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Note

Influence of sample ions on the isotachophoretic separation of cerebrospinal fluid

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The isotachophoretic analysis of many samples of human cerebrospinal fluid (CSF) has consistently shown three front running peaks (FRPs)[1] which lie well ahead of albumin. The presence of these components has also been noted by Kjellin *et al.*[2] and Del Principe *et al.*[3]. The first and third of these highly mobile components have now been identified as folic acid and uric acid respectively[4] (Fig. 1).

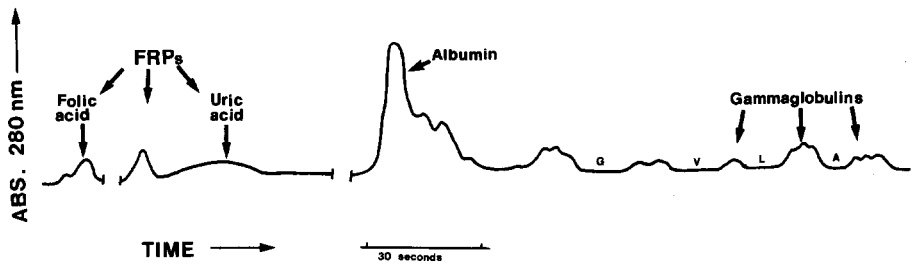

Fig. 1. Isotachopherogram of 10 μl of normal CSF with spacers. CSF (30 μl) and amino acid solution (4 μl) [6 mg each of glycine (G), valine (V), leucine (L) and β-alanine (A) dissolved together in 10 ml distilled water] and 0.6 μl ampholyte solution [equal volumes of 1% (v/v) pH 6–8, pH 7–9, pH 9–11]. Leading electrolyte, 5 m*M* morpholinoethanesulphonic acid (Sigma, St. Louis, MO, U.S.A.), 10 m*M* Ammediol, (Sigma) and 0.5% (m/v) hydroxypropylmethylcellulose (Sigma), pH 9.1; terminating electrolyte, 5 m*M* ε-aminocaproic acid (Sigma), adjusted to pH 10.6 with saturated aqueous solution of barium hydroxide (BDH, Poole, U.K.). Separation conditions started in the constant current mode at 200 μA and reduced to 50 μA when the voltage had risen to 10 kV. Chart speed, 6 cm/min. ABS = Absorbance, FRPs = front running peaks.

The concept that the FRPs move zone electrophoretically in an "out of stack" configuration[5] ahead of the isotachophoretically separated "in stack" proteins has led to certain problems. In particular, these components appear to be concentrated. It is known that 10 μl of sample occupies approximately 50 mm in the 0.5-mm diameter capillary. Thus any component moving electrophoretically must occupy a zone at least equivalent to 50 mm and probably longer due to the effects of diffusion and convection. Yet the FRPs are observed to be concentrated into a zone less than 1 mm long and must therefore be migrating isotachophoretically (in stack) and obeying the Kohlrausch regulating function[6].

To investigate this problem the morpholinoethanesulphonic acid (MES)–Ammediol leading electrolyte[7] was exchanged for a chloride leading electrolyte (HCl–

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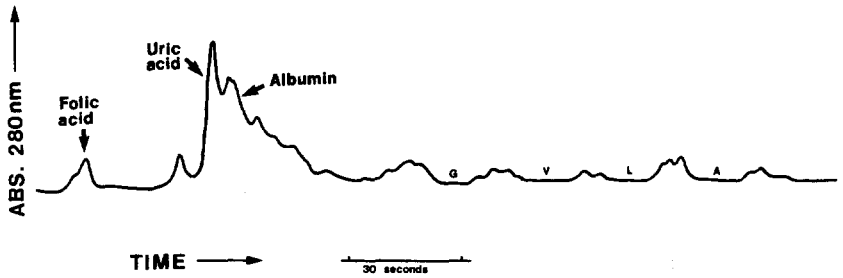

Fig. 2. Isotachopherogram of normal CSF. Leading electrolyte, 5 m*M* hydrochloric acid, and 0.5% (m/v) hydroxypropylmethylcellulose adjusted to pH 9.1 with solid Ammediol; terminating electrolyte and separation conditions as in Fig. 1.

Ammediol). The terminating electrolyte was ε-aminocaproic acid. The same CSF was analysed in the new leading electrolyte system (Fig. 2).

As can be seen from Fig. 2, all the components appear to be moving isotachophoretically as the mobility of the FRPs now fits the leading–terminating frame and therefore they are stacked. There is more efficient concentration of the FRPs and the zones are in contact with each other and with albumin. It may be concluded that the FRPs, in particular folic acid and uric acid, have mobilities greater than the MES ion and less than the chloride ion.

A possible explanation of the behaviour of the FRPs in the MES system is that they are being regulated by the chloride ions which are present as sodium chloride in the CSF sample. Although this regulation is not optimum, it could result in the concentration effect expected from isotachophoresis.

To investigate this hypothesis, the CSF sample was dialyzed for 18 h at 4°C against distilled water using a dialysis membrane with a molecular weight cut-off of 1000 daltons (Spectrum Medical Industries) in order to remove the sodium chloride. A 10-μl sample of the dialyzed CSF with spacers was analyzed in the MES system (Fig. 3a).

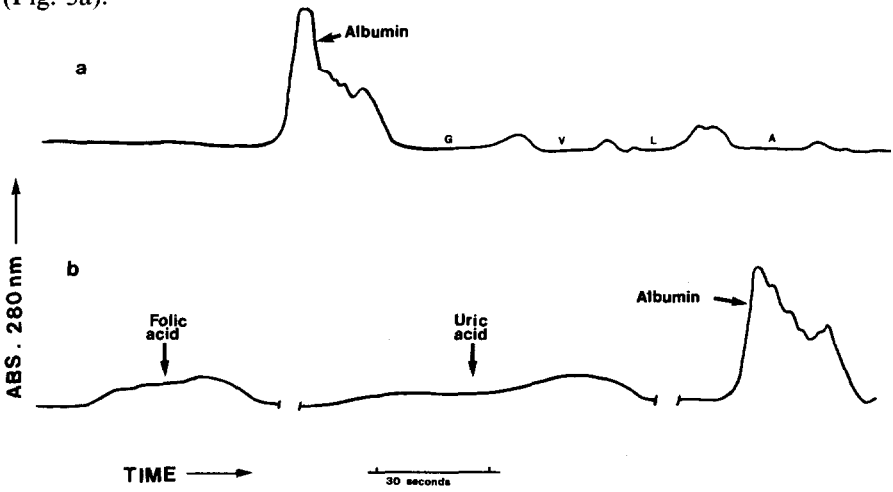

Fig. 3. (a) Isotachopherogram of dialyzed CSF analysed in the MES leading electrolyte system as described in Fig. 1. (b) Isotachopherogram of dialyzed CSF analysed in the MES leading electrolyte system with 1 μl saturated folic acid and 1 μl saturated uric acid in distilled water added.

The isotachopherogram shows that the FRPs are absent while the pattern of the protein peaks is normal. The absence of FRPs indicates that their molecular weight must be less than 1000 daltons. However, as the identity of two of the FRPs is now known, folic acid and uric acid were added to the dialyzed CSF and a sample analyzed in the MES system (Fig. 3b).

The isotachophoretic pattern obtained in the absence of chloride ions of the dialyzed CSF with added folic acid and uric acid, clearly indicates that the FRPs have migrated zone electrophoretically. The peaks are broad and have a reduced peak height and the components these peaks represent occupy at least 50 mm in the capillary.

A further consequence of the hypothesis that the chloride ions derived from the sample are acting as leading ion, is that the MES present in the system would take the role of a spacer.

This was confirmed by first analysing a dialyzed CSF with folic acid and uric acid added in the chloride leading electrolyte system and finding a typical "in stack" isotachopherogram (Fig. 4a). This sample was re-run with 1 μl of 5 m*M* MES added to the sample (Fig. 4b). It may be seen that the MES has indeed slotted in, between the uric acid and albumin and is obviously acting as a spacer.

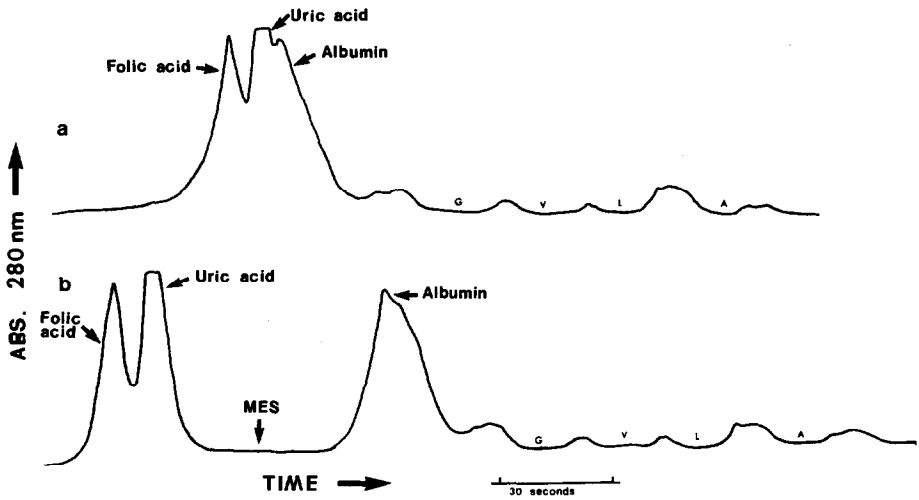

Fig. 4. (a) Isotachopherogram of a dialyzed CSF sample with 1 μl saturated folic acid and 1 μl saturated uric acid analyzed in the chloride leading electrolyte system as described in Fig. 2. Terminating electrolyte as in Fig. 1. (b) Isotachopherogram of a dialyzed CSF sample as in (a) with 1 μl 5 m*M* MES added.

These results indicate that the sample ions have a great influence on the isotachophoretic resolution of a sample. In the present study, the chloride ion as NaCl present in the CSF sample takes over the role of the MES as the leading ion. The portion of the MES electrolyte that has been overtaken by the chloride ions acts as a spacer.

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