Measurement of Ultrafiltrable Calcium in Serum with Use of the "Worthington Ultrafree Anticonvulsant Drug Filter"

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We describe a method for ultrafiltrable calcium involving the use of Worthington Ultrafree Anticonvulsant Drug Filters. When measured at 37 °C, with mineral oil covering the sample to prevent loss of CO2, values for ultrafiltrable calcium correlated reasonably well (r = 0.91) with those for ionized calcium as measured with an ion-selective electrode. All patients' samples with significantly high or low values for ionized calcium were identified by the ultrafiltration method, including one specimen for which the McLean-Hastings protein correction could not explain the discrepancy between ionized and total calcium. The method requires about 2 mL of serum, yields about 100 μL of protein-free ultrafiltrate, and with it any laboratory with a semi-micro calcium method can measure ultrafiltrable calcium.

Additional Keyphrases: Ca2+ • ion-selective electrodes • methods for the small laboratory

Much of the calcium in serum is inactive physiologically; ordinarily, about 40 to 50% of it is "protein-bound," primarily to albumin, and another 10–15% "complexed" to various anions such as phosphate, bicarbonate, and citrate (1-3). The rest is in the form of the free, divalent cation, often referred to as the "ionized" calcium. Because the free calcium cation is the physiologically active form, various methods have been used to estimate it. McLean and Hastings, in their pioneering work (1, 2), introduced the first biological assay for ionized calcium and presented a means of estimating physiologically active calcium, based on a relationship to serum protein concentration. Numerous methods (4-7) have been proposed for measuring "diffusible" calcium (ionized plus complexed) by dialysis or ultrafiltration through semipermeable membranes, but they are difficult and time consuming and require precise control of factors that affect calcium binding to protein and anions, such as pH and temperature (6, 7). Thus they are not well suited to clinical laboratory use. Toffaletti et al. (8, 9) introduced a continuous-flow (AutoAnalyzer) method for measuring dialyzable calcium that is much easier than the previous manual methods, but the method requires equipment (and laboratory space) that is dedicated to that one determination. Selective-ion electrodes that directly measure ionized calcium have also gained in popularity; but again, specialized and costly equipment is required.

In this paper, we describe a simple, rapid, inexpensive method for ultrafiltrable calcium that requires no specialized equipment and can be performed by any laboratory that can measure total calcium in 100 μL of protein-free ultrafiltrate.

Materials and Methods

Collect blood in "serum separator" evacuated blood collection tubes (SST Vacutainer; Becton, Dickinson and Co., Rutherford, NJ 07070). Allow the blood to clot for 30 to 60 min, and centrifuge the capped tubes at 2000 x g for 10 min to position the serum separator material (a polyester gel). Once the serum separator is positioned by centrifugation, there is no demonstrable change in ultrafiltrable calcium concentration for at least 24 h if the tubes are kept capped and refrigerated.

Obtain serum samples for measurement of total, ionized, and ultrafiltrable calcium anaerobically through the rubber cap, by using a syringe and needle. Measure serum ultrafiltrable and total calcium by an o-cresolphthalein complexone colorimetric method (we used a discrete analyzer for this, the Abbott ABA-100 and Abbott "A-Gent" reagents and aqueous standards; Abbott Laboratories, Pasadena, CA 91030). Measure ionized calcium with a Space-Stat 20 Ionized Calcium Analyzer (Orion Research, Cambridge, MA 02139). Measure total protein (we used a Multistat III microcentrifugal analyzer with the manufacturer's reagents and standards; Instrumentation Laboratory, Lexington, MA 02173). We used lyophilized human serum as control material (Hyland Division, Travnel Labs., Costa Mesa, CA 92626).

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Table 1. Ultrafiltrable, Measured Ionized, Calculated Ionized, and Total Calcium for Healthy Individuals

<table>
<thead>
<tr>
<th>Ultrafiltrable</th>
<th>Measured Ionized</th>
<th>Calculated &quot;Ionized&quot;a</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.46</td>
<td>1.24</td>
<td>2.46</td>
</tr>
<tr>
<td>SD</td>
<td>0.075</td>
<td>0.049</td>
<td>0.102</td>
</tr>
<tr>
<td>Reference intervalb</td>
<td>1.31–1.61</td>
<td>1.14–1.34</td>
<td>0.95–1.21</td>
</tr>
</tbody>
</table>

* Calculated by using the McLean–Hastings total protein correction (2, 12).

b Mean ± 2 SD.

Use Ultrafree Anticonvulsant Drug Filters (Worthington Diagnostic Division, Millipore Corp., Freehold, NJ 07728) to prepare ultrafilters of serum with package insert instructions modified only as follows. Place about 2 mL of serum in the filter reservoir and cover it with 1 mL of mineral oil, to minimize loss of CO₂. Place the syringe-filter assembly in the rack provided by Worthington into an incubator set at 37 °C, and after it has pre-warmed for 30 min apply suction to the lower side of the filter housing by use of a 1-mL tuberculin syringe. After about 20 min, when 100 to 200 μL of ultrafiltrate has accumulated in the syringe, remove it and assay the ultrafiltrate as described for total calcium. The ultrafiltration is thus carried out anaerobically at 37 °C. The mean pH change during the ultrafiltration process was found to be +0.08 (SD = 0.008) with the mineral oil covering, as it compared with +0.56 (SD = 0.08) without mineral oil.

**Results and Discussion**

Table 1 shows the ultrafiltrable, measured ionized, calculated “ionized,” and total calcium for a group of 22 healthy ambulatory outpatients, who were receiving a routine physical examination. Our reference intervals are comparable to those reported by Toffaletti and Bowers (9): 2.20–2.58 mmol/L for total calcium and 1.30–1.47 mmol/L for dialyzable. Our reference interval for ionized calcium is substantially higher than the 1.00 to 1.12 mmol/L that Toffaletti et al. originally reported (8). However, Orion has since changed their Space-Stat 20 calcium sensor design, leading to higher values for ionized calcium (10). Table 2 shows the day-to-day precision for total and ultrafiltrable calcium. Analyzing split samples from patients on successive days, and using range statistics (11), we obtained a comparable estimate of day-to-day precision (CV = 2.3%). Both estimates are comparable to that reported by Toffaletti et al. for their AutoAnalyzer method (8).

Figure 1 show the relation between ultrafiltrable calcium and total calcium for this group of outpatients plus 16 hospitalized patients with various disorders of calcium hemostasis.

**Table 2. Day-to-Day Precision for Total and Ultrafiltrable Calcium as Measured in Hyland Lyophilized Control Serum**

<table>
<thead>
<tr>
<th>Total calcium</th>
<th>Ultrafiltrable calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, mmol/L</td>
<td>Mean, mmol/L</td>
</tr>
<tr>
<td>Level I</td>
<td>Level II</td>
</tr>
<tr>
<td>2.46</td>
<td>2.30</td>
</tr>
<tr>
<td>0.043</td>
<td>0.078</td>
</tr>
<tr>
<td>1.7%</td>
<td>3.4%</td>
</tr>
</tbody>
</table>

**Discussion**

These patients were selected for ultrafiltrable and ionized calcium analysis by screening over 3000 specimens submitted for Technicon SMA 12/60 assay of calcium and total protein from a 690-bed Veterans Administration Hospital during one month. As shown in Figure 2, with the ultrafiltration method we could identify all patients with significantly high or low values for ionized calcium. In all cases with high and all but one case with low total calcium, the calculated “ionized” calcium (2, 12) agreed with the ultrafiltrable and measured ionized calcium. In one patient with low values for total, ultrafiltrable, and ionized calcium (1.85, 1.02, and 0.81 mmol/L, respectively) the calculated “ionized” calcium value (0.96 mmol/L) was normal. Patients receiving citrated blood products were not included, but in such situations, measured or calculated ionized calcium theoretically should be more useful than ultrafiltrable calcium. Such a discrepancy was encountered with the Hyland lyophilized control serum, which is prepared from pooled citrated human plasma. In both “Level I” and “Level II,” over 85% of the total calcium was
ultrafiltrable, as shown in Table 2. However, the measured values for ionized calcium were only 0.3 mmol/L for Level I and 0.7 mmol/L for Level II.

We conclude that the ultrafiltration method we describe here should allow any laboratory using a semi-micro calcium method to determine ultrafiltrable calcium and that results of this determination correlate well with ionized calcium as determined by ion-selective electrode. In most cases, for samples not collected just after a blood transfusion, a calculated “ionized” calcium based on the total protein will predict a patient’s approximate ionized or ultrafiltrable calcium. However, as Ladenson et al. (13) recently demonstrated, no algorithm accurately predicts the measured ionized calcium in all cases. Thus, a laboratory may occasionally wish to measure ionized or, lacking the necessary specialized equipment, ultrafiltrable calcium. The eventual clinical utility of ultrafiltrable calcium in diagnosis and treatment of disease is yet to be established, and further investigations by use of methods such as that described here will be needed to answer this question.

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References