Photometric Determination of Cerebrospinal Fluid Total Globulin and A/G Ratio with a Tryptophan Reaction

Abraham Saifer and Shirley Gerstenfeld

A previously described colorimetric tryptophan procedure for determination of serum total globulin and A/G ratio was modified so as to increase its sensitivity and suitability for determination of the small amount of globulins present in cerebrospinal fluid. Twenty-seven normal CSF specimens analyzed with the tryptophan method yielded a mean total globulin value of 14.0 ± 3.03 mg./100 ml., and a mean A/G ratio of 1.45 ± 0.25. These data are in good agreement with those reported for normal CSF specimens with the paper-electrophoretic method.

To demonstrate its clinical application in the diagnosis of neurologic disorders, the tryptophan method was used in the analysis of 51 CSF specimens from neurologic patients. Elevated total globulin values were obtained in 57 per cent. Six patients with normal total protein values showed decreased A/G ratios.

While the determination of the cerebrospinal fluid (CSF) total protein (TP) is considered a routine laboratory procedure in the diagnosis and investigation of neurologic disorders, the albumin-globulin (A/G) ratio of an individual CSF is rarely investigated. This is the consequence of the large amounts of CSF required per determination.

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and/or the laborious and time-consuming procedures employed, e.g., electrophoresis, immunochemical methods, and the like. Indeed, recently published articles have not only pointed out the large number of protein fractions present in CSF and their general similarities to serum proteins (1–3) but also the discrepancies between “normal values” obtained with different procedures for both TP and A/G ratios (4, 5). Earlier investigators (6–8), using salt-fractionation methods reported A/G ratios for CSF of 2.2–6.6. These A/G values are markedly elevated in comparison with the normal range of 0.48–1.48 obtained with an immunochemical procedure (9) and of 1.01–1.87, with the electrophoretic method (4, 10). Since discrepancies of this magnitude do not occur in determinations of the serum A/G ratio, the CSF values cited serve to demonstrate the inadequacy of the salt-fractionation procedure for biologic fluids of low protein content.

In a previous report from this laboratory (11), a method was described for the photometric determination of the A/G ratio of serum proteins without salt fractionation. The procedure is based upon the experimental fact that albumin has a low tryptophan content (about 0.2%) as compared to the various globulin fractions (about 3.0%). A quantitative determination of the tryptophan content of the precipitated TP would then constitute an accurate measure of its total globulin (TG) value. The specific tryptophan reaction previously applied to the unhydrolyzed serum proteins (11) was modified in order to increase its sensitivity and thus make it more suitable for the analysis of the small amount of globulins present in CSF.

**Experimental**

Reagents

1. *Trichloracetic acid (TCA).* 10% solution in distilled water.
2. *Glyoxylic acid.* 0.25% solution in glacial acetic acid (Mallinckrodt).
3. *Perchloric acid, 70% (Merck)*
4. *Potassium persulfate, saturated solution.* Add 25 ml. distilled water to 2 g. of c.p. potassium persulfate (Merck) and shake well for 5 minutes, and use the clear supernatant solution. Keep refrigerated when not in use and prepare fresh weekly.
5. *Thioglycolic acid (Fisher Scientific Company).* Stock solution. Add 1 ml. of thioglycolic acid (kept refrigerated) to 19 ml. of glacial acetic acid and prepare fresh weekly. Working solution. Dilute
the stock solution 1:10 by volume with glacial acetic acid. Prepare fresh for each day’s run.

6. Standards. Dried Serum Standard (Versatol-Warner-Chilcott) or pooled serum of known total globulin content is diluted 1:100 with 0.9% NaCl containing 1:10,000 Merthiolate as a preservative. Human serum albumin (50 mg./100 ml.) and human gamma globulin (20 mg./100 ml.) can also be used as additional standards for the TP and TG determinations, respectively.

Procedure

CSF TG

Pipet, in duplicate, 1.0-ml. aliquots of CSF and standards into 15 X 115 mm. centrifuge tubes. Add 1.0 ml. of 10% trichloracetic acid to each tube and mix. Let tubes stand at room temperature for 10 min. and then centrifuge at 3000 rpm for 10 min. Decant supernatant fluid. Dissolve or suspend the precipitate in 0.25 ml. of distilled water. Into a separate tube pipet 0.25 ml. of distilled water as a reagent blank. Add 1.25 ml. of 0.25% glyoxylic acid solution in glacial acetic acid and mix by tapping tubes. Let stand 5 min. at room temperature. Add 0.05 ml. of saturated potassium persulfate (cold) and mix. Let stand 1 min. at room temperature. Add 0.05 ml. of 0.5% thioglycolic acid in acetic acid and mix. Let stand 5 min. at room temperature. Centrifuge at 3000 rpm for 5 min. Read in a spectrophotometer at 560 mμ or in a Klett industrial-type photoelectric colorimeter (using a long path cell with a No. 56 filter) against the reagent blank set at zero.

Calculation

\[
\text{CSF TG (mg./100 ml.)} = \frac{\text{OD of unknown}}{\text{OD of standard}} \times \text{TG standard (mg./100 ml.)}
\]

CSF TP

The procedure for the determination of the CSF TP employed in these studies was that described previously (13). In instances in which it was necessary to conserve CSF, the TP was determined with the more sensitive Lowry method (13), as follows: 0.25 ml. of CSF was precipitated with an equal volume of 10% TCA and the centrifuged precipitate was dissolved in 1.0 ml. of distilled water with addition of 1–2 drops of 10% NaOH solution. The remainder of the procedure was performed exactly as described by Lowry et al. (13), except that the tubes were heated at 50° for 10 min. to enhance final color development and to obtain better stoichiometry.
removed through prior precipitation of proteins, the TP results obtained with both procedures were equivalent. The normal range with this method is 19.0–50.0 mg./100 ml.

Results

Normal Subjects

Twenty-seven CSF specimens were obtained by lumbar puncture from nonneurologic patients prior to operation or other medical treatment. The samples consisted of a random selection without regard to either sex or age among adult patients. The TP and TG were determined in duplicate, with the procedures described above, and the albumin and A/G ratios were calculated. A statistical analysis of results obtained for the normal group is presented in Table 1.

Subjects with Neurologic Disease

Fifty-one CSF specimens were obtained from patients on the neurologic wards and the same determinations performed as for the normal group above. The neurologic group included patients with cerebrovascular accidents (17), primary or secondary brain tumors (9), cerebral arteriosclerosis (5), convulsive disorders of unknown etiology (3), internal hydrocephalus (2), tabes dorsalis (3), and epilepsy, multiple sclerosis, and Guillain-Barré syndrome (1 each). The results for the neurologic patients are analyzed in Table 1.

Discussion

The present procedure for determination of the A/G ratio of CSF is based on direct determination of the TG content of precipitated proteins by means of a specific colorimetric tryptophan reaction. Since

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<th>Table 1. Data for CSF Proteins with the Tryptophan Reaction</th>
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<td><strong>Normal subjects (N = 27)</strong></td>
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<tr>
<td><strong>Mean ± S.D.</strong></td>
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<tr>
<td>Total protein (mg./100 ml.) (Biuret)</td>
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<td>Albumin (mg./100 ml.)</td>
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<td>Globulin (mg./100 ml.)</td>
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<td>A/G ratio</td>
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*Six patients with normal TP had A/G values less than 0.95.
albumin is obtained by subtraction of the CSF total globulin from the TP, the latter value must be precisely determined. Published figures for the normal range of the TP content of CSF have varied widely, depending on the method used or whether the protein was precipitated prior to its determination. Such procedures as the Lowry et al. (14, 15) modification of the Folin tyrosine method or U.V. absorption measurements (16) have been used for the direct determination of CSF total protein. Other investigators (17, 18) have stressed the fact that CSF contains sufficient amounts of nonprotein substances, in comparison with the amount of protein present, so that such direct methods yield results which are too high. For this reason a protein precipitation step was incorporated into our earlier procedure for CSF total protein (12) as well as in our present TG method. In agreement with the findings of Sethna and Tsao (17), removal of interfering substances by means of prior protein precipitation yields equivalent CSF total protein values with different procedures. The normal range of 18.8-49.8 mg./100 ml. found for our group of 27 normal individuals checks closely with the normal range of 15-50 mg./100 ml. suggested by Plum and Fog (4) on the basis of a comprehensive survey of the literature.

The reported total globulin content of normal CSF is also very much dependent on the procedures employed. Kabat et al. (9), using a specific anti-albumin serum, determined the albumin content of 32 normal spinal fluids with an immunochemical method. The total globulin value of each fluid was determined as a difference from the TP as determined with a sulfosalicylic acid turbidimetric procedure (18). The mean TG value of 19 ± 0.22 mg./100 ml. is somewhat higher, and the mean A/G value of 0.91 ± 0.22 correspondingly lower, than those obtained with the tryptophan method. However, the mean value of 14.0 ± 3.03 mg./100 ml. obtained with the colorimetric tryptophan method for TG is in good agreement with the values obtained by means of paper electrophoresis (4, 19), as are the mean A/G ratios of 1.45 ± 0.25.

A simple, accurate procedure for the A/G ratio, requiring only small amounts of CSF, has been mentioned in the literature (20, 21) as an important diagnostic aid in neurologic conditions. Unlike the immunochemical or electrophoretic procedures, which are laborious and require a high degree of technical skill, expensive equipment, and reagents, the tryptophan procedure is simple enough to be performed routinely in any clinical chemistry laboratory.
Neurologic disorders frequently result in changes in the distribution of the various protein fractions of the CSF although corresponding changes do not occur in the serum protein fractions. Diseases which fall into this category include multiple sclerosis (22), cerebral tumors (23), cerebral atrophies, and other brain diseases (24). The A/G ratio results for the 51 neurologic spinal fluids given in Table 1 provide additional evidence for the clinical usefulness of the proposed procedure in the diagnosis of neurologic disorders. These data are part of a more extensive clinical study which is now in progress.

References