Modern Methods for Determining Cerebrospinal Fluid Protein

Derek Watson

A simple and rapid micro method for the determination of albumin and total protein in cerebrospinal and other body fluids is presented. It is based on the UV spectrophotometric measurements of neutral and acid-alcohol-treated spinal fluid and is shown to be accurate and reproducible. Using spectrophotometry at 220 m\(\mu\) and two other recently improved technics, 84 specimens of spinal fluid from infants were analyzed for total protein. The results obtained showed good agreement over the range 30–200 mg./100 ml.

Suggested improvements in two technics widely used clinically for estimating spinal fluid protein stimulated the inception of a comparative study which is reported below.

Meulemans (1) demonstrated the necessity of adding sufficient sodium sulfate to salicylsulfonic acid (SSA) in order to eliminate an error in protein estimation caused by albumin and globulin giving unequal amounts of turbidity with aqueous SSA (2). The turbidity method of Meulemans is presently studied because of its simplicity. The Folin-Ciocalteu copper technic as originally adapted by Doughaday et al. (3) for spinal fluid protein estimation has been shown to be unreliable unless a blank of deproteinized spinal fluid is analyzed at the same time (4, 5). The latter suggestion has now been followed.

Rieder (6) studied the ultraviolet spectral curves of cerebrospinal fluid and concluded that direct spectrophotometry in the region of 260–280 m\(\mu\) was unreliable for the determination of protein. In the present work, ultraviolet spectrophotometry according to Tombs et al. (7) has been successfully applied and shown to be capable of excellent
recoveries when albumin and globulins are added to spinal fluid. This rapid method has also been extended to include measurement of albumin in deglobulinized spinal fluid.

**Materials and Methods**

The cerebrospinal fluids examined were those submitted to the laboratory for routine testing. These were 84 specimens obtained from 82 infants who showed signs of cerebral anoxia or had respiratory or cyanotic "episodes"; none had inflammatory disease. The infants were 1–26 days old.

Each specimen was counted for cells by the usual methods and then centrifuged for 5 min. at 500 g. Protein analyses were carried out on the supernatant fluid. In a few cases, corrections for total protein were made by subtracting 1 mg./100 ml. for every 1000 cells per cubic millimeter. Solutions of crystalline human albumin, Cohn globulin fraction IV, and pure γ globulin (Commonwealth Serum Laboratories, Melbourne, Australia) were used in concentrations of 30, 60, 90, and 120 mg./100 ml. in order to standardize the analyses. Controls were run with each batch of tests. Spectrophotometry was performed with an Optica (Milano) instrument. Measurement of turbidity and color was made in 1.5-cm. tubes using an EEL colorimeter.

**Spectrophotometry at 220 mμ**

**Reagents**

1. *Isopropyl alcohol (analytic grade)*
2. *Trichloroacetic acid-isopropanol* 5 ml. trichloroacetic acid (20% w/v aq.) are made up to 100 ml. with Solution 1
3. *Aqueous NaCl 0.9% w/v*

**Method**

Place 3.8 ml. of Solution 1 in Tube 1, 3.8 ml. Solution 2 in Tube 2, and 3.8 ml. Solution 3 in Tube 3. To each of the 3 tubes add 0.20 ml. spinal fluid. After mixing the contents of each tube, centrifuge the alcohol-containing tubes for 10 min. at 750 g. Measure the absorbance of the saline mixture (due to total protein) and that of the supernatant from the acid-alcohol mixture (due to albumin) at 220 mμ using the supernatant fluid from the neutral alcohol tube as blank solution. Convert absorbances to mg. protein/100 ml. by means of a factor derived from the readings given by the standard solutions.
Turbidimetry with SSA
Reagent
3% w/v salicylsulfonic acid (SSA) containing 7% w/v anhydrous sodium sulfate

Method
Conduct the test exactly according to Meulemans (3).

Colorimetry with the Folin-Ciocalteu Reagent
Reagents
1. Alkaline copper solution 5 ml. 0.5% w/v CuSO₄·5H₂O added to 45 ml. of a solution containing 2% w/v Na₂CO₃ and 0.05% w/v potassium tartrate in 0.1N NaOH
2. Folin-Ciocalteu's phenol reagent Diluted so that 1 ml. neutralizes 9 ml. 0.1N NaOH

Method
Prepare a control blank by heating at 56° for 4 min. a mixture of 0.1 ml. trichloroacetic acid (20% w/v) and 0.4 ml. spinal fluid. Analyze according to Doughaday et al. (2) 0.25 ml. of the supernatant fluid obtained after centrifuging and 0.20 ml. of the original spinal fluid.

Results and Discussion
The results shown in Fig. 1 indicate that the absorbances of the protein solutions at 220 mμ obey Beer’s Law, and that their extinction coefficients do not differ appreciably from each other.
The results of 8 recovery experiments are shown in Table 1. These demonstrate not only that satisfactory recovery of albumin and total protein is possible, but also that the treatment with alcoholic TCA, first used by Delaville et al. (9), quantitatively precipitates the globulins in spinal fluid.

A sample of ventricular fluid containing 32 mg. protein per 100 ml. was analyzed 20 times, together with a blank, for total protein. From the results, a standard deviation of 1.8 mg./100 ml. was calculated. A specimen of spinal fluid containing 85 mg./100 ml. was similarly analyzed 12 times; the S.D. was 2.2 mg./100 ml.

Initially, the method of Jacobs (10), which is based on "the protein error of indicators," was studied. In this laboratory, the technic proved to be unsatisfactory and was therefore discontinued. The results of protein analyses on 82 spinal fluid specimens by the other three methods (Table 2) showed very good agreement, and it is concluded

### Table 1. Recovery by UV Spectrophotometry of Albumin and Globulins Added to Ventricular Fluid

<table>
<thead>
<tr>
<th>Human crys. albumin (mg./100 ml.)</th>
<th>Cohn globulin fraction IV-6 (mg./100 ml.)</th>
<th>Pure γ-globulin (mg./100 ml.)</th>
<th>Total protein added (mg./100 ml.)</th>
<th>Protein recovered</th>
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<td>Total</td>
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<td>Albumin</td>
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<td>mg./100 ml.</td>
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<td>40</td>
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<td>42</td>
<td>105</td>
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### Table 2. Comparison of Results (mg./100 ml.) Obtained by Three Methods of Protein Analysis on 84 Specimens of Spinal Fluid

<table>
<thead>
<tr>
<th>Method</th>
<th>51 Infants, 1–6 days</th>
<th>87 Infants, 7–26 days</th>
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<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
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<tr>
<td>Tombs et al. (7), present modification</td>
<td>26–180</td>
<td>77.0</td>
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<tr>
<td>Kingsbury et al. (8), modified by Meulemans (1)</td>
<td>25–180</td>
<td>77.4</td>
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<tr>
<td>Doughaday et al. (9), modified by Zondag and Van Boetzelaar (5)</td>
<td>28–195</td>
<td>79.7</td>
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that each of these modified technics is a reliable clinical method. The
difference between the slightly higher figures given by the colorimetric
method and those of the other methods was not statistically significant
($P = >0.05$). The mean spinal fluid protein level of the group of pre-
mature infants was similar to that of the term infants of comparable
age.

References

   (1955).
   39, 663 (1952).
   (1926).