of serum specimens contaminated with heparin (from indwelling lines) into heparin-containing Vacutainer Tubes (Becton Dickinson, Rutherford, NJ), and (b) by adding heparin (final concentration, 25–50 KIU/L) to the diluent used on the Tosoh AIA1200. The results are shown in Table 1. Interference was judged to be eliminated if triplicates were no longer discordant. Addition of excess heparin did appear to end or reduce the interference, except for specimen 17. Notice that spurious results were either high (specimens 12, 13, and 16), or low (specimens 11, 14, 15, and 19). For specimen 10, however, results for the replicates themselves were not discordant; rather, the interference was revealed by the change in results due to heparin treatment. This indicates that discordance among replicates does not necessarily reveal this problem. In fact, among the other 18 triplicate results, most have pairs that would be considered acceptable duplicates (e.g., specimens 1, 7, 9, 13, 14, 16, 19).

Serum protein electrophoresis indicated that fibrinogen was present in every specimen we examined that exhibited interference. It is possible that clotting in the sample cup (rather than within the instrument) could cause short sampling. However, this would produce results that are spuriously decreased, not increased (we observed both), and would not be remedied by addition of heparin to the Tosoh diluent as we found. We conclude that fibrin formation within the Tosoh reaction vessel was the most likely cause of the artificially high or low results observed with that assay system. Because of the limited data we have for the IMx, we can only note that interference from fibrin may occur but we cannot suggest a mechanism or location.

Although we encountered this interference in patients’ specimens infrequently (our estimate of prevalence is ≤1%), the problem cannot be ignored. A laboratory receiving a few hundred specimens per day may risk several spurious results daily if using susceptible instrumentation.

The wider significance of our observation is that, in addition to tests with completely clotted and completely anticoagulated plasma specimens, incompletely clotted serum specimens—whether from coagulation factor deficiencies or the presence of anti-coagulants—should also be considered when evaluating new methods and instruments.

Mark H. Zweig
Janice Glickman
Gyorgy Csako

Clin. Chem. Service
W.G. Magnuson Clinic. Center, Room 2C407
National Institutes of Health
Bethesda, MD 20892

1 Author for correspondence.

Glucose Interference in Jaffé Creatinine Method: Effect of Calcium from Peritoneal Dialysate

To the Editor:

Measurement of creatinine in peritoneal dialysate is used in the peritoneal equilibration test to assess the performance of ambulatory peritoneal dialysis (1). Because peritoneal dialysate solutions often contain glucose, which interferes in the Jaffé creatinine measurement, Farrel and Bailey proposed the following correction equation to account for the interference (J):

\[
\text{corr. Cr} = (\text{meas. Cr} – F \times \text{gluc})
\]

where \(F\) is an analyzer-dependent constant.

We found, however, that the correction equation failed to predict the spurious creatinine results produced by unused peritoneal dialysate solutions. Moreover, we found significant discrepancies between creatinine results for the unused peritoneal dialysate solutions and for glucose solutions with the same glucose concentration; e.g., spurious creatinine values were 86 \(\mu\)mol/L for 236 mmol/L glucose solution and 154 \(\mu\)mol/L for unused dialysate solution at the same glucose concentration.

Trying to uncover the cause of this discrepancy, we further analyzed the interference of the constituents of the unused peritoneal dialysate solutions. The dialysate Dianeon™ (with glucose 42.5 g/L) was from Baxter Healthcare (Miami, FL) and included glucose 236 mmol/L, lactate 35 mmol/L, NaCl 132 mmol/L, MgCl₂ 0.75 mmol/L, and CaCl₂ 1.75 mmol/L. To prepare comparable solutions, we obtained reagent-grade glucose, CaCl₂, pyruvate (Sigma Chemical Co., St. Louis, MO), acetone, and lactate (Wako, Osaka, Japan).

We measured creatinine with a Hitachi 7450 automated analyzer (Hitachi, Tokyo, Japan), using alkaline picrate reagents (Iatron Laboratory, Tokyo, Japan). The analyzer settings for this measurement were, according to the manufacturer’s guidelines: reagent 1 (NaOH 105 mmol/L) 280 \(\mu\)L, reagent 2 (picric acid 38 mmol/L) 70 \(\mu\)L, and sample volume 14 \(\mu\)L. The kinetic measurement of optical absorbance was determined between points 30 and 35 (360–420 s after the addition of reagents), with a main wavelength of 505 nm and secondary wavelength of 600 nm.

All analytes were measured on the same day. The between-run quality-control data (CVs, n = 4) were 6.06% and 1.87%, respectively, for Wako control serum I (mean creatinine 117 \(\mu\)mol/L) and control serum II (mean 567 \(\mu\)mol/L).

Measurements of the constituents of the peritoneal dialysate solution and of their combinations revealed that no single constituent other than glucose interfered directly with the measurement. However, calcium enhanced the interference caused by glucose. In the presence of 1.75 mmol/L calcium, the spurious creatinine measurement caused by

Table 1. Triplicate FSH measurements in specimens exhibiting apparent interference in assays performed on the Tosoh AIA 1200.

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>No heparin added</th>
<th>Heparin added after specimen collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine “sera” with continued fibrin formation after centrifugation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>130, 87, 134</td>
<td>130, 87, 134</td>
</tr>
<tr>
<td>2</td>
<td>1, 86, 155</td>
<td>1, 86, 155</td>
</tr>
<tr>
<td>3</td>
<td>62, 72, 94</td>
<td>62, 72, 94</td>
</tr>
<tr>
<td>4</td>
<td>13, 12, 6</td>
<td>13, 12, 6</td>
</tr>
<tr>
<td>5</td>
<td>147, 154, 57</td>
<td>147, 154, 57</td>
</tr>
<tr>
<td>6</td>
<td>141, &lt;1, 200</td>
<td>141, &lt;1, 200</td>
</tr>
<tr>
<td>7</td>
<td>15, 7, 8</td>
<td>15, 7, 8</td>
</tr>
<tr>
<td>8</td>
<td>82, 1, 46</td>
<td>82, 1, 46</td>
</tr>
<tr>
<td>9</td>
<td>22, 11, 10</td>
<td>22, 11, 10</td>
</tr>
</tbody>
</table>

Sera potentially contaminated with heparin during collection

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>No heparin added</th>
<th>Heparin added after specimen collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>21, 19, 19</td>
<td>123, 119, 120</td>
</tr>
<tr>
<td>11</td>
<td>9, 35, 34</td>
<td>60, 61, 82</td>
</tr>
<tr>
<td>12</td>
<td>200, 200, 2</td>
<td>3, 3, 3</td>
</tr>
<tr>
<td>13</td>
<td>3, 3, 27</td>
<td>3, 3, 3</td>
</tr>
<tr>
<td>14</td>
<td>34, 34, 34</td>
<td>33, 35, 34</td>
</tr>
<tr>
<td>15</td>
<td>39, 78, 57</td>
<td>80, 76, 82</td>
</tr>
<tr>
<td>16</td>
<td>12, 8, 8</td>
<td>8, 8, 8</td>
</tr>
<tr>
<td>17</td>
<td>67, 50, 28</td>
<td>53, 91, 41</td>
</tr>
<tr>
<td>18</td>
<td>78, 84, 43</td>
<td>—</td>
</tr>
</tbody>
</table>

Serum from patient with prolonged PT

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>No heparin added</th>
<th>Heparin added after specimen collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>&lt;1, 5, 5</td>
<td>6, 6, 6</td>
</tr>
</tbody>
</table>

* Serum was added to heparin-containing tube before sampling by analyzer. For samples 12–19, heparin was added to diluent.
236 mmol/L glucose solution was increased from 86 to 105 μmol/L, close to that caused by the unused peritoneal solution. The other constituents and their combinations had no effect on the glucose interference. Thus, the enhancing effect of calcium on the glucose interference with creatinine measurement accounted for the creatinine discrepancies between unused peritoneal dialysate solution and glucose solution.

We then studied the effects of various combinations of different concentrations of glucose and calcium on the Jaffe creatinine measurement. At a fixed calcium concentration, the interference was in proportion to the glucose concentration. At a fixed glucose concentration, the presence of calcium produced an additive spurious creatinine concentration in proportion to the calcium concentration. Therefore, the spurious creatinine results might be expressed as follows (all concentrations in mmol/L):

\[
[\text{corr. Cr}] = [\text{meas. Cr}] - F_1[\text{gluc}] (1 + F_2[\text{Ca}])
\]

The factors F1 and F2 depended on the analyzers and settings used. In our setup, \(F_1 = 0.36 \, (SE = 0.01)\) and \(F_2 = 0.49 \, (SE = 0.03)\). The interference of two other known interferents in the Jaffe creatinine measurement, acetone and pyruvate, was not affected by the presence of calcium.

A previous study by Larpent and Verger (2) showed that creatinine in peritoneal dialysate solutions was appropriately measured only by enzymatic assays. Most of the interference from dialysate solutions was thought to come from the high concentration of glucose (1). The interference effect of calcium, which they had thought was unpredictable (2), we found to be in proportion to its concentration. At a calcium concentration of 2 mmol/L, the magnitude of spurious increase in creatinine was \(1.5 \times 2\) times that caused by glucose alone.

Two categories of creatinine interference have been proposed (3, 4): substances such as glucose, which slowly reduce the alkaline picric acid to picramate; and those such as acetocetate, pyruvate, and other \(\alpha\)-keto acids, which react with alkaline picric acid to form colored complexes. Calcium probably acts as a cofactor in the reduction of alkaline picric acid and amplifies the glucose interference effect, whereas the reaction of \(\alpha\)-keto acids to form complexes with the picric acid seems not to be influenced by calcium.

The low concentrations of calcium in serum and urine might be insufficient to amplify the glucose effect on creatinine concentrations. However, in samples with a high concentration of glucose and containing calcium, the indirect interferent effect of calcium should be taken into account. In our laboratory, the used peritoneal dialysate may still contain high concentrations of glucose and calcium, 40 and 1.4 mmol/L, respectively, after 4 h of peritoneal incubation, for a dialysate with an initial concentration of 138.8 mmol/L glucose and 1.75 mmol/L calcium. Creatinine measurements obtained under such conditions should be interpreted with caution.

We recommend that the previously established correction equation for glucose interference should be modified as reported here. Alternatively, the enzymatic method for creatinine measurement, which is unaffected by glucose, should be considered.

This work was supported by a grant (NSC-82-0412-8002-156-MO2) from the National Science Council, Republic of China.

References


Shyh-Chyi Le
Keh-Sung Tsai

Dept. of Lab. Med.  
National Taiwan Univ. Hosp.  
No. 7, Chung-Shan S. Rd.  
Taipei, Taiwan, R.O.C.

1 Author for correspondence.