A Procedure for the Determination of Cerebrospinal Fluid Total Protein and Gamma Globulin in Neurologic Disorders

N. M. Papadopoulos, W. C. Hess, D. O'Doherty, and J. E. McLane

Elevation of the $\gamma$-globulin concentration in cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS) and other neurologic disorders has been reported (1-3). Expression of the $\gamma$-globulin values in milligrams per hundred milliliters does not reveal whether this increase is due to a general elevation of total protein or $\gamma$-globulin alone. Determinations of both $\gamma$-globulin and total protein may further distinguish MS from other neurologic disorders with high $\gamma$-globulin. To avoid any error that may result from using two different methods for their determination, one method that determines both is desirable. The purpose of this report is to describe a simple chemical procedure for the determination of both $\gamma$-globulin and total protein, and its application for the estimation of their ratio in CSF of patients with MS, other neurologic and non-neurologic disorders.

MATERIALS AND PROCEDURE

REAGENTS

1. Zinc sulfate in barbital buffer, pH 7.3:

   1.75 Gm. zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$),
3.60 Gm. barbital,
1.50 Gm. sodium barbital.
Dissolve the barbital and sodium barbital in warm water in a one liter volumetric flask, then add the zinc sulfate and bring to volume with water.
2. 12.5% aqueous sodium carbonate.
3. 1.25% aqueous sodium carbonate.
4. 0.05% copper sulfate (CuSO₄·5H₂O) aqueous solution.
5. Phenol reagent (Folin and Ciocalteau), diluted 1:4.
6. γ-Globulin* standard, 0.1 mg./ml in 1.25% aqueous sodium carbonate.
7. Total protein standard† reconstituted according to the directions.

PROCEDURE FOR TOTAL PROTEIN
Place 0.4 ml. CSF in a test tube and dilute to 2 ml. with 1.25% sodium carbonate solution. Add 2 ml. of copper sulfate solution and 5 ml. 12.5% sodium carbonate solution. Mix well after each addition. Maintain the mixture at 37° for 30 minutes. Then add 1 ml. of the phenol reagent, mix well, and allow to stand for 15 minutes at room temperature for complete color development. Read in a Coleman or similar photoelectric colorimeter at 540 mμ, and calculate the protein concentration from a standard calibration curve.

PROCEDURE FOR γ-GLOBULIN
Pipette 2 ml. CSF into a 15 ml. centrifuge tube, add an equal volume of the zinc sulfate reagent, cover with parafilm, shake well, and place in a refrigerator overnight (about 15 hours) for the precipitation of γ-globulin. Next day centrifuge for 10 minutes and decant the supernatant. Dissolve the precipitate with 2 ml. of 1.25% sodium carbonate solution and follow the same procedure as for total protein described above.

RESULTS AND DISCUSSION
The colorimetric method for the determination of γ-globulin in CSF reported from this laboratory (3), which is based on the use of the Folin and Ciocalteau phenol reagent (4), was adapted and modi-

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*Protein Foundation Laboratories, Jamaica Plain, Mass.
†Versatol, a trade name for dialyzed dried serum, Warner-Chilcott Laboratories, Morris Plains, N. J.
fied for the determination of both γ-globulin and total protein in CSF. With the use of γ-globulin and total protein standards, reference curves were prepared that followed linearity and approached each other closely in the range of from 0 to 20 mg./100 ml. (Fig. 1). By diluting the CSF, for the determination of total protein, an optical density range is obtained similar to that in the γ-globulin determination. The finding that serum and CSF proteins give similar electrophoretic patterns (5) justifies the use of the serum protein standard for the preparation of the reference curve for total protein. Although the tyrosine contents of γ-globulin and total protein may not be the same, the curves prepared from standards of γ-globulin and total protein showed that any error produced by the difference is not great enough to change the results appreciably (Fig. 1). The tyrosine equivalence method was compared with the Kjeldahl nitrogen procedure as well as with other protein tests in CSF, and good agreement was reported (6). Also, its sensitivity and accuracy compares very well with the Daughaday et al. (7) method; in fact, the average value they report for CSF total protein is the same as reported here.
Lumbar spinal fluids obtained from patients with MS and other neurologic and non-neurologic disorders were analyzed for γ-globulin and total protein, and the ratio of γ-globulin to total protein was calculated as per cent γ-globulin. As is shown in Table 1, in the controls (patients with no neurologic symptoms), the mean values of γ-globulin and total protein were 3.0 and 39.0 mg./100 ml. respectively, and their ratio 8.3%. Results in Table 2 show that in MS the total protein concentration was similar to that in the control, while a marked increase of γ-globulin was found, resulting in a high mean ratio of 30.0%. Table 3 represents values from patients with neurologic diseases such as brain and spinal cord tumors, pernicious anemia, and Parkinsonism. In these conditions an elevation of γ-globulin concentration in the presence of high total protein resulted in a lower mean ratio than in MS.

The significance of these results lies in the characteristically high γ-globulin to total protein ratio found in the MS cases. This unusually high ratio differs from the control and other neurologic conditions studied. The differentiation of MS from some of the other conditions might not have been possible by determination of γ-globulin alone, because in some of the latter cases γ-globulin was elevated to the same extent as in MS. Thus the importance of the determination of both γ-globulin and total protein and estimation of their ratio is evident for the differential diagnosis of MS. The simplicity and sensitivity of the chemical test and the small amount of CSF required for the determination of the proteins make this procedure practical for routine analysis.

Table 1. CSF from Control Patients

<table>
<thead>
<tr>
<th>Total protein (mg./100 ml.)</th>
<th>γ-Globulin (mg./100 ml.)</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.0</td>
<td>2.2</td>
<td>5.8</td>
</tr>
<tr>
<td>38.0</td>
<td>3.1</td>
<td>8.2</td>
</tr>
<tr>
<td>45.0</td>
<td>3.5</td>
<td>7.8</td>
</tr>
<tr>
<td>38.0</td>
<td>4.2</td>
<td>11.0</td>
</tr>
<tr>
<td>40.0</td>
<td>4.0</td>
<td>10.0</td>
</tr>
<tr>
<td>35.0</td>
<td>3.5</td>
<td>10.0</td>
</tr>
<tr>
<td>38.0</td>
<td>2.0</td>
<td>5.3</td>
</tr>
<tr>
<td>44.0</td>
<td>3.7</td>
<td>8.4</td>
</tr>
<tr>
<td>39.0*</td>
<td>3.0*</td>
<td>8.3*</td>
</tr>
<tr>
<td>± 3.4*</td>
<td>± .85*</td>
<td>± 2.0*</td>
</tr>
</tbody>
</table>

*Mean. *Standard Deviation.
A procedure for determinations of both γ-globulin and total protein is described. Its application for the analysis of samples of cerebrospinal fluid from non-neurologic, multiple sclerosis, and other central nervous system disorders, revealed a characteristically high ratio of γ-globulin to total protein in multiple sclerosis. These findings suggest that this simple chemical test may be used routinely as an aid in the diagnosis of multiple sclerosis.
REFERENCES

ERRATUM
An error appeared in Abstract No. 21, ‘‘The Paper Chromatographic Separation of Human Serum Lipoproteins,’’ by McDonald, Banaszak and Kissane, Clin. Chem. 5, 270, (1959). In the sixth line of the abstract, the phrase ‘‘13.3 Gm. sodium veronal’’ should read ‘‘10.3 Gm. sodium veronal.’’