MEASUREMENT OF BLOOD/CSF PERMEABILITY COEFFICIENTS AND OF INTRATHECAL SYNTHESIS OF IgG BY CAPILLARY ISOTACHOPHORESIS

P. Delmotte

National Center for Multiple Sclerosis, Melsbroek, Belgium

Although a considerable amount of time and effort has been spent to study the immunological response on the cellular level in multiple sclerosis, no clear-cut pattern has emerged until now. The difficulties to standardize the cellular tests and the overlapping of results between multiple sclerosis and other neurological diseases, have hampered their use for diagnostic purposes.

On the contrary, it is a well established fact, that the qualitative and quantitative study of the humoral immunological response within the central nervous system, remains, until now, one of the most important parameters for the diagnosis of inflammatory diseases of the central nervous system, and this is especially true for multiple sclerosis, (1).

For this purpose, several techniques have been proposed and used: zone electrophoresis, immunoelectrophoresis, rocket electrophoresis, isoelectric focusing, to only name some of them.

During the last two years, we have been experimenting with capillary isotachophoresis of the serum and cerebrospinal fluid proteins. We have been able to show that this new electrophoretic technique offers some interesting advantages:

1) exactly controlled working conditions lead to very reproducible results and sample solutions can be quantitatively injected.
2) protein fractions are detected by their UV absorbance under dynamic equilibrium conditions.
3) peak areas of separated protein fractions are a direct measure of the absolute amount of protein present.

A detailed description of the entire experimental set-up is beyond the scope of this presentation. For technical details see Delmotte (2).

The lower limit of detection lies around 10 nanograms of protein.

Unconcentrated cerebrospinal fluid can be used, but the high salt content adversely influences the separation results.

We concentrate the cerebrospinal fluid about 10 times and 4 microliter of this concentrate are injected. For serum only 0.6 microliter are used.

Figure 1 shows the isotachophoretic separation patterns of the serum and cerebrospinal fluid of the same normal individual. By manipulation of the composition of the spacer mobility gradient, one can get a clear cut separation of albumin and at the same time a mobility subfractionation of the immunoglobulin G fraction. The non-UV absorbing zones are due to amino-acids injected together with the sample.

Figure 1 : a-(upper fig.) Capillary isotachophoresis of serum.
   b-(lower fig.) Idem for cerebrospinal fluid from same individual.
Marker amino-acids: A=beta-alanine; V=valine; G=glycine.