Azide Interference with Bilirubin Determinations in Which Ehrlich's Reagent is Used

To the Editor:
A recent letter by Eckfeldt et al. (1) recalled some of our unpublished findings of a few years ago, showing the effect of sodium azide on continuous-flow (SMA 12/60) measurements (Table 1).

The azide interference with total bilirubin determinations they reported can be also be seen in our results, although not conspicuously. As can be seen in Table 1, measurements other than bilirubin may be affected, and quite possibly, creatine kinase and alkaline phosphatase are depressed and apparent calcium is elevated with increased azide concentrations. Tests that were unaffected include lactate dehydrogenase, aspartate aminotransferase, creatinine, uric acid, phosphorus, albumin, protein, and cholesterol. The analysis of lyophilized sera (Versatol) reconstituted with identical concentrations of sodium azide gave similar, confirming results.

Reference

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Ed. note: Readers of this journal are reminded of the explosive propensities of azides.

Optimizing Measurement of Glycosylated Hemoglobins

To the Editor:
Measurement of glycosylated hemoglobin is an important factor in the long-term regulation of the carbohydrate metabolism of patients suffering from diabetes mellitus.

Several techniques for measuring these hemoglobins have been described. Column chromatography is the simplest and can be applied in any laboratory. However, in practice, separation of the glycosylated hemoglobins raises certain problems. According to a recent report (1) this applies to a commercial method and, in our experience, also to other published methods involving column chromatography (2, 3).

The column packing used is: Bio-Rex 70, 100–200 mesh, Na+ form (Bio-Rad Laboratories).

In our experiments we found that successful separation of glycosylated hemoglobins from other hemoglobins largely depends on pH and phosphate buffer concentration. As a measure for the concentration of the phosphate buffer, the osmolality of the buffer was determined. The great interdependence of pH and osmolality can be seen in the following experiments.

Separation is good at pH 6.80 and with a buffer concentration of 97 mosm/kg. It is also good at pH 6.85 and with a buffer concentration of 87 mosm/kg. At a pH of 6.80 and a buffer concentration of 97 mosm/kg, some of the Hb A1 is left in the column. At pH 6.85 and a buffer concentration of 97 mosm/kg, some of the main fraction of the hemoglobins is carried along with the glycosylated hemoglobins. For satisfactory separation the correct combination of pH and buffer concentration is therefore of the greatest importance.

A pH of 6.85 and a buffer concentration of 87 mosm/kg produced optimum separation.

It is advisable to have a good pH meter and a good osmometer for testing the columns for measuring the glycosylated hemoglobins.

References

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Problems in Measuring Total Protein in Cerebrospinal Fluid with the Amylase-Lipase Analyzer

To the Editor:
Use of the Perkin-Elmer Amylase-Lipase Analyzer Model 91 (Perkin-Elmer Corp., Oak Brook, IL 60521) has been recommended by Murray (1) as a precise and inexpensive method for determination of total protein in cerebrospinal fluid (CSF).

The instrument is minimally modi-
Table 1. CSF Protein and Nephelos Reading Compared for Five Pooled Human Sera with Different Albumin/Globulin Ratios

<table>
<thead>
<tr>
<th>Total protein</th>
<th>Albumin</th>
<th>Albumin/globulin ratio</th>
<th>Albumin/globulin</th>
<th>y(protein) = mx(neophelos) + c</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td>37</td>
<td>1.00</td>
<td>1.00</td>
<td>y = 0.0212x + 0.0397</td>
</tr>
<tr>
<td>68</td>
<td>36</td>
<td>1.13</td>
<td>1.13</td>
<td>y = 0.0192x + 0.0342</td>
</tr>
<tr>
<td>71</td>
<td>39</td>
<td>1.22</td>
<td>1.22</td>
<td>y = 0.0190x + 0.0263</td>
</tr>
<tr>
<td>74</td>
<td>41</td>
<td>1.24</td>
<td>1.24</td>
<td>y = 0.0195x + 0.0119</td>
</tr>
<tr>
<td>74</td>
<td>44</td>
<td>1.47</td>
<td>1.47</td>
<td>y = 0.0174x + 0.0160</td>
</tr>
</tbody>
</table>


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Normal Range for Serum Sodium by Ion-Selective Electrode Analysis Exceeds That by Flame Photometry

To the Editor:

We wish to report our experience with a NOVA 1 Ion-Selective Electrode Analyzer for Sodium and Potassium (Nova Biomedical, Newton, MA) recently marketed in the United Kingdom by American Hospital Supply (U.K.) Ltd., Didcot, Oxon. An instrument supplied for a trial period in our laboratory produced results for plasma and serum sodium that differed significantly from those obtained by flame photometry.

In the NOVA 1, ion-selective electrodes measure sodium and potassium in whole-blood plasma, separated plasma or serum, or urine. A microprocessor automatically measures sodium and potassium in an aqueous standard with each specimen and performs a two-point calibration of the electrodes at 2-h intervals.

During a trial period of 14 days we evaluated the instrument by measuring sodium and potassium in serum and plasma and comparing the results with those obtained when the same samples were analyzed with a Technicon SMA 6/60 analyzer. The analytical precision of the NOVA 1, assessed by replicate analyses of a commercial control serum, was found to be acceptable. For 92 measurements each during 14 days, sodium values were 142.77 ± 1.15 mmol/L (mean ± SD), with a coefficient of variation (CV) of 0.81%; potassium values were 3.92 ± 0.062 mmol/L (mean ± SD), with a CV of 1.33%.

However, when we analyzed 84 specimens of heparinized plasma from hospital patients, the difference between the plasma potassium means for the NOVA 1 and the SMA 6/60 was +0.008 mmol/L, but the difference between the plasma sodium means was +5.6 mmol/L. For sodium, the differences ranged from +0 to +11 mmol/L in individual specimens.

These differences between the sodium values from the NOVA 1 and the SMA 6/60 were larger than either we or Nova Biomedical had expected. Specimens of serum and plasma from seven patients and six normal subjects were sent to Nova Biomedical in the United States, who confirmed the large sodium differences between ion-selective electrode analysis and flame photometric analysis, and excluded the possibility that the differences were due to any instrument or standardization error.

The wide range of sodium differences in patients' samples led us to investigate the comparison between NOVA 1 and the SMA 6/60 for normal serum specimens. Blood was collected from 22 members of the laboratory staff in apparent good health, allowed to clot for 1 h, centrifuged, and the serum was separated. The difference between the serum potassium means for the NOVA 1 and the SMA 6/60 was +0.049 mmol/L, and between the sodium means was +4.7 mmol/L. The differences in serum sodium in individual specimens ranged from +3 to +6 mmol/L.

The current edition of the NOVA 1 operating manual states that the values for serum sodium and potassium are similar to those obtained by other methods of measurement, such as flame photometry, and the sodium reference range quoted applies to sodium determined by flame photometry. Our results, obtained from normal serum samples, give the following ranges (mmol/L) for sodium and potassium (mean ± 2SD):

<table>
<thead>
<tr>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA 6/60</td>
<td>137–144</td>
</tr>
<tr>
<td>NOVA 1</td>
<td>142–148</td>
</tr>
</tbody>
</table>

Our finding of a higher normal range for serum sodium confirms the finding of Patal and O’Gorman (1), who evaluated another ion-selective electrode analyzer (Space Stat 30; Orion Biomedical).

It is important that these differences between the ranges for normal values for sodium as measured with ion-selective electrodes and flame photometry be known, particularly in a laboratory where both types of instrument are used.

We have informed Nova Biomedical of our findings of a significant difference in values obtained for plasma and serum sodium when measured by ion-selective electrodes as compared to flame pho-