We thank Margaret Villiers, Department of Medicine, University Hospital, Nottingham, for carrying out all the column assays of glycosylated hemoglobin.

References

Assessment of the Benzenthionium Chloride Method for Routine Determination of Protein in Cerebrospinal Fluid and Urine

Elisabeth Flachaire,1 Odile Damour,2 Jacques Bienvenu,2 Tarak Aouliti,2 and Roger Later1

We have tested the characteristics of the method of Iwata and Nishikaze (Clin Chem 25: 1317, 1979). The linearity, sensitivity, and precision are satisfactory and the reactivity of benzenthionium chloride with various proteins (albumin, immunoglobulins) is the same. The method has been compared with Meulemans’s technique (Clin Chim Acta 5: 757, 1960), routinely used in our laboratories, by analysis of 82 samples of cerebrospinal fluid (CSF) and 119 samples of urine. Our results for cerebrospinal fluid agree well with those of Iwata and Nishikaze (r = 0.976; y = 0.992x – 0.013), but we find their method unsuitable for urinary protein determination, probably because of interfering compounds in urine.

Additional Keyphrase: turbidimetry

We have been very interested in the new turbidimetric method described by Iwata and Nishikaze (1) for determination of proteins in cerebrospinal fluid (CSF) and urine. Benzenthionium chloride in alkaline is used as a flocculation reagent. Iwata and Nishikaze say that this method has a higher sensitivity than the classic method of Lowry et al. (2) and shows satisfactory reproducibility and recovery, and that the turbidity produced is the same for albumin and gamma-globulins, and is more stable than in Meuleman’s method (3) or in the method of Bossak et al. (4).

We have evaluated their method, and we have compared the results with those by the method we routinely use in our laboratories, the sulfosalicylic acid method (3).

Materials and Methods

Preparation and Storage of Specimens

CSF and urine specimens were promptly centrifuged after collection. Protein was determined concurrently by the two methods, either immediately or after storage at −20 °C (the specimen being centrifuged just before analysis). Spectrophotometers used were the Model 25 Beckman (at Pierre Benite5) and Model 1800 Philips (at Lyon6).

Reagents

Iwata/Nishikaze (I/N) method: Benzenthionium chloride (Sigma Chemical Co., St. Louis, MO 63178) 2 g/L, sodium hydroxide 0.5 mol/L in 33 mmol/L tetrasodium ethylenediaminetetraacetate (EDTA) (Prolabo, Paris, France).

Meuleman’s method: (a) Dissolve 6 g of sulfosalicylic acid in 100 mL of distilled water. (b) Dissolve 14 g of anhydrous sodium sulfate in 100 mL of water. Mix equal volumes of a and b for use in the protein determination.

Standard and control. Human albumin (crystallized and lyophilized; Sigma A9511 and human globulin (Gamma 16; Institut Merieux, Marcy l’Etoile, France).
Methods

Protein in urine is systematically tested for by two techniques: Albustix strips, used as indicated by the manufacturer, Miles Laboratories, and Heller's nitric acid ring test, read at 3 min.

*I/N method*: Mix 0.1 mL of sample and 4.0 mL of the NaOH/EDTA mixture and immediately add 1 mL of a 2.0 g/L solution of benzethonium chloride. Shake the mixture well two to 10 times by hand or by vortex-mixing for 5 s. After 50 to 60 min measure the absorbance vs water, at 450 nm for cerebrospinal fluid or 600 nm for urine.

*Meulemans's method*: To 1 mL of urine add 4 mL of the reagent mixture and mix well. After 10 min measure the absorbance (A), at 450 nm for CSF and 600 nm for urine.

*Standard*: Working standards of human serum albumin were prepared in the range of 0.10 to 2 g/L by dilution with physiological saline. The same working solutions were used in the two techniques.

Results

Analytical Variables

*Linearity*: the calibration curve is linear from 0.25 to 1 g/L at 450 nm and from 0.25 to 1.5 g/L at 600 nm.

*Sensitivity*: For an absolute expression, sensitivity, determined in the linear part of the standard curve, is expressed as the change in absorbance per milligram of protein per liter of final mixture. The I/N method is about sevenfold as sensitive as Meulemans's method (Figures 1, 2).

*Precision*: Precision within- and between-runs is shown in Table 1. For evaluation of between-run precision, 30 aliquots were stored at −20 °C and one was used daily.

*Reactivity of benzethonium chloride with albumin and immunoglobulins*: To test this we used different mixtures containing various proportions of pure albumin and immunoglobulins. Their purity was previously confirmed by electrophoresis on cellulose acetate and immunoelectrophoresis, and the protein content of solutions of them was measured by gravimetry (6) and by the biuret technique (7). Proteins as measured in these various mixtures by use of the I/N method were identical with that theoretically expected.

*Comparison of the two techniques*: Using Meulemans's method as our comparison method, we assayed 82 samples of CSF. The correlation is shown in the Figure 3.

Again using Meulemans's method as the comparison method, we assayed 119 samples of urine with protein concentrations ranging from 0 to 1.40 g/L by the two techniques. The results are presented in Figure 4.

With urine, 89 specimens (at Lyon) and 49 specimens (at Pierre Benite), selected on the basis of negative results with "Albustix" strips, Heller's nitric ring test, and the sulfosalicyclic acid method (<0.10 g/L), were further analyzed with the I/N method. The results are presented in Table 2, classed in four ranges of concentrations.

Discussion

Our results confirm most of the general conclusions of Iwata and Nishikaze. We found the same sensitivity, preci-

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**Table 1. Precision of the Iwata/Nishikaze Method**

<table>
<thead>
<tr>
<th></th>
<th>Within-run</th>
<th></th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF</td>
<td>Urine 1</td>
<td>Urine 2</td>
</tr>
<tr>
<td>n</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Mean concn, g/L</td>
<td>0.637</td>
<td>0.443</td>
<td>0.920</td>
</tr>
<tr>
<td>SD, g/L</td>
<td>0.016</td>
<td>0.013</td>
<td>0.020</td>
</tr>
<tr>
<td>CV, %</td>
<td>2.48</td>
<td>2.93</td>
<td>2.17</td>
</tr>
</tbody>
</table>

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344 CLINICAL CHEMISTRY, Vol. 29, No. 2, 1983
Table 2. Results by the I/N Technique for Some Urines Negative by Other Methods

<table>
<thead>
<tr>
<th>Institution 1</th>
<th>Institution 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of selected negative urines</td>
<td>89</td>
</tr>
<tr>
<td>No. (and %) positive by the I/N technique</td>
<td>63 (71%)</td>
</tr>
<tr>
<td>Range, g/L</td>
<td></td>
</tr>
<tr>
<td>0.10–0.20</td>
<td>41</td>
</tr>
<tr>
<td>0.20–0.30</td>
<td>9</td>
</tr>
<tr>
<td>0.30–0.50</td>
<td>9</td>
</tr>
<tr>
<td>0.50–0.80</td>
<td>4</td>
</tr>
</tbody>
</table>

ters we used. With CSF, our results agree well with those of Iwata and Nishikaze, but for urines our results are not in agreement with theirs (Figure 4). Moreover, for a group of urine specimens, we found a number of “positive” results (>0.10 g/L) with the I/N technique that were not confirmed by the other methods (Table 2). We saw no case in which urine “negative” by the I/N method was found “positive” with any of the other techniques.

Furthermore, our findings suggest that most of the pseudoproteinurias revealed by Heller’s nitric acid ring test (precipitation ring above the interface) also react with benzethonium chloride. Because interfering substances present in urine evidently cause falsely high values with the I/N technique, a new protocol must be found if benzethonium chloride is to be used as the precipitating agent. Our results make it clear that no simple chemical method yet devised for protein in urine, especially in normal urine, is likely to give accurate values. We are currently investigating this problem in our laboratories. The most nearly definitive method (8) is too cumbersome for routine use.

References