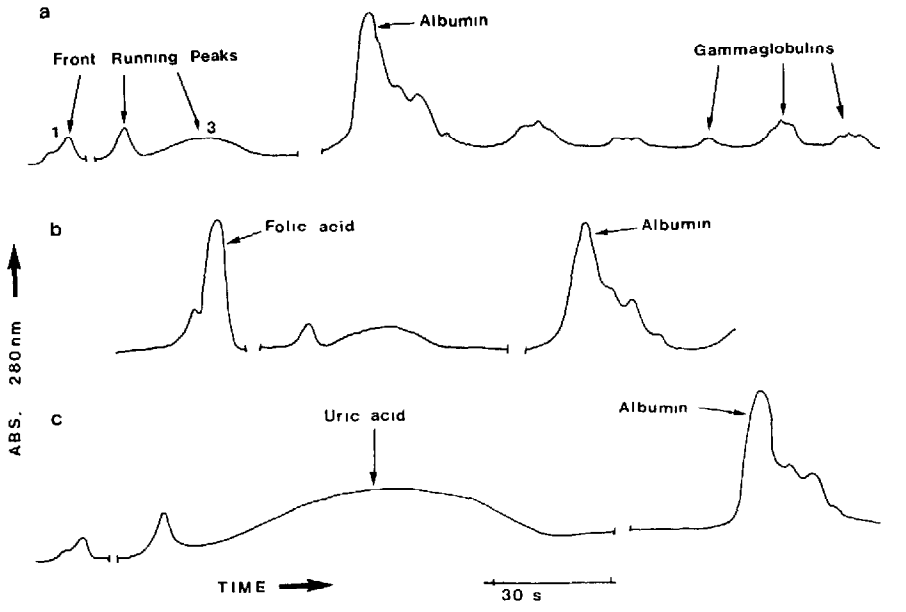

Fig. 1. Isotachopherogram of a normal CSF sample showing the positions of the FRPs, albumin and gammaglobulins (a). The addition of 1 μl of a saturated aqueous solution of folic acid (b) and uric acid (c) increases the area of FRP 1 and FRP 3, respectively.

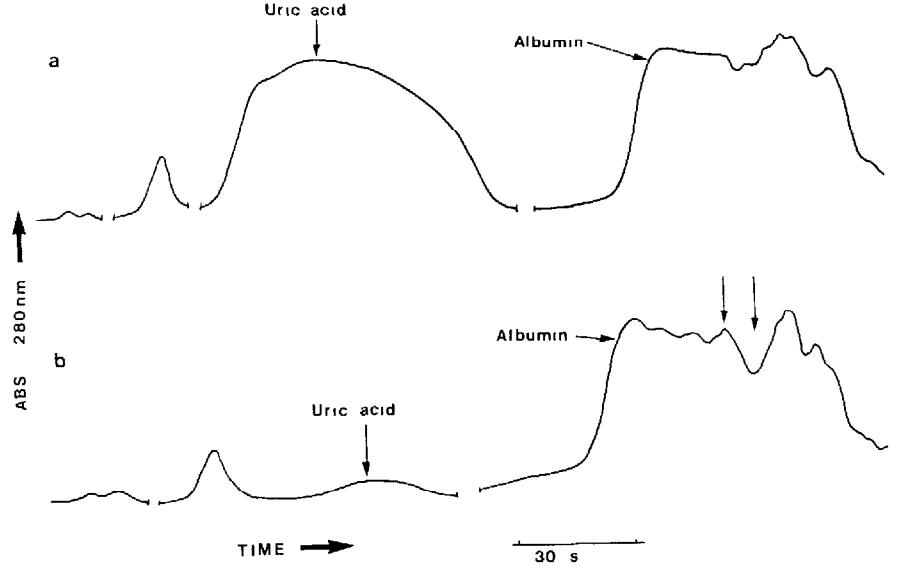

Fig. 2. (a) Isotachopherogram of a CSF sample from a patient with meningitis showing an enlarged FRP 3. Incubation of the CSF sample with uricase results in a reduction of FRP 3; two changes to the CSF profile are indicated by arrows (b).

One would expect to see evidence of the breakdown product of uric acid, allantoin and the enzyme in the isotachopherogram. Two changes in the CSF profile were noted in Fig. 2b (arrows). Firstly, an extra UV-absorbing peak appeared which was confirmed to be the enzyme when more of the enzyme

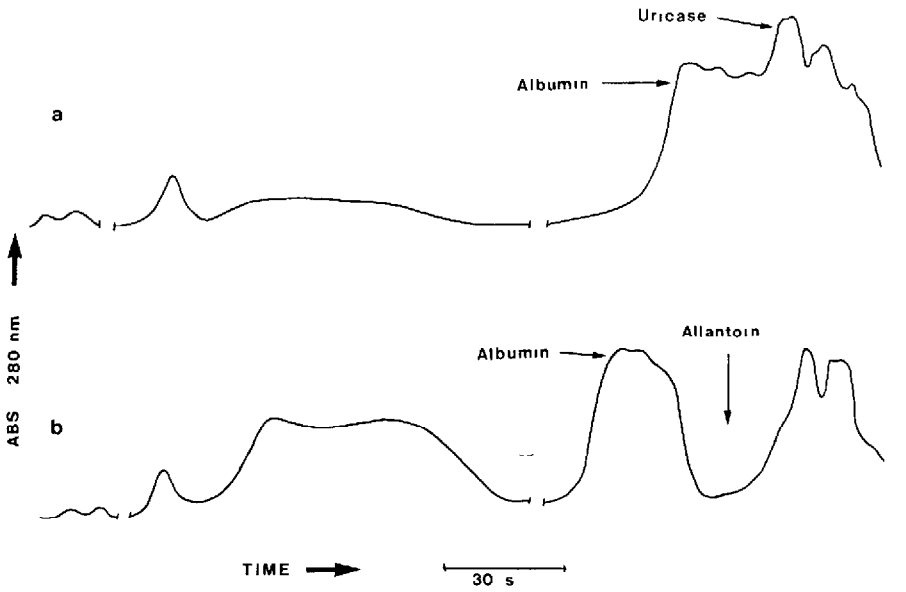

Fig. 3. Isotachopherogram of the same CSF sample as in Fig. 2a with 1 μl of 1 mg/ml allantoin (a) and 1 μl of 2 mg/ml uricase (b) added separately.

was added to the CSF (Fig. 3a). A non-UV-absorbing zone also appeared and this was proved to be allantoin using the spiking method (Fig. 3b).

DISCUSSION

Uric acid and folic acid are both naturally occurring substances in the CSF of normal healthy subjects. The normal CSF folic acid concentration is 17.6—67 ng/ml [10]. The brain, however, cannot utilize folic acid in this form and it is reduced to the methyltetrahydrofolate form which is actively transported from the blood into the CSF via the choroid plexus [11—13]. This concentration is 2.5—5 times higher than in the serum [13]. Reduced levels of serum folate have been found in multiple sclerosis, peripheral neuropathy, organic brain disease and myelopathy [14].

It may be noted that methotrexate, a drug used in the treatment of acute lymphocytic leukaemia, also migrates as an FRP in a position close to the folic acid peak (unpublished observation). As methotrexate has a molecular structure almost identical to folic acid, this would be expected. It was in fact this observation that led to the idea that folic acid may be the first FRP.

Uric acid, a breakdown product of purine metabolism, occurs in normal CSF at a concentration of 0.25 mg/dl [13]. Elevated levels of CSF uric acid have been found in meningitis [15], with increasing age [16], alcohol withdrawal states [17], early and definite atrophy [18]. The CSF uric acid concentration of the patient with meningitis examined by isotachophoresis in this study was 4 mg/dl, 16 times higher than the normal concentration.

Elevated levels of serum uric acid may also be reflected in the CSF [13]. An increase in serum uric acid is seen in gout, leukaemia, haematological disorders and where there is decreased renal excretion induced by drugs [19].

The size and shape of the uric acid curve, as seen on an isotachopherogram, may vary greatly. This peak has been noted to be increased in CSF samples where blood–brain barrier damage has occurred. CSF uric acid also appears to be increased in some multiple sclerosis patients and in patients with other neurodegenerative diseases (unpublished observations). This increase is probably due to neuronal damage with resultant nucleic acid catabolism.

Due to the variation in the amount of folic acid and uric acid in the CSF, the possibility exists that there may be some connection between the quantity of these substances and pathology of the central nervous system.

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