Effect of Storage on Measurement of Ionized Calcium in Serum of Uremic Patients


We studied, in 70 acidotic and non-acidotic uremic patients, the analytical variance in serum ionized calcium as related to duration and temperature of storage. Storage of serum or whole blood at 4 °C for as long as 6 h did not significantly alter the measured concentration of ionized calcium in the serum. Storage at room temperature for 6 h, or longer at 4 °C or −20 °C, resulted in inaccuracies in 39 to 79% of the samples of serum and in 38 to 92% of the samples of whole blood. These errors were not negated by correcting the values for ionized calcium to a pH of 7.40. Indeed, corrected values for calcium were even more unreliable in acidic patients. We conclude that samples from uremic patients should be analyzed for ionized calcium within 2 h, or within 6 h if stored at 4 °C.

Additional Keyphrases: variation, source of • acidosis • sample handling • ion-selective electrodes • acid–base disorders • kidney disease • dialysis therapy

Calcium homeostasis is better reflected by the concentration of ionized than of total calcium. The former depends in part on plasma protein concentrations, but changes in acid–base status alter protein binding of calcium and hence the proportion present in ionized form (1). The Radiometer ICA1 analyzer (Radiometer A/S, Copenhagen, Denmark) measures ionized calcium and pH simultaneously, and also applies a correction for pH. However, the physiological relevance of pH correction in the measurement of ionized calcium is unclear when applied to sera from patients with acid–base disorders.

Patients with chronic renal failure have abnormal calcium metabolism, and regular monitoring of serum calcium is important in their management. Before measurement of total calcium is replaced by estimation of ionized calcium in these patients, further studies on methods of handling specimens are required. Here we report the effects of storage and temperature on the concentration of ionized calcium in sera from acidic and non-acidotic uremic patients.

Patients and Methods

We studied 70 patients who were undergoing dialysis therapy, including 13 with acidosis (pH < 7.32). Blood was collected by venepuncture into syringes, then injected into evacuated tubes containing no additive (Sherwood Medical, St. Louis, MO 63103). After the clotted sample had been centrifuged, the serum was aspirated into syringes, which were promptly sealed without an air gap to maintain anaerobic conditions until analysis.

To assess the effects of storage of the uncentrifuged sample, we collected blood from each patient into three evacuated tubes. Serum was separated from the first of the three and analyzed immediately, from the second after 6 h (stored at room temperature), and from the third after 24 h (stored at 4 °C).

To assess the effects of storage of serum, we analyzed serum from samples immediately after clotting and centrifugation and replicate serum samples after 2, 6, and 24 h of storage at room temperature, 4 °C, or −20 °C.

We measured serum ionized calcium, pH, and pH-corrected ionized calcium simultaneously with a Radiometer ICA1 analyzer according to the manufacturer’s instructions. Correction of the calcium value to what it would be at pH 7.4 is based on a multiplication factor, corrected calcium = ionized calcium × [1 − 0.53 (7.4 − actual pH)] that is said to be valid within the pH range 7.4 through 7.6 (2). The electrode was calibrated with two aqueous solutions: solution 1 contained 1.4 mmol of calcium chloride, 106.3 mmol of sodium chloride, and 83.7 mmol of N-tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid per kilogram of water. Solution 2 contained 2.8 mmol of calcium chloride, 102 mmol of sodium chloride, and 107.9 mmol of N,N-tris(2-hydroxyethyl)-2aminoethanesulfonic acid per kilogram of water. At 37 °C the respective concentrations of ionized calcium and the pH values are 1.25 and 2.50 mmol/L and 7.383 and 6.841.

For quality control we used Qualichek “high” and “low” (Radiometer) buffered-saline solutions containing about 1.75 (pH 7.25) and 0.80 (pH 7.55) mmol of ionized calcium per liter, and plasma QAP1 (Dade Lab) containing 0.90 mmol of ionized calcium per liter. The electrode was recalibrated every 2 h. With the plasma QAP1 used as a control in our laboratory during a year, the precision of the instrument was ±0.02 mmol/L, similar to that reported by Sigggaard-Andersen et al. (3).

Analysis of results. The significance of the difference between the mean ionized calcium concentrations in sera from uremic patients and a normal population was calculated by use of the unpaired Student’s t-test. The difference between the mean ionized calcium concentrations for groups of samples before and after the same conditions of storage was called the “mean increment,” and its significance was determined by the paired Student’s t-test. We also calculated the change in ionized calcium concentration, expressed as a positive value, for each pair of samples before and after storage and defined a value exceeding 0.02 mmol/L as an “accuracy error.” The mean of these values for a group of samples undergoing the same conditions of storage was called the “mean change.”

Results

Table 1 compares results for sera from uremic patients with those for 95 healthy adults as measured with the same instrument.

We found such accuracy errors for serum extracted from stored samples of whole blood in 36% of cases for ionized and 100% of cases for corrected calcium (Table 2). Storage of whole blood from non-acidotic uremic patients resulted in accuracy errors in serum ionized and corrected calcium in 20 to 92% of samples (Table 2).
Storage of serum at room temperature for as long as 2 h and at 4 °C for as long as 6 h did not alter the concentrations of ionized calcium in more than 90% of samples (Table 3). Longer storage of serum resulted in accuracy errors in 39 to 79% of cases. Although the pH after storage remained within the range 7.2–7.6, corrected calcium values were inaccurate in 46 to 75% of samples.

Discussion

These results contradict the findings for healthy adults in previous studies. For example, using the same instrument for samples from five normal subjects, Brouman et al. (2) found no change in ionized calcium concentrations after 24 h for sera stored at −20 °C and no change in corrected calcium for sera stored for seven days. Smith et al. (4) stored sera aerobically and found no change in values for corrected calcium.

Toffaletti et al. (5) recently studied the stability of ionized calcium and pH in samples that had been stored at different temperatures. The samples were from ostensibly healthy volunteers. Their data indicate that ionized calcium is stable whether or not the blood is separated, especially if it is stored at 4 °C. A previous study of normal subjects from our laboratory (in which a different instrument was used) showed no significant change in concentrations of ionized calcium after 24-h storage at 4 °C (6).

Uremic serum possibly is less stable than serum from normal subjects.

Table 2. Effect of Storage of Whole Blood from Uremic Patients on Concentrations of Ionized Calcium in the Serum

<table>
<thead>
<tr>
<th>Temp. interval, h</th>
<th>No. samples</th>
<th>&quot;Mean change&quot; from 0-h value measured (pH corrected), Ca²⁺, μmol/L</th>
<th>% of samples differing by &gt;20 μmol/L from 0-h value</th>
<th>pH after storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room</td>
<td>6</td>
<td>11</td>
<td>6 (NS)</td>
<td>36</td>
</tr>
<tr>
<td>4 °C</td>
<td>24</td>
<td>13</td>
<td>15 (NS) &lt;0.001</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Non-acidotic</td>
<td>6</td>
<td>31</td>
<td>15 (NS &lt;0.001)</td>
<td>20 (71)</td>
</tr>
<tr>
<td>4 °C</td>
<td>6</td>
<td>20</td>
<td>5 (NS &lt;0.001)</td>
<td>17 (65)</td>
</tr>
<tr>
<td>4 °C</td>
<td>24</td>
<td>20</td>
<td>18 (NS &lt;0.001)</td>
<td>26 (92)</td>
</tr>
</tbody>
</table>

*Values in parentheses are corrected to pH 7.40. NS, not significant (p>0.01).

Table 3. Effect of Temperature of Storage of Serum on Concentrations of Ionized Calcium

<table>
<thead>
<tr>
<th>Storage interval, h</th>
<th>No. samples</th>
<th>&quot;Mean increment&quot; from 0-h value measured (pH corrected), Ca²⁺, μmol/L</th>
<th>Significance</th>
<th>% of samples differing by &gt;20 μmol/L from 0-h value</th>
<th>pH after storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>2 (−3 NS)</td>
<td>17 (NS)</td>
<td>10</td>
<td>7.42–7.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12)</td>
<td>(35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>25 (−2 NS)</td>
<td>29 (NS)</td>
<td>46</td>
<td>7.40–7.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 °C</td>
<td>28</td>
<td>−28 (−13 NS &lt;0.001)</td>
<td>9 (NS (36))</td>
<td>46</td>
<td>7.40–7.41</td>
</tr>
<tr>
<td>24</td>
<td>28</td>
<td>20 (−2 NS)</td>
<td>9 (NS (40))</td>
<td>7</td>
<td>7.40–7.43</td>
</tr>
<tr>
<td>−20 °C</td>
<td>28</td>
<td>20 (−5 NS)</td>
<td>50 (NS (80))</td>
<td>79</td>
<td>7.41–7.51</td>
</tr>
<tr>
<td>24</td>
<td>23</td>
<td>20 (−30 NS &lt;0.001)</td>
<td>28 (NS (35))</td>
<td>39</td>
<td>7.38–7.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(−23)</td>
<td>(65)</td>
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</table>

NS, not significant (p>0.01). *Values in parentheses are corrected to pH 7.40.
normal subjects, accounting for the contrasting findings in this report. However, we suggest that it may also arise because of the method that we used to interpret these data. Our results demonstrated large positive and negative changes in ionized calcium concentrations in sera from different patients after storage. Thus the mean ionized calcium concentrations for a large number of samples may change little after the same conditions of storage, though many individual changes occur that well exceed the precision of the method. Some of the previous investigators did not find significant individual changes after storage (5), but others did not report on this, and considerably fewer subjects were involved in all studies than in the present report.

Further studies are needed to confirm the stability of ionized calcium concentration in the serum of normal subjects, but our results suggest that, at least in uremic patients, storage of blood or serum is associated with significant changes in ionized calcium and pH-corrected values for it. We recommend that samples from such patients stored at room temperature be analyzed within 2 h, or within 6 h if stored at 4 °C. Changes occurring on longer storage may not invalidate the clinical usefulness of ionized calcium measurement, but they are likely to exceed the precision attainable with the analyzer.

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References


Detection of Thyroid Disorders by Use of Basal Thyrotropin Values Determined with an Optimized “Sandwich” Enzyme Immunoassay

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To measure the concentrations of thyrotropin (thyroid-stimulating hormone), we used the components of a commercially available two-step “sandwich” enzyme immunoassay (Enzymun-Test® TSH, Boehringer Mannheim) based on the specific binding of the β-subunit of thyrotropin by monoclonal antibodies coated on polystyrene tubes. By modifying the original assay protocol, we lowered the limit of detection to 0.16 milli-int. units/L, using a total incubation period of 22 h. With this modification we could differentiate between patients responsive to administration of thyroliberin (thyrotropin-releasing factor) and those who were non-responders, by measuring only the basal concentration of thyrotropin. Furthermore, we demonstrated a correlation between the basal concentration of thyrotropin and its increase after administration of thyroliberin (r = 0.77, n = 48).

Additional Keyphrases: thyroliberin stimulation test – thyroid status – monoclonal antibodies

The key role of thyrotropin in differentiating hyperthyroidism from euthyroidism has focused on sensitive methods for measuring it in human serum (1–3). The diagnostic value of basal concentrations of thyrotropin as measured with commercially available kits has been limited to discriminating primary hypothyroidism and euthyroidism. Assessment of euthyroidism, hyperthyroidism, and borderline hypothyroidism, however, requires additional measurement of thyrotropin after administration of thyroliberin.

Recently Seidel et al. (4–6) developed a sensitive radioimmunoassay for thyrotropin, capable of detecting basal thyrotropin concentrations for predicting the susceptibility of the pituitary gland to stimulation by thyroliberin. This method, however, based on a commercially available double-antibody radioimmunoassay, required considerably prolonged incubation periods and tracer-purification procedures to attain the necessary increase in sensitivity.

Recently a new method for determination of thyrotropin has become available (Enzymun-Test® TSH; Boehringer Mannheim GmbH, Mannheim, F.R.G.), a two-step "sandwich"-type enzyme immunoassay. Our modifications of this assay resulted in good sensitivity without loss of practicability and demonstrated a good correlation between basal and thyroliberin-stimulated thyrotropin values.

Materials and Methods

Principle

Polystyrene tubes are coated with a monoclonal antibody against the β-subunit of thyrotropin. During incubation with standard or serum, thyrotropin is bound to the tube.