plasma

Fig. 2. Relation between ultrafiltrate volume and apparent binding (drug concentration in ultrafiltrate) for theophylline (●) and acetaminophen (■) at 10 mg/L in plasma. Bars indicate SD (n = 7).

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References


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Tris Carbonate Interferes with Certain Methods for Protein and CO2

To the Editor:

Evaluating a lot of Fisher "Serachem" normal human control material (lot no. 311-010), reconstituted with Fisher abnormal diluent II (no. 293576; Fisher Scientific Co., Orangeburg, NY 10902), we found discrepancies in the bicarbonate and total protein concentrations, as determined with different analytical systems. The diluent contains 0.02 mol/L of distilled water and an unspecified concentration of tris(hydroxymethyl)aminomethane (Tris) carbonate. A second diluent used in this study was distilled water and tetramethyl ammonium bicarbonate (Hyland, Costa Mesa, CA 92626).

Total carbon dioxide was determined with a SMAC (Technicon Instruments Corp., Tarrytown, NY 10591), in which the carbon dioxide released after addition of acid is absorbed by an alkaline buffered solution containing phenolphthalein (1). The acid in the red color of the phenolphthalein is proportional to the total carbon dioxide content of the sample. We also used a PVA-4 electrolyte analytical system (Photovolta Corp., New York, NY 10010), which determines bicarbonate as follows: a strong acid is added to the sample, liberating carbon dioxide, and an amount of acid equivalent to the carbon dioxide released is used to neutralize the sample. The residual free acid is backtitrated to pH 7.4 and the bicarbonate concentration computed.

We determined total serum protein with the SMAC, which includes the biuret reaction and measurement of absorbance of the resulting complex at 550 nm (1); an aca (Du Pont Co., Instrument Products Div., Wilmington, DE 19898), which also involves the biuret reaction but measures the absorbance of the complex at 540 nm (2); a manual
biuret method (3); the method of Lowry et al. (4); the Coomassie Blue dye method (5); and a spectrophotometric method based on the difference in absorbance at 235 and 280 nm (6).

We found a significant difference between the SMAC and the PVA-4 determinations of total carbon dioxide (bicarbonate) when the lyophilized control material was diluted with Fisher abnormal diluent II: 35 (SD 2) mmol/L vs 55 (SD 2) mmol/L (n = 80). Use of the Hyland diluent, however, produced no such difference, the respective means being 24 and 25 mmol/L (n = 80).

During preparation of control materials, the carbon dioxide content is frequently depleted. Many manufacturers supply a diluent that contains an additive that will increase the total carbon dioxide content of the control material. If a carbonate ion rather than a bicarbonate ion is used to enhance the total carbon dioxide content, a problem may result. The method for the determination of bicarbonate with a PVA-4 requires two hydrogen ions to react with each carbonate ion to form carbonic acid. Carbonic acid is converted to water and carbon dioxide, which is liberated as a gas. The back titration to a fixed endpoint of pH 7.4 results in measurement of each carbonate ion in the control material as two bicarbonate ions. On the other hand, in the SMAC method each carbonate ion releases one carbon dioxide molecule when acid is added. Thus, the addition of either a carbonate ion or a bicarbonate ion to the control material will have the same effect on the total carbon dioxide content with the SMAC method.

Apparently, Tris causes a positive interference with the biuret method for the determination of protein. There was a significant difference in the protein concentration determined on the SMAC or the aca when the control material was diluted with the Fisher diluent II, as compared with dilution with the Hyland diluent or distilled water (Table 1). The apparent protein concentration in the Fisher diluent alone exceeded 4.6 g/L when analyzed for protein with the SMAC or aca, or by the manual biuret method, but was insignificant when determined by the Lowry, Coomassie, or absorbance methods. A 40 mmol/L solution of Tris gave about the same results for protein concentration as the Fisher diluent by all six methods. On the other hand, the Hyland diluent had an apparent protein concentration of 0.02 g/L or less, as determined by these methods. When five different concentrations of Tris (from 10 to 50 mmol/L) were analyzed in triplicate with the SMAC and the aca, the apparent protein concentration (g/L) was directly proportional to the Tris concentration (mmol/L); regression equations for the two analytical systems were as follows:

\[
\text{aca: Protein} = 198 \times \text{Tris} + 2.63 \\
\text{SMAC: Protein} = 307 \times \text{Tris} - 0.33
\]

We also noted that the Fisher abnormal diluent II seemed to contain protein when analyzed with the SMAC or the aca. When the manufacturer informed us that the diluent contained Tris carbonate, we demonstrated that Tris causes a positive interference with the biuret method for the determination of protein, as has also been documented previously (7–9). Tris forms a complex with the cupric ion and the maximum absorbance for the complex is quite similar to that of the protein-cupric complex (8). Robson et al. (7) showed that Tris affected both the intercept and the slope of the biuret calibration curve, the slope of the calibration curve decreasing with increasing concentrations of Tris. Unless the concentration of Tris is known and standardized with the biuret method, it thus would be very difficult to adjust appropriately for the interference caused by Tris. For this reason, Tris carbonate is not a suitable additive to control materials or diluents when the control material will be used for either the determination of total serum protein or bicarbonate.

Tris also induces a positive bias in the determination of protein by the method of Lowry et al. (4), although it is about 1000-fold less than the interference noted with the biuret method. Gellert et al. (10) previously noted a significant positive interference with the Lowry method when the Tris concentration exceeded 0.1 mol/L. We found a very slight positive interference by Tris with the spectrophotometric method and no interference with the Coomassie Blue method.

Accordingly, we recommend avoiding the use of Tris in any solutions being considered for the determination of protein and avoiding addition of Tris carbonate in bicarbonate determinations.

**Table 1. Apparent Protein Concentration of Three Diluents as Measured with Six Different Analytical Systems**

<table>
<thead>
<tr>
<th>Apparent protein, g/L</th>
<th>Tris, 0</th>
<th>Fisher</th>
<th>Hyland</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMAC</td>
<td>0.02</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>aca</td>
<td>0.07</td>
<td>0.05</td>
<td>0.3</td>
</tr>
<tr>
<td>Manual biuret</td>
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<td>0</td>
</tr>
<tr>
<td>Lowry et al.</td>
<td>0.07</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>Coomassie Blue</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spectrophotometric</td>
<td>0.02</td>
<td>0.01</td>
<td>0</td>
</tr>
</tbody>
</table>

References


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**Microcomputer Program for Generating Beckman ASTRA-8 Reports**

To the Editor:

We have interfaced a microcomputer to a Beckman ASTRA-8 chemistry analyzer for the purpose of collating the data and printing reports. The computer system is a Radio Shack TRS-80 Model III 48K with one-disc drive and an Epson MX-80 printer (total cost $2500). The BASIC program features two modes of data transfer from the ASTRA-8, several methods of examining and printing the data, and built-in diagnostic tests for