Our data for cells from 10 normal human subjects (Table 2) agree well with other published data (5–7). Typical published ranges, in micrograms per milliliter of packed cells, for alpha-tocopherol are 1.4–2.9 (n = 9) (5), 1.5–2.1 (n = 5) (6), and 1.3–2.6 (n = 12) (7); and for gamma-tocopherol, <0.1–0.12 (6) and 0.14–0.58 (7). Concentrations of tocopherols in plasma from these same subjects are also in Table 2. Tocopherols in erythrocytes and plasma are not directly related (8), and their divergence in Table 2 for different subjects is not unexpected.

The procedure described here is rapid and direct, but its accuracy depends on fluorescence detection with an instrument that is sufficiently sensitive and stable (3).

Blood was generously donated by employees at the Beltville Human Nutrition Research Center and Doctors Hospital of Prince Georges County. Plasma and erythrocytes from pediatric patients were graciously supplied by Dr. Lois Johnson (University of Pennsylvania Hospital).

We used a Model 650-105 spectrophotofluorometer (Perkin-Elmer Corp., Norwalk, CT 06856).

References

CLIN. CHEM. 29/10, 1842–1844 (1983)

A Unique Protein in Normal Human Cerebrospinal Fluid
Nicholas M. Papadopoulos, Peter A. LeWitt, Richard P. Newman, Marc I. Raphaelson, and Thomas N. Chase

On analysis of cerebrospinal fluid (CSF) samples from normal volunteer donors by high-resolution zone electrophoresis on agarose gel, an electrophoretically homogeneous protein band consistently appeared in the \( \gamma \)-globulin region. Application of immunofixation electrophoresis in attempts to identify the band with use of monospecific antibodies against individual human serum proteins and against heavy- and light-chain immunoglobulins as well as polyvalent antisera did not produce a positive immunoprecipitation reaction with the protein band. The serum samples from these subjects did not show similar bands. Therefore, we conclude that this protein band is a normally occurring protein that is unique to CSF.

Additional Keyphrases: high-resolution zone electrophoresis immunofixation

An improved high-resolution agarose gel electrophoretic technique was used for the analysis of serum samples from healthy individuals. The resulting patterns showed a higher incidence of electrophoretically homogeneous protein bands in the \( \gamma \)-globulin region than did other zone electrophoretic methods (1). The same technique was combined with immunofixation to demonstrate and identify an occult myeloma paraprotein (2). Here we describe its use with cerebrospinal fluid (CSF) samples from normal subjects and some patients with defined neurological disorders. The protein electrophoretic patterns for serum and CSF of normal subjects differ distinctly in the \( \gamma \)-globulin region.

Materials and Methods
CSF was sampled by routine lumbar puncture from 24 normal volunteer donors (14 men, 10 women; ages 30 to 60 years) after informed consent. Samples from patients were obtained in the course of diagnostic evaluations. Analysis was performed the same day or after overnight storage at 4 °C. Five milliliters of CSF was concentrated 50-fold in a Minicon Concentrator B-15 (Amicon Corp., Lexington, MA), and 5 \( \mu \)L of the concentrated CSF was used for electrophoresis on microscope slides coated with agarose gel. After electrophoresis for 13 min, the proteins were fixed and stained with Amido Black as previously described (1, 3). Electrophoresis of serum proteins was performed by the same procedure.

Immunofixation electrophoresis was used to identify protein bands in duplicate analyses of the CSF sample, run simultaneously. After electrophoresis, immunofixation was performed by overlaying a strip of cellulose acetate, soaked in monospecific antiserum, on the gel, directly on top of the protein band. After the slide was incubated in a humidified chamber for 15 min at 37 °C, to allow diffusion and immunoprecipitation to occur, the strip was removed and the slide immersed in an 8.5 g/L saline solution for 2 h to wash out unprecipitated proteins. The slide was dried by placing filter paper on top of the gel. The dried gel was stained with Amido Black 10-B in dilute (25 mL/L) acetic acid and destained in dilute acetic acid (3).

1 Clinical Chemistry, Clinical Center, and 2 Experimental Therapeutic Section, NINCDS, National Institutes of Health, Bethesda, MD 20205.
Received Jan. 31, 1983; accepted July 5, 1983.

1842 CLINICAL CHEMISTRY, Vol. 29, No. 10, 1983
The following monospecific antisera against human serum proteins were used: for heavy-chain immunoglobulins, IgG, IgA, IgM, IgD, and IgE; for light chains, \( \kappa \) and \( \lambda \); for the individual serum proteins \( \alpha_1 \)-acid glycoprotein, \( \alpha_1 \)-antitrypsin, C-reactive protein, haptoglobin, \( \alpha_2 \)-macroglobulin, transferrin, and ceruloplasmin. Polyvalent antiserum to normal human serum was also applied. All these antisera were from Kallestad Labs., Inc., Austin, TX 78701.

Results

Figure 1 illustrates the variety of protein electrophoretic patterns that can be demonstrated by this technique in CSF from normal subjects and also from patients with certain defined neurological disorders. The CSF protein electrophoretic pattern for a patient with leukemia (A) has a diffuse zone in the \( \gamma \)-globulin region. Electrophoretic pattern B, with a weak but discrete protein band in the \( \gamma \)-globulin region, is characteristic of most normal CSF donors; pattern C, with two protein bands, is from a patient with malignancy of the central nervous system. Pattern D, with three bands, was found in patients with Alzheimer’s disease (4). Electrophoretic pattern E, with four bands, is from a patient with active multiple sclerosis.

These CSF protein electrophoretic patterns, with two, three, and four protein bands in the \( \gamma \)-globulin region, demonstrate the clear resolution and diversity of patterns obtained by our technique. These abnormal patterns will serve as a basis for future detailed studies of other such pathological cases. The example of the normal pattern, included for comparison, is typical of all our normals with the single band.

An unexpected finding in this study was the presence of a single protein band in the \( \gamma \)-globulin region of the CSF electrophoretic patterns for 20 of the 24 normal subjects. Of the remaining four, two bands were detected in the CSF of three volunteers, and four bands in one.

We used immunofixation electrophoresis in an attempt to identify the single band. A typical example of electrophoretic and immunofixation electrophoretic analysis of the same CSF sample from a normal subject is shown in Figure 2, where electrophoretic pattern A shows the protein band in the \( \gamma \)-globulin region; pattern B shows that, after immunofixation electrophoresis, the diffuse protein zones were demonstrable in the \( \gamma \)-globulin region with the IgG \( \kappa \) and \( \lambda \)-chain monospecific antisera, but no discrete band was visible, unlike the corresponding CSF protein electrophoretic pattern. Results were the same when we used the polyvalent antiserum. None of the other monospecific antisera mentioned above produced precipitation in the \( \gamma \)-globulin region corresponding to the protein band. Thus, this band was not identified by the antiserum used in the immunofixation procedure.

The bands in the CSF electrophoretic patterns of four of the 24 normal persons were weak and did not react with any of the various antisera. We could not determine whether their concentration was below the sensitivity of the immunoprecipitation technique or whether they were non-immunoglobulin proteins.

Only a diffuse \( \gamma \)-globulin zone was visible in the serum electrophoretic patterns from the normal subjects.

In support of this finding are previous reports of the presence of protein bands in the CSF of patients and the recent report (5) that there may be CSF proteins different from those in serum, as determined by two-dimensional electrophoresis.

Discussion

The consistently demonstrable single protein band in the \( \gamma \)-globulin region of the CSF electrophoretic patterns of normal subjects by our electrophoretic test system raised the question of whether the band is the result of a methodological artifact or is ascribable to a protein unique to CSF. Neither diluted normal serum nor the serum samples from the same normal subjects, analyzed with the same electrophoretic system, showed a similar single band.
During the last 20 years several authors, using different techniques, have reported proteins "apparently specific" for CSF or "discrete proteins" in the γ-globulin region of electrophoretic patterns of CSF from patients. These were variously named slow-γ, fast-γ, post-γ, γ-trace, and δ-αT. These reports and a recent one by Link and Kostulas (6) support our finding of a discrete band in normal CSF electrophoretic patterns that is unique to CSF and is not a methodological artifact. This band cannot represent a protein with a relative molecular mass of <15 000, because these are removed by the Minicon B-15 concentrators. Previous reports have described protein bands in electrophoretic patterns of CSF from patients. The present study shows one band in the CSF samples of normal volunteers.

The pattern for protein in normal CSF that is obtained by agarose gel electrophoresis, used routinely in many clinical laboratories, will serve as a reference against which CSF samples of patients can be evaluated—a need also suggested by others (6).

Demonstration of the band does not interfere with the diagnosis of multiple sclerosis and other neurological conditions because in these conditions the term "oligoclonal immunoglobulin bands" is used; i.e., several immunoglobulins are present. Our work suggests that the finding of a single band in the γ-globulin region should prompt further testing to differentiate between an immunoglobulin and non-immunoglobulin protein because immunoglobulins have been associated with pathological conditions, i.e., multiple sclerosis.

Clinical examination of the 24 volunteers revealed no neurological abnormality. One of these subjects was a 40-year-old woman whose CSF showed four discrete bands in the γ-globulin region, a pattern similar to what we have seen in cases of definite multiple sclerosis. When this woman was re-evaluated several months later, the tests, including visual testing, evoked responses, but there were no findings consistent with demyelinating disease. Electrophoresis of her serum protein produced no evidence of any γ-globulin bands, and immunoglobulin concentrations in her serum and CSF were normal. Is this pattern transient? Will this patient ultimately develop symptoms and signs consistent with multiple sclerosis? Multiple sclerosis is occasionally diagnosed at postmortem examination in individuals who have had innocuous symptoms and a benign course of the disease (7).

References