Journal of Chromatography, 569 (1991) 281–296
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5957

Review

Analysis of cerebrospinal fluid proteins by electrophoresis

F. WIEDERKEHR

Institute of Clinical Chemistry, University Hospital Zürich, 8091 Zürich (Switzerland)

(First received December 29th, 1990; revised manuscript received January 24th, 1991)

ABSTRACT

The cerebrospinal fluid (CSF) is a specific ultrafiltrate of plasma, which surrounds the brain and spinal cord. The study of its proteins and their alteration may yield useful information on several neurological diseases. By using various electrophoretic separation techniques, several CSF proteins have been identified derived from plasma or from brain. Different one-dimensional methods, such as agarose gel electrophoresis and isoelectric focusing, are of similar value in identifying the non-specific oligoclonal bands, which are mainly helpful in the diagnosis of multiple sclerosis and other inflammatory diseases. Isoelectric focusing has a greater resolution than other one-dimensional methods, and it yields additional data about disease-associated proteins occurring in Alzheimer's disease, Huntington's chorea and amyotrophic lateral sclerosis. Silver-stained two-dimensional gels provide more information about the complex protein composition of CSF, particularly about proteins produced in the brain, such as apolipoprotein E and neuron-specific enolase. For the detection of oligoclonal antibodies, the investigation of protein changes revealed by Parkinson's disease, schizophrenia and Creutzfeldt–Jakob disease, and the analysis of CSF immune complexes, two-dimensional electrophoresis has a greater sensitivity.

CONTENTS

0378-4347/91/$03.50

LIST OF ABBREVIATIONS

AGE	Agarose gel electrophoresis
ALS	Amyotrophic lateral sclerosis
Apo	Apolipoprotein(s)
CSF	Cerebrospinal fluid
CNS	Central nervous system
2-DE	Two-dimensional electrophoresis
IC	Immune complex(es)
Ig	Immunoglobulin(s)
IEF	Isoelectric focusing
MS	Multiple sclerosis
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecylsulphate
SSPE	Subacute sclerosing panencephalitis

1. INTRODUCTION

Cerebrospinal fluid (CSF) is normally a clear fluid that surrounds the brain and spinal cord, providing cushioning against trauma. It is produced by ultrafiltration of proteins, ions, water and other constituents through the vascular epithelium, basement membrane, and epithelium of the choroid plexus [1]. After circulation around the brain and spinal cord, it is reabsorbed into the venous circulation through the arachnoid granulations on the superior surface of brain. The CSF affords the central nervous system (CNS) a unique and highly specialized environment. Products of metabolism from various sites within the brain are excreted into the interstitial space, and subsequently via bulk flow or net diffusion into the CSF. In this manner, the metabolites are rapidly diluted. The CSF is produced at a rate of some 500 ml per day and occupies a volume of *ca.* 135 ml in the normal adult. Consequently, the CSF has an average turnover time of 6 h [1].

Most proteins contained in the CSF normally come from the blood, their concentrations in the CSF being determined largely by their molecular size and the relative impermeability of the blood–CSF barrier to large molecules [2,3]. A small proportion of proteins are locally produced in the CNS. The normal concentration of CSF total proteins is 150–450 mg/l. Infants and older individuals show higher concentrations; ventricular and cisternal CSF have lower protein concentrations than lumbar CSF. However, ventricular fluid has a higher proportion of low-molecular-mass proteins than lumbar fluid. This is due to the progressive equilibration of CSF with plasma through the capillary walls during the passage down the spinal canal. The selectivity of the barriers for albumin and immunoglobulin G (IgG) is, however, the same [4].

The CSF reflects the state of the nervous system in the diseased state as well as in health. Pathological conditions of the brain or spinal cord often produce qualitative or quantitative alterations in the protein compositions. Therefore, examinations of CSF proteins is an important diagnostic aid in neurology. The CSF is usually obtained by lumbar puncture from the lumbar subarachnoid space, in the region of cauda equina. Normal CSF is colourless and clear, but xanthochromia (yellow colour) may occasionally occur from a traumatic lumbar puncture, which prohibits reliable diagnostic information. When blood has been present in the CSF for more than 4 h, a pale orange xanthochromia appears due to haemoglobin pigment. Also, a very high protein content or severe jaundice may produce a faint yellowish colour that can stimulate xanthochromia. Turbidity in the CSF may result from large numbers of leucocytes or bacteria.

For protein studies, the measurement of CSF total proteins is a part of routine CSF examination. Various values have been suggested as a normal protein content in the CSF, but such values depend largely on the method of testing. Low levels are relatively rare, but elevation of total proteins occurs in many diseases, especially in cases of inflammation, infection and tumours. There is often an increase in the permeability of the blood–CSF barrier, leading to elevated concentrations of CSF proteins. Some acute neurological conditions, such as bacterial meningitis, can be fatal, and speedy diagnosis is essential. More information than merely the measurement of CSF total proteins is expected from the determination of certain protein fractions. The determination of albumin is a good marker of barrier functions [5]. Many other individual proteins have also been examined. The quantitative analysis of high-molecular-mass proteins can be a more reliable indicator of mild barrier disturbances [6]. One difficulty of this approach, however, is that large proteins, *e.g.* fibrinogen and α2-macroglobulin, are present in the CSF in very low concentrations. Immunoglobulins are not normally synthesized in the CNS, and enter the CSF by diffusion. They comprise *ca.* 10% of the total proteins. The predominant Ig class is IgG; only very small amounts of IgM and IgA are present owing to their larger molecular size.

Following the earlier studies of Kabat *et al.* [7], it has become generally accepted that selective elevation of γ-globulins in the CSF indicates local production of proteins within the CNS. In inflammatory conditions, the migration of lymphocytes into the CNS tissue mainly results in synthesis and release of Ig into the extracellular space and subsequently the CSF. This increase is predominantly due to IgG, although increases if IgM and IgA can be detected [8,9]. Selective elevation of Ig in the CSF has been especially found in multiple sclerosis (MS), as well as in other CNS diseases, such as neurosyphilis, neuroborreliosis, or in subacute sclerosing panencephalitis (SSPE), a rare, demyelinating disease of childhood.

2. FRACTIONATION OF CSF PROTEINS

For the investigation of CSF proteins in health and disease, several protein

separation techniques have been employed, in addition to methods used for the immunological identification and quantification of single proteins. The most common separation technique for demonstrating individual protein fractions is, at present, electrophoresis. Electrophoresis of CSF proteins has been carried out on a variety of supporting media (cellulose acetate, agarose, polyacrylamide, etc.), mainly in order to define so-called oligoclonal IgG, which is suitable for the clinical laboratory. Each of these methods has certain advantages and disadvantages, and different resolving power.

2.1. Agarose gel electrophoresis

An overview of CSF protein composition can be obtained by performing agarose gel electrophoresis (AGE) on a sample that has been concentrated to *ca.* 10–15 mg/ml. CSF proteins are resolved according to charge, resulting in several zones containing from a few to many nearly equally charged polypeptides. The method is well established in the laboratory and has a reasonably good reproducibility. The AGE pattern of a normal adult reveals a prominent prealbumin fraction that migrates slightly faster than plasma prealbumin. Prealbumin is synthesized by the choroidal epithelium [10]. A slight disturbance of the blood–CSF barrier is frequently indicated by a slight decrease of the prealbumin band. Albumin is the major fraction on AGE, comprising 55–70% of the CSF proteins. The α1-band consists primarily of α1-antitrypsin. An increase of this band can indicate unspecific damage to nervous cells: an acute vessel syndrome, an epileptic attack, an acute relaps of MS or Tay–Sachs disease [11]. The α2-region contains large proteins, such as the polymeric haptoglobin phenotypes. Transferrin is detected in the β1-region, and the major β2-region contains the τ-protein. τ-Protein is a desialylated transferrin present in significant amounts in the CSF, but essentially absent from the blood because of its rapid removal by asialo-receptors present on reticuloendothelial cells [12]. An increased proportion of τ-protein relative to transferrin can be seen when there is release of lysosomal hydrolases into the CSF due to cell death. This may be enhanced if there is also a reduction in the flow of CSF, as seen, for example, in vertebral disc protrusion [12]. Transferrin can also be locally synthesized within the CNS. Messenger RNA coding for transferrin has been identified in choroid epithelial cells [10].

The band pattern found in the γ-region consists almost exclusively of IgG. The banding is very faint in normal samples. In some samples, however, γ-trace protein may be present at the cathodal end of the γ-region. It is a cysteine proteinase inhibitor (also called cystatin C) of low molecular mass, which is synthesized within the CNS, probably in the astroglia [13]. So far, it is of clinical interest only, inasmuch as its CSF concentration has been found to be subnormal at an early age in patients with hereditary amyloidosis of a type where amyloid depositions in cerebral vessels follow in middle age [14]. AGE has been found to be highly sensitive for evaluation of alterations in the γ-globulin region. Of the procedures

used for determination of oligoclonal antibodies, it is at present the most common. Abnormal electrophoretic patterns of a γ-globulin increase, with or without total protein increase, have been reported in cases of MS, neurosyphilis, meningitis, SSPE and a few others, as described above. These oligoclonal antibodies have been most investigated in MS and are now considered useful in supporting the clinical diagnosis of MS [15]. Oligoclonal antibodies are found in over 90% of MS patients. They occur, however rarely, in patients with non-inflammatory neurological diseases, such as amyotrophic lateral sclerosis (ALS), epilepsy, vertigo, Parkinson's disease, neoplasms and Alzheimer's disease [16,17]. An increase of the γ-region in the CSF is also observed in association with systemic diseases in which there is elevated serum γ-globulin, *e.g.* multiple myeloma, liver cirrhosis, sarcoidosis and collagen disease [18]. Therefore, it is recommended that serum electrophoresis be carried out in conjuction with CSF electrophoresis.

2.2. Electrophoresis on polyacrylamide gels

Modern electrophoretic techniques, such as sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF) in PAGE, and a combination of these two techniques, two-dimensional gel electrophoresis (2-DE), offer a greater reproducibility and a higher resolving power for CSF proteins, on both the analytical and preparative scales.

2.2.1. SDS-PAGE

Separation based directly on molecular mass differences was developed by Maizel [19], who treated proteins with the highly charged detergent SDS, to allow them to separate in continuous or gradient pore-size gels based solely on molecular mass. More theoretical aspects of the separation of proteins in polyacrylamide are described in special monographs [20,21]. Using this method, Carson *et al.* [22] separated CSF proteins according to molecular mass. SDS-PAGE has also been used by other authors in the diagnosis of MS [23,24]. It reveals a larger number of bands in the IgG region than AGE does. The heterogeneity demonstrated by this technique presumably reflects molecular mass differences in the CSF Ig in MS. An increase in the passage of plasma proteins into the CSF as a result of damage to the barriers can also be detected by this method [25]. It is a more sensitive method than various protein estimations because it enables the identification of impaired barrier function or CSF flow problems when the CSF total protein concentration is still within the reference range. The advantage of SDS gels is that a large volume of sample (200–300 μl) can be applied, thus obviating the need for concentration. On the other hand, with the introduction of silver staining in the electrophoretic analysis of CSF proteins, an enhancement of sensitivity is gained. SDS-PAGE with silver staining can detect up to 40 bands of CSF proteins [26].

2.2.2. Isoelectric focusing

IEF is a technique with a high resolving capacity whereby proteins are separated according to their isoelectric points. The method was made practicable by the introduction of carrier ampholytes. Proteins with differences in their isoelectric points as small as 0.01–0.02 p*I* units can be separated from each other [27]. The potential of IEF for the separation of CSF proteins was pointed out by Fossard *et al.* [28]. Since then, extensive experience has been accumulated and numerous publications written. Most of the studies have been devoted to the detection of oligoclonal bands in order to support the diagnosis of demyelinating diseases in general, and of MS in particular (*e.g.* refs. 29–32).

The principal abnormalities of the oligoclonal IgG are cathodic in location [33]. They manifest themselves as discrete bands against the polyclonal background. They represent the product of a limited number of clones of plasma cells. The normal amount of background polyclonal IgG is relatively low in the CSF because all the γ-globulins are derived from serum, so locally synthesized clonal products are easily visible even in very low concentrations. IgG that are too basic may migrate into the cathode wick as a consequence of the so-called cathodic drift. Mainly in CSF samples taken from patients with MS, proteins are present with abnormally high isoelectric points. The IgG bands are found between pH 8.6 and 9.5. In contrast, serum IgG are focused as multiple bands on IEF between pH 4.7 and 8.6 [34]. With the introduction of the electrophoretic technique of the immobilized pH gradient, it is possible to detect the highly alkaline IgG bands present in CSF. The method gives higher resolution and is less prone to interference from artefacts [35]. However, potential drawbacks in the immobiline methodology are the laborious and time-consuming preparation of the gels and the long analysis time [36]. For routine clinical work, a semi-automated microsystem (Phast System, Pharmacia-LKB, Uppsala, Sweden) with precast IEF gels has been recently developed. It permits rapid, reproducible IEF separation of CSF samples within 3 h and automated staining with Coomassie Blue or silver [37,38].

The use of a general protein stain after IEF can pose problems of interpretation. In particular, the CSF-specific β-trace and γ-trace proteins can be confused with oligoclonal bands; they change their mobility after storage. To avoid such difficulties of interpretation, immunoblotting with enzyme-labelled antiserum must be used to identify true oligoclonal bands [39]. The influence of the blood–CSF barriers should always be taken into account when looking for oligoclonal bands. In the presence of an impaired barrier, locally synthesized oligoclonal bands may be completely eclipsed by transudated polyclonal plasma Ig. Such effects may occur in cases of Guillain–Barré syndrome and in severe bacterial meningitis [40]. Fig. 1 shows IEF patterns of the CSF from a patient without clinical evidence of an inflammatory process in the CNS and from a patient suffering from MS.

In addition to the study of oligoclonal Ig fractions, considerable attention has

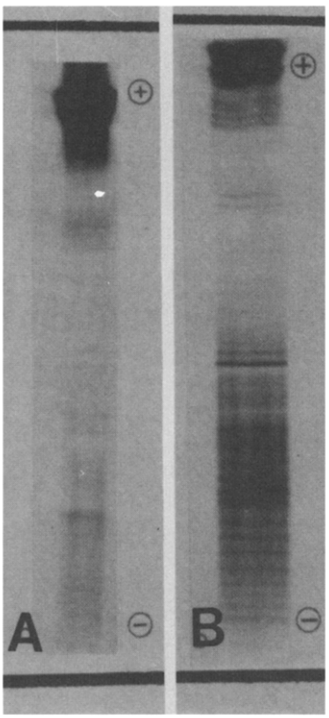

Fig. 1. Isoelectric focusing of CSF proteins carried out on a 2117 Multiphor (LKB). The polyacrylamide gel was 12.5 × 13 cm with a thickness of 1 mm, and consisted of 40 g/l acrylamide and 20 g/l ampholytes (3.5–10). Separation was carried out at 800 V for 4 h at 2°C. The gels were stained with silver diamine. (A) Normal CSF without oligoclonal bands; the polyclonal Ig form a diffuse background. (B) CSF from a patient with MS; several pathological bands can be seen in the alkaline region between pH 6.4 and 8.8.

been paid to the study of proteins in CSF derived from the CNS. Various non-plasma proteins have been demonstrated. In several studies, IEF has been adapted in order to demonstrate such non-plasma proteins or characteristic protein changes in several CNS disorders. Kjellin and Stibler [41] examined CSF samples in muscular dystrophies and in ALS. In ALS, abnormal CSF protein fractions occurred mainly in the alkaline pH range. Using IEF, Stibler [42] also noticed that, in 70% of patients with Huntington's chorea, an unidentified protein with an isoelectric point of 7.2 was present. To increase the sensitivity of CSF protein investigation by IEF, Wikkelsø and co-workers [43–45] removed serum proteins from the CSF by using immobilized antibodies to serum proteins, thus increasing the possibility of detecting very small changes in the minor CSF-specific fraction, which are normally hidden by serum proteins. The method has been tested on CSF from a group of human patients suffering from transient ischemic attack, since they represent a relatively homogenous group of patients without signs of brain damage [43]. When this method was used to study CSF proteins in dement-

ed patients, a characteristic protein pattern could be demonstrated in dementia of the Alzheimer type [44]. The changes were restricted to the CSF-specific fractions. None of these protein changes was observed in an age-matched group suffering from multi-infarct dementia. In a further study, these authors were able to detect additional bands at pH 4–5 in the CSF-specific fraction from patients with Huntington's chorea, where glioses are a predominant feature of the pathological changes [45]. Although the IEF results on CSF proteins other than oligoclonal antibodies are slightly contradictory at present, it can be concluded from these studies that several disease states lead to changes in CSF proteins.

2.2.3. Two-dimensional gel electrophoresis

There is no doubt that the highest resolution of complex protein mixtures such as CSF can be achieved only by combining two independent separation systems. The technique described by O'Farrel [46] is the basis for all 2-DE analysis of CSF proteins. A combination of IEF under denaturing conditions in cylindrical gels in the first dimension with SDS slab gels in the second dimension is usually chosen. In this manner, proteins are separated according to two independent characteristics; one is the charge, which is reflected by the isoelectric point, and the other is the molecular mass, which determines the mobility of the SDS–protein complexes. The introduction of a highly sensitive silver-staining method allows the detection of very small amounts of proteins at the nanogram level [47–49]. Silver stains generally provide more than a 100-fold increase in sensitivity over that attained by the most commonly used organic protein stain, Coomassie Blue. The 2-DE technique and silver staining have been comprehensively reviewed [50,51].

Utilizing 2-DE and silver staining, Goldman *et al.* [52] have shown that more than 300 protein fractions in the CSF can be made visible. By comparing CSF samples from twenty unrelated individuals with the corresponding plasma, they found six clusters of proteins that are more prominent in the CSF. These may represent proteins deriving mainly from the brain and spinal cord. The existence of nervous tissue-specific proteins in the CSF has been suggested for many years (*e.g.* refs. 43 and 53; for review see ref. 54) (Figs. 2 and 3).

For a better distinction between serum proteins and CSF-specific proteins on the 2-DE gel, some researchers have used immobilized antibodies against serum proteins [55–58], as described above for IEF [43]. Another method for increasing the sensitivity for detection of CSF-specific proteins consists of prefractionation of CSF proteins by size-exclusion gel permeation chromatography [58,59]. With this technique, CSF proteins are separated into two or three fractions, and each fraction can be analysed by 2-DE. For identification, CSF proteins are electrophoretically transferred from 2-DE gels to nitrocellulose sheets and subsequently detected by immunological procedures (immunoblotting) as described by Towbin *et al.* [60]. The principles methodological aspects and applications of protein blotting have been reviewed [61,62]. Some prominent CSF protein groups have been identified such as a multiple form of apolipoprotein (apo) E, apo-D, neuron-

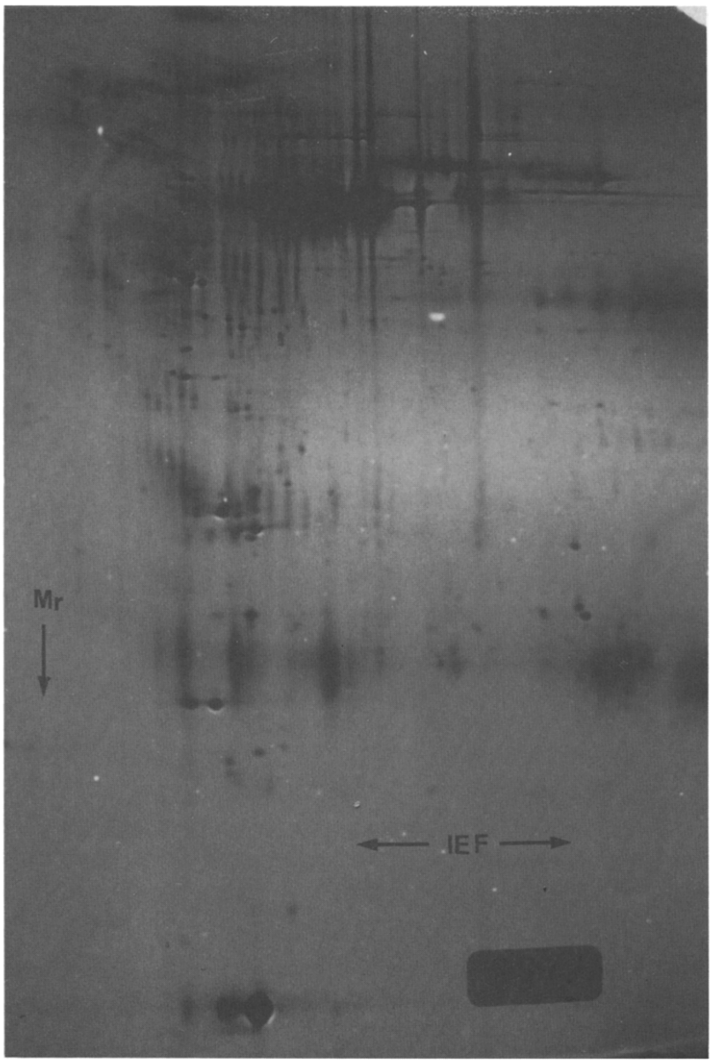

Fig. 2. Silver-stained 2-DE gel of CSF proteins with a total loading of 15 μg of protein. The acidic side is to the left and the higher-molecular-mass proteins are at the top. IEF was performed in capillary gel columns (14 mm × 1.5 mm I.D.) containing, per litre, 40 g of acrylamide, 9 mol of urea, 2% Nonidet P-40, 20 g of pH 3.5–10 ampholytes and 5 g of pH 5–7 ampholytes. The anolyte was 50 m*M* phosphoric acid, and the catholyte was 100 m*M* NaOH. The separation was done for 10 500 V h. The second dimension was performed in slab gels (180 × 160 × 1.5 mm), containing 12 g/l polyacrylamide.

specific enolase, β-trace protein, and a transthyretin-related protein. The enrichment of apo-AI, -E, -CII and -CIII, and the absence of apo-B, in human CSF have been demonstrated in an earlier report [63]. In two studies it has been shown that apo-E is a secretory product of the astrocytes [64,65]. In the analysis of ventricular fluid by 2-DE, apo-E is not detectable [66]. These apolipoproteins

probably perform important functions in the brain, the human organ richest in lipids.

Another point of interest is the high concentrations of transthyretin and transthyretin-related protein, which is probably a dimer of transthyretin [86]. An extremely high level of transthyretin mRNA (prealbumin mRNA) in choroid plexus tissue suggests very active synthesis of transthyretin in this region [87,88]. The functional significance of the high rate of intrathecal synthesis of transthyretin is not fully understood. It might also transport thyroid hormones and the retinol-binding protein in the CSF. The nature of the interaction between transthyretin and the cells of the CNS is not clear; however, it has been shown that transthyretin binds to specific high-affinity receptors on human astrocytoma cells. It is likely that similar receptors are present on normal human astrocytes [89]. The β-trace protein is split into several fractions. It consists of several populations of molecules with different net charges, but with similar molecular masses. It is thought to be of glial cell origin [90]. β-Trace protein behaves differently in CSF samples from different patients; its intensity on the 2-DE gel is increased in MS cases [71]. These observations correlate with those of Olsson *et al.* [90], who found an increase of β-trace protein in the CSF from severely disabled MS patients as a consequence of CNS tissue damage.

Myelin basic protein is found in extremely low amounts in the CSF and may be increased in patients with acute MS or stroke [91]. It shows a very high isoelectric point and is, therefore, not detectable on the 2-DE map. A potential alternative for detecting myelin basic protein would be a non-equilibrium system [92]. In all published 2-DE patterns of CSF, many proteins that were absent in serum have been observed. Their identities remain at the moment unknown. Some serum proteins, such as the subcomponents C1s of the complement protein C1 and transferrin, are intrathecally modified. The modification is a highly specific removal of sugar residues. The isoforms with the lowest sialic acid content have the most basic isoelectric points.

Besides plasma proteins, which can cross the blood–brain barrier, the CSF contains specific proteins, which might be increased, decreased or altered in certain neurological disorders. The Ig fractions are resolved into heavy and light chains under the dissociation conditions in 2-DE. The light chains are distributed over nearly the entire pH range as diffuse spots. CSF samples from patients with oligoclonal antibodies show an oligoclonal zone in the region of the Ig light chains. The novel Ig appear as sharply edged spots because of their limited heterogeneity [66–71]. In cases of extremely high γ-globulin values in CSF, oligoclonal zones can also be detected in the heavy chain region [62]. Several authors have reported that the pattern of oligoclonal bands in AGE and IEF remains constant during the course of a disease [72,73]. Using the more sensitive method of 2-DE, these findings could be confirmed with different diseases producing Ig clones [66]. The diagnostic value for the detection of oligoclonal antibodies is better than that of AGE and IEF. Many artefacts occur during IEF, which can lead to misin-

terpretation, are eliminated in the 2-DE system. Therefore it is possible to demonstrate oligoclonal Ig in cases with presumptive MS with the 2-DE method only [67,71].

Little is known about changes in the CSF in non-inflammatory diseases of the CNS. Fifteen out of twenty patients with typical idiopathic Parkinson's disease show a protein with a molecular mass of $25 \cdot 10^3$ and a charge similar to that of albumin. It is not detectable in the 2-DE pattern in any of 91 normal volunteers

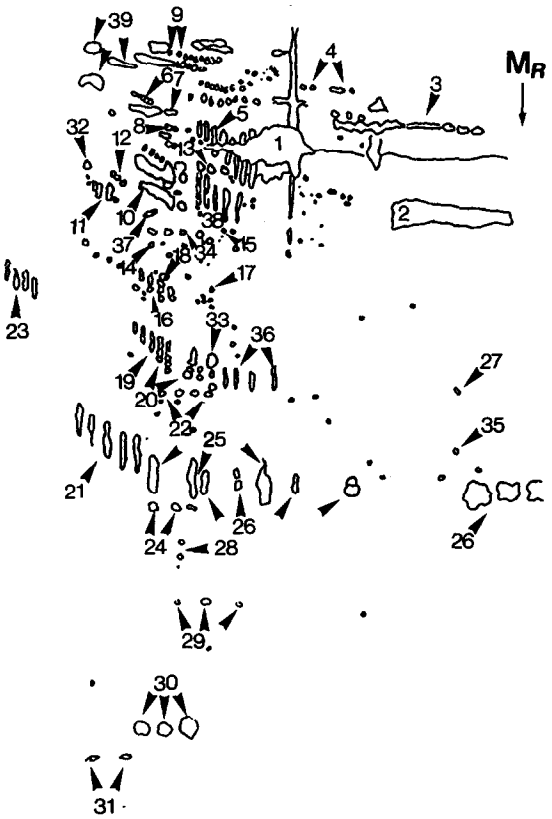

Fig. 3. Schematic standard 2-DE map for CSF proteins. The construction of the map is based on an examination of *ca.* thirty normal CSF samples. The spots are numbered from 1 to 39: 1 = albumin; 2 = IgG heavy chain; 3 = transferrin; 4 = C3 activator monomer; 5 = haemopexin; 6 = intrathecal modified C1s factor; 7 = prothrombin; 8 = α1B-glycoprotein; 9 = α1-antitrypsin dimer; 10 = α1-antitrypsin; 11 = α2HS-glycoprotein; 12 = α1-antichymotrypsin; 13 = IgA heavy chain; 14 = neuron-specific enolase; 15 = fibrinogen α-chain; 16 = haptoglobin β-chain; 17 = actin; 18 = apolipoprotein A IV; 19 = G4-glycoprotein; 20 = CSF-specific apolipoprotein E; 21 = apolipoprotein D; 22 = transthyretin-related protein (transthyrethin dimer?); 23 = α1-acid glycoprotein; 24 = apolipoprotein A I; 25 = β-trace protein; 26 = Ig light chains; 27 = C4 α-chain; 28 = retinol-binding protein; 29 = haptoglobin α2-chains; 30 = transthyretin (prealbumin); 31 = haptoglobin α1s and α1f chains; 32–39 = unidentified CSF-specific proteins, which are not found on the 2-DE pattern of serum/plasma; 32–35 and 39 do not react with antibodies to serum proteins, and 36–38 reveal a clear reaction with antibodies to serum proteins.

[74]. The study of CSF proteins by 2-DE was also extended to the clinically heterogeneous disorder of schizophrenia. Schizophrenia is diagnosed and monitored primarily by its psychological symptoms and manifestations. Lumbar puncture is often carried out on these patients, when inflammatory diseases or tumours are suspected, since such organic disorders sometimes give rise to symptoms resembling schizophrenia. Two additional proteins of M_r $40 \cdot 10^3$ have been found in 32% of patients with schizophrenia [75]. Another study confirmed these results by finding these proteins in 48% of patients with schizophrenia [76]. In both studies, the proteins also appear in patients with affective and other psychiatric disorders, and in patients with MS, but not in neurological controls. The proteins are believed to have originated within the CNS, as they could not be detected in ten-fold concentrates of CSF from normal volunteers, or in patients' serum. It is also unlikely that the appearance of these proteins is drug-related, as there was no drug common to the treatment of all the patients who displayed the abnormal proteins. In Creutzfeldt–Jakob disease, which is sometimes difficult to distinguish from other types of dementia, two proteins appear on the 2-DE gel [77]. These CSF proteins, with molecular masses of $29 \cdot 10^3$ and $26 \cdot 10^3$ and isoelectric points of 5.1 and 5.2, were found in 100% of the patients with Creutzfeldt–Jakob disease, but also in 50% of the patients with herpes simplex encephalitis. On the other hand, CSF samples from patients with senile dementia of the Alzheimer type and multi-infarct dementia show no differences compared with CSF samples from aged non-demented individuals. However, in contrast to the silver-stained gels described in other reports, the gels were stained with the less sensitive Coomassie Blue dye [78].

A special method of CSF research is the isolation of circulating immune complexes (IC) from the CSF and their electrophoretic analysis. In both infectious and autoimmune disorders, IC may contain diagnostically important antigens and antibodies. Many investigators have now documented that IC are found in both the serum and the CSF of MS patients [79–81], in patients with acute inflammatory polyneuritis [82] and in patients suffering from progressive rubella panencephalitis, a rare slow viral disease of the CNS [83]. Isolated IC have been analysed by one-dimensional [84] and by two-dimensional gel electrophoresis [85]. With both techniques, in a small number of MS patients, additional proteins were noticed that are not present in control CSF. Above all, the 2-DE technique permits an excellent survey of all IC present in the CSF, and it should be capable of providing leads to antigens of unknown identity in a number of neurological disorders.

In all these investigations, 2-DE combined with silver staining has been shown to resolve many CSF disease-associated proteins. Those in MS patients especially in the Ig light chain region, are far better resolved than with any one-dimensional system. The changes revealed in Parkinson's disease, schizophrenia and Creutzfeldt–Jakob disease would never have been detected through the use of AGE, SDS-PAGE of IEF. Application of this sensitive methodology to other neurolog-

ical diseases would probably be informative. On the other hand, its application to routine clinical analysis, especially for determination of oligoclonal antibodies, is limited by the expense and technical difficulties.

2.2.4. Additional studies with 2-DE

If 2-DE of the CSF is to be used as a diagnostic tool, it should be judged in the same way as any other test with regard to specificity and sensitivity. The 2-DE technique should be more efficient than other tests. It could be possible that 2-DE is able to demonstrate more abnormalities than a single test when CSF is examined. On the other hand, it is unlikely that 2-DE will become the procedure of choice when a single component only is to be measured, even if the problems of quantitation can be solved. In such cases, 2-DE might be of more value as a tool to develop new laboratory tests. A certain protein in the CSF, which is specific for a disease, could be isolated. The preparation of a specific antibody and the development of a radio- or enzyme-immunoassay should allow the measurement of the specific protein on a routine base.

An additional approach is an exact characterization of interesting CSF proteins. For a long time, the purification of small amounts of proteins from complex mixtures and the limited sensitivity of protein sequence analysis was a problem. The combined use of 2-DE and protein sequence analysis has partially alleviated the limitations in protein analysis. Since its introduction in 1985, the technique of protein electroblotting and microsequencing has become one of the most important tools in obtaining partial sequence information on gel-separated proteins [93,94]. An excellent review of blotting and sequencing is given in a special collection of publications [95]. Information on N-terminal sequences can be obtained from as little as 10 pmol of highly purified protein. In this manner, partial amino acid sequences have been obtained for six CSF proteins [86]. One of these proteins has been identified as the transthyretin molecule with double the molecular mass of the usual protein described above. The other five proteins were not homologous with any of the known sequences in the protein databases. Of these five partially sequenced proteins, two were diagnostically altered in Creutzfeldt–Jakob disease, one was reduced in schizophrenia, and two others were increased in MS. They can also be used to synthesize oligopeptides and oligonucleotides to study the genetic and cellular origin and function of such proteins.

3. CONCLUSION

The differentiation and analysis of CSF proteins has substantially contributed to our knowledge of physiological and pathophysiological processes in the CNS. The electrophoretic separation of CSF proteins is carried out for at least three different purposes:

(1) For the routine laboratory, one-dimensional electrophoretic techniques, such as AGE and IEF, are of great value in identifying oligoclonal antibodies, which are helpful in the diagnosis of MS and other inflammatory diseases of the CNS.

(2) In biochemical research, 2-DE is very useful for the investigation of the complete range of proteins circulating in the CSF. With the help of the sensitive silver staining, trace amounts of proteins are still detectable. Besides oligoclonal antibodies, 2-DE yields additional information about probable alterations in the complex CSF protein composition. The availability of affordable and sophisticated automated computer systems could facilitate the evaluation of great amounts of information. A particular problem in 2-DE analysis of CSF proteins is the high relative abundance of certain proteins, particularly albumin. One solution to this problem is the use of affinity chromatography or the fractionation of CSF proteins by gel permeation chromatography prior to 2-DE.

(3) Identification and characterization of CSF proteins by different biochemical and immunological methods. The procedure of immunoblotting using commercial antisera allows the location of specific proteins in the gel pattern. This may be interesting for proteins derived from the brain, such as neuron-specific enolase, myelin proteins, glial fibrillary acidic protein, and special forms of transthyretin, or for a better characterization of lipoproteins in the CSF, such as apo-E, which perform important functions in the brain, the organ richest in lipids. An additional approach is an exact characterization of CSF proteins directly from the 2-DE gels by partial amino acid sequencing using the refined method of microsequencing. Interesting proteins would be disease-associated CSF proteins. Special attention should be devoted to the antigens which can be isolated from the CSF in association with the corresponding antibodies as IC. Mainly in MS, such antigens could yield important information about the still unknown etiology of this disease. Comparison of the protein sequences of these antigens with known sequences in the protein databases could yield information concerning whether these antigens represent brain, viral or lymphocytic components.

4. ACKNOWLEDGEMENTS

We thank the Neurology Department of the University Hospital in Zürich for the CSF samples and the corresponding diagnosis. Our work in CSF research is supported in part by the National Multiple Sclerosis Society.

REFERENCES

1 R. W. P. Cutler, L. Page, J. Calicich and G. W. Watters, *Brain*, 91 (1968) 707.
2 K. Felgenhauer, *Klin. Wochenschr.*, 52 (1974) 1158.
3 G. Schliep and K. Felgenhauer, *J. Neurol.*, 218 (1978) 77.
4 B. Weissner and W. Bernhardt, *J. Neurol. Sci.*, 37 (1978) 205.
5 C. R. Hornig, O. Busse, W. Dorndorf and M. Kaps, *J. Neurol.*, 229 (1983) 11.
6 G. Schliep and K. Felgenhauer, *J. Neurol.*, 207 (1974) 171.
7 E. A. Kabat, D. H. Moore and H. Landow, *J. Clin. Invest.*, 21 (1942) 571.
8 S. T. Nerenberg, R. Prasad and M. E. Rothman, *Neurology*, 28 (1978) 988.
9 D. E. McFarlin, E. S. Mingioli, W. Strober, W. W. Tourtelotte and J. N. Whitaker, *Neurology*, 28 (1978) 991.

10 D. R. Soprano, A. Makover, D. S. Goodman and E. A. Zimmerman, *Neurology*, 36 (1986) 900.
11 D. Karcher, L. Kevenyl and A. Lowenthal, in T. O. Kleine (Editor), *Neue Labormethoden für die Liquordiagnostik*, G. Thieme Verlag, Stuttgart, New York, 1980, pp. 44–46.
12 E. J. Thompson and G. Keir, *Ann. Clin. Biochem.*, 27 (1990) 425.
13 C. B. Laurell, in E. J. Thompson (Editor), *Advances in CSF Protein Research and Diagnosis*, MTP Press, Lancaster, 1987, p. 123.
14 A. Grubb and H. Löfberg, *Scand. J. Clin. Lab. Invest.*, 117 (Suppl. 45) (1985) 7.
15 C. M. Poser, D. W. Paty, L. Scheinberg, D. W. I. Mc Donald, F. A. Davis, G. C. Ebers, K. P. Johnson, W. A. Sibley, D. H. Silberberg and W. W. Tourtellotte, *Ann. Neurol.*, 13 (1983) 227.
16 H. Link and V. Kostulas, *Clin. Chem.*, 29 (1983) 810.
17 M. G. Low, M. A. J. Ferguson, A. H. Futerman and I. Silman, *Trends Biochem. Sci.*, 11 (1986) 212.
18 R. H. Kelly and T. J. Hardy, *Int. Arch. Allergy Appl. Immunol.*, 69 (1982) 56.
19 L. Maizel, *Science*, 151 (1966) 988.
20 B. D. Hames and D. Rickwood (Editors), *Gel Electrophoresis of Proteins: A Practical Approach*, IRL Press, Oxford, 1981.
21 A. T. Andrews (Editor), *Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications*, Oxford University Press, New York, 1986.
22 J. H. Carson, E. Barabarese, P. E. Braun and T. A. McPherson, *Proc. Natl. Acad. Sci. U.S.A.*, 75 (1978) 1976,
23 F. H. Hochberg and L. Wolfson, *Sci. Tools*, 26 (1979) 44.
24 P. K. Coyle and C. Johnson, *Neurology*, 33 (1983) 510.
25 M. H. Johnson and E. J. Thompson, *J. Clin. Pathol.*, 35 (1982) 1328.
26 M. G. Harrington and C. R. Merril, *J. Chromatogr.*, 429 (1988) 345.
27 O. Vesterberg and H. Svensson, *Acta Chem. Scand.*, 20 (1966) 820.
28 C. Fossard, G. Dale and A. L. Latner, *J. Clin. Pathol.*, 23 (1970) 586.
29 P. Delmotte, *Clin. Chem. Clin. Biochem.*, 9 (1971) 334.
30 J. L. Trotter, G. Banks and P. Wang, *Clin. Chem.*, 23 (1977) 2213.
31 J. E. Olsson and K. Nilsson, *Neurology*, 29 (1979) 1883.
32 R. D. Davenport and D. F. Keren, *Clin. Chem.*, 34 (1988) 764.
33 A. Siden, *J. Neurol.*, 224 (1980) 133.
34 W. W. Tourtellotte, A. R. Potvin, I. Booe, R. W. Baumhefner, M. J. Walsh, P. Dickstein, T. Ingram, T. Cowan, P. Shapshak and P. Delmotte, *Neurology*, 32 (1982) 261.
35 G. Cowdrey, B. Gould, J. Rees and G. Firth, *Electrophoresis*, 11 (1990) 813.
36 T. Pirttilä, H. Frey, K. Matila and A. Siden, *Electrophoresis*, 9 (1988) 582.
37 I. Wybo, M. Van Blerk, R. Malfait, P. Goubert and F. Gorus, *Clin. Chem.*, 36 (1990) 123.
38 H. R. Achermann, R. Gallusser, S. Haab and S. Stoecklin, *Lab. Med.*, 14 (1990) 153.
39 R. W. H. Walker, G. Keir, M. M. Johnson and E. J. Thompson, *J. Neuroimmunol.*, 4 (1983) 141.
40 P. Gallo, F. Bracco and T. Tavolato, *J. Neurol.*, 84 (1988) 87.
41 K. G. Kjellin and H. Stibler, *J. Neurol. Sci.*, 27 (1976) 45.
42 H. Stibler, *Thesis*, Karolinska Hospital, Stockholm, 1978.
43 C. Wikkelsø, C. Blomstrand and L. Rönnbäck, *J. Neurol. Sci.*, 44 (1980) 247.
44 C. Wikkelsø, C. Blomstrand and L. Rönnbäck, *J. Neurol. Sci.*, 49 (1981) 293.
45 C. Wikkelsø and C. Blomstrand, *Acta Neurol. Scand.*, 66 (1982) 199.
46 P. H. O'Farrel, *J. Biol. Chem.*, 250 (1975) 4007.
47 R. C. Switzer, C. R. Merril and S. Shilfrin, *Anal. Biochem.*, 98 (1979) 231.
48 C. R. Merril, R. C. Switzer and M. L. van Keuren, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 4335.
49 B. R. Oakley, D. R. Kirsch and N. R. Morris, *Anal. Biochem.*, 105 (1980) 361.
50 M. J. Dunn in A. Chrambach, M. J. Dunn and B. J. Radola (Editors), *Advances in Electrophoresis*, Vol. 1, VCH, Weinheim, 1987.
51 T. Rabilloud, *Electrophoresis*, 11 (1990) 785.
52 D. Goldman, C. R. Merril and M. H. Ebert, *Clin. Chem.*, 26 (1980) 1317.
53 E. C. Laterre, J. F. Heremans and A. Carbonara, *Clin. Chim. Acta*, 10 (1964) 197.

54 E. R. Einstein, *Proteins of the Brain and CSF in Health and Disease*, C. C. Thomas, Springfield, IL, 1982.
55 G. B. Dermer, L. M. Silverman and J. F. Chapman, *Clin. Chem.*, 28 (1982) 759.
56 G. B. Dermer and J. J. Edwards, *Electrophoresis*, 4 (1983) 212.
57 M. J. Walsh, L. Limos and W. W. Tourtellotte, *J. Neurochem.*, 43 (1984) 1277.
58 F. Wiederkehr, A. Ogilvie and D. J. Vonderschmitt, *J. Neurochem.*, 49 (1987) 363.
59 F. Wiederkehr, M. Büeler, M. Wacker and D. J. Vonderschmitt, *Electrophoresis*, 10 (1989) 480.
60 H. Towbin, T. Staehlin and J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 4350.
61 U. Beisiegel, *Electrophoresis*, 7 (1986) 1.
62 O. J. Bjerrum and N. H. H. Heegaard, *Handbook of Immunoblotting of Proteins*, Vol. I, CRC Press, Boca Raton, FL, 1988.
63 P. S. Roheim, N. Cavey, T. Forte and G. L. Vega, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 4646.
64 J. K. Boyles, R. E. Pitas, E. Wilson, R. W. Mahley and J. M. Taylor, *J. Clin. Invest.*, 76 (1985) 1501.
65 G. J. Snipes, C. B. McGuise, J. J. Norden and J. A. Freemann, *Proc. Natl. Acad. Sci. U.S.A.*, 83 (1986) 1130.
66 F. Wiederkehr, A. Ogilvie and D. J. Vonderschmitt, *Clin. Chem.*, 31 (1985) 1537.
67 M. G. Harrington, C. R. Merril, G. Goldman, X. H. Xu and D. E. McFarlin, *Electrophoresis*, 4 (1984) 236.
68 M. J. Walsh, W. W. Tourtellotte, J. Roman and W. Dreyer, *Clin. Immunol. Immunopathol.*, 35 (1985) 313.
69 M. J. Walsh and W. W. Tourtellotte, *J. Exp. Med.*, 163 (1986) 41.
70 F. Wiederkehr, A. Ogilvie and D. J. Vonderschmitt, *Electrophoresis*, 7 (1986) 89.
71 F. Wiederkehr, J. Imfeld and D. J. Vonderschmitt, *J. Clin. Chem. Clin. Biochem.*, 24 (1986) 1017.
72 J. E. Olsson and H. Link, *Arch. Neurol.*, 28 (1973) 392.
73 K. Nilsson and J. E. Olsson, *Clin. Chem.*, 24 (1978) 1134.
74 M. G. Harrington and C. R. Merril, *Clin. Chem.*, 30 (1984) 1933.
75 M. G. Harrington, C. R. Merril and E. F. Torrey, *Clin. Chem.*, 31 (1985) 722.
76 D. B. Wildenauer, W. Hoechtlen, K. H. von Hoch and M. Achenheil, in A. T. Endler and S. Hanash (Editors), *Two-Dimensional Electrophoresis*, VCH, Weinheim, 1989, p. 212.
77 M. G. Harrington, C. R. Merril, D. M. Asher and D. C. Gajdusek, *N. Engl. J. Med.*, 315 (1986) 279.
78 L. Alafuzoff, R. Adolfsson, G. Bucht, E. Jellum, P. D. Mehta and B. Windblad, *Eur. Neurol.*, 25 (1986) 285.
79 T. G. Tachovsky, R. P. Lisak, J. Koprowski, A. N. Theofilopoulos and F. J. Dixon, *Lancet*, ii (1976) 997.
80 N. Kasui, A. R. Pachner and R. K. Yu, *J. Neurol. Sci.*, 75 (1986) 33.
81 P. K. Coyle, *Neurology*, 35 (1985) 429.
82 J. C. Antoine, D. Mickel, J. P. Lamelin, B. Laurent and B. Schott, *Acta Neurol. Scand.*, 73 (1986) 477.
83 P. K. Coyle and J. S. Wolinsky, *Ann. Neurol.*, 9 (1981) 557.
84 P. K. Coyle, *J. Neuroimmunol.*, 15 (1987) 97.
85 F. Wiederkehr, M. Wacker and D. J. Vonderschmitt, *Electrophoresis*, 10 (1989) 473.
86 C. R. Merril and M. G. Harrington, in A. T. Endler and S. Hanash (Editors), *Two-Dimensional Electrophoresis*, VCH, Weinheim, 1989, pp. 243–255.
87 B. Weisner and U. Kauerz, *J. Neurol. Sci.*, 61 (1983) 27.
88 P. W. Dickson, A. R. Aldred, J. G. T. Menting, P. D. Marley, W. H. Sawyer and G. Schreiber, *J. Biol. Chem.*, 261 (1987) 3475.
89 C. M. Divino and G. C. Schussler, *J. Clin. Endocrinol. Metab.*, 71 (1990) 1265.
90 J. E. Olsson, H. Link and R. Müller, *J. Neurol. Sci.*, 27 (1976) 233.
91 S. R. Cohen, R. M. Herndon and G. M. McKhan, *N. Engl. J. Med.*, 295 (1976) 513.
92 P. Z. O'Farrel, J. M. Goodman and P. H. O'Farrel, *Cell*, 12 (1977) 1133.
93 J. Vandekerckhove, G. Bauw, M. Puype, J. Van Damme and M. Montagu, *Eur. J. Biochem.*, 152 (1985) 9.
94 R. H. Aebersold, D. B. Teplow, L. E. Hood and S. B. H. Kent, *J. Biol. Chem.*, 261 (1986) 4229.
95 J. Klose (Editor), Symposium Papers on Blotting and Sequencing, in *Electrophoresis*, 11 (1990) pp. 515–594.