Facilitated Determination of Ionized Calcium
Phillip Urban, Barbara Buchmann, and Daniel Scheidegger

Using a calcium-containing heparin preparation for anticoagulation, we determined [Ca\(^{2+}\)], the mean concentration of ionized calcium, in whole blood of 120 healthy blood-donors to be 1.23 (SD 0.04) mmol/L. Similarly, for 50 intensive-care patients selected without conscious bias, the correlation between [Ca\(^{2+}\)] in serum (mean 1.15, SD 0.10 mmol/L) and in whole-blood samples anticoagulated with the same heparin preparation (mean 1.15, SD 0.09 mmol/L) was very good (r = 0.95). Storing samples anaerobically on ice for as long as 2 h did not alter whole-blood [Ca\(^{2+}\)]. On the other hand, various concentrations of calcium-free heparin preparations all induced a significant decrease in measured [Ca\(^{2+}\)]. By using whole-blood samples, rather than plasma or serum, for [Ca\(^{2+}\)] determination with a calcium-selective electrode, repetitive measurements can be made with simple handling procedures, facilitating rapid implementation of appropriate therapeutic measures for critically ill patients.

Additional Keyphrases: whole blood vs plasma or serum samples  
- reference interval  
- ion-selective electrode  
- sample handling  
- variation, source of  
- calcium-containing heparin  
- critical-care patients

Calcium is present in plasma in three different forms: ionized, bound to protein, and complexed with molecules such as lactate or citrate (1). Although the ionized fraction Ca\(^{2+}\) has long been considered to represent the biologically active part of total plasma calcium (2), only during the last decade has the availability of calcium-selective electrodes made possible its routine determination.

The lack of a consensus concerning the optimal procedure for determining Ca\(^{2+}\) has led to "normal values," ranging from 0.96 to 1.27 mmol/L (3). This is largely because of differences in the nature of the sample (whole blood, plasma, or serum), the use of various concentrations of heparin, the type of electrode used, and the calibration solution used.

For clinical use—and particularly for acutely ill patients—the need for fast and dependable results for small blood samples is best met by using anticoagulated whole-blood samples. This would facilitate, in selected cases, monitoring [Ca\(^{2+}\)] during, e.g., the course of surgery or an acute illness (4) and promptly taking appropriate therapeutic measures.

Using a Nova-2 Ca\(^{2+}\)-selective electrode, we determined the "normal" reference intervals for [Ca\(^{2+}\)] in whole blood anticoagulated with a calcium-containing heparin preparation. We also compared the effect of various preparations and concentrations of heparin on [Ca\(^{2+}\)] and studied the effects on samples of storage either on ice or at room temperature.

---

Materials and Methods

Procedure

Venous blood was sampled from 120 apparently healthy nonfasting volunteer blood-donors: 83 men and 37 women. The samples were collected at different times of the day (5), after phlebotomy of about 400 mL of blood, with slight or no tourniquet pressure. We used 2-mL disposable plastic syringes, the dead space of which was filled with a calcium-containing heparin preparation (S-4500 Heparin; Radiometer, Copenhagen, Denmark), containing, per milliliter, 875 int. units of heparin, 155 μmol of NaCl, and 3 μmol of CaCl₂. The final heparin concentration in the sample was about 20 int. units per milliliter of blood.

After expelling any air bubbles, we capped the syringe, mixed its contents by rotation, and set it on ice. [Ca\(^{2+}\)] was measured within 2 h of sampling, in triplicate, with a Nova-2 calcium-selective electrode (Nova Biomedical, Newton, MA 02164). For calibration we used two standard aqueous Ca\(^{2+}\) preparations, 1 and 2 mmol/L, respectively, and a reference solution containing KCl, 2 mol/L, before each set of measurements. After every five samples we measured a control containing 1.5 mmol of Ca\(^{2+}\) per liter.

Comparison Studies

Use of calcium-containing heparin (S-4500, Radiometer) vs serum. We obtained 50 paired samples of arterial blood from patients in a surgical intensive-care unit. The samples to be used for measuring [Ca\(^{2+}\)] in whole blood were drawn as described above. To determine [Ca\(^{2+}\)] in serum, we collected 10 mL of blood into a Vacutainer Tube (Terumo Europe, Leuven, Belgium) under anaerobic conditions, left it to clot and then centrifuged the blood in the closed Vacutainer Tube and aspirated the serum into a syringe. We measured [Ca\(^{2+}\)] in serum and whole blood as described above.

Effect of different heparin preparations. Arterial blood samples from 20 of the patients on a surgical intensive-care unit were collected as five sequential 2-mL samples into plastic syringes that contained 50 μL of the following heparin preparations, 5000 int. units/mL each: Liqueumin-Roche (Hoffmann-La Roche, Basel, Switzerland), Kabi-Vitrum (Kabi AB, Stockholm, Sweden), Novo (Novo Industri A/S, Copenhagen, Denmark), Liquemin Organon (Organon, West Orange, NJ), and Elkins-Sinn (Elkins-Sinn Inc., Cherry Hill, NJ). The final concentration of heparin in each sample was 125 int. units/mL. Two more samples were collected into 2-mL plastic syringes of which the dead space was filled with a dilution of sodium heparin (Liquemin-Roche) to give a final concentration in the blood sample of about 10 and 25 int. units, respectively. An eighth sample was collected in a Vacutainer Tube and used to determine serum [Ca\(^{2+}\)] as described above. [Ca\(^{2+}\)] was measured as described above.

---

Surgical Intensive-Care Unit, Department of Anesthesia, University of Basel/Kantonsspital, CH-4031 Basel, Switzerland.

Received July 24, 1984; accepted November 7, 1984.
**Effect of storage.** Three arterial blood samples were drawn sequentially from 14 patients in a surgical intensive-care unit and collected into 2-mL disposable plastic syringes containing the calcium/heparin preparation (S-4500, final concentration in the sample about 20 int. units/mL). One sample was analyzed for Ca\(^{2+}\) without delay, the second was stored on ice for 2 h before assay, and the third was left at room temperature for 2 h before assay.

**Results**

**Reference interval.** The mean normal value for [Ca\(^{2+}\)] as determined with venous whole blood from healthy adult blood donors anticoagulated with about 20 int. units of heparin per milliliter was 1.23 (SD 0.04) mmol/L. The mean ± 2 SD was 1.15–1.31. The coefficient of variation for triplicate measurements of 120 samples was 3.25%.

**Use of calcium-containing heparin vs serum.** Our mean values for [Ca\(^{2+}\)] in serum or in whole blood anticoagulated with S-4500 heparin were identical (1.15 ± 0.10 and 1.15 ± 0.09 mmol/L, respectively, mean ± SD), and the correlation between the paired values was good (r = 0.95). Figure 1 shows the individual data.

**Effect of use of different heparin preparations and different concentrations of heparin.** All the calcium-free heparin preparations decreased [Ca\(^{2+}\)] by about 20% as compared with its concentration in serum (Table 1). There were slight differences in the extent of the [Ca\(^{2+}\)] decrease induced by different heparin preparations; these were statistically significant (p = 0.001) when analyzed with a two-way analysis of variance, but they appear too small to be of any clinical importance.

Whole-blood samples anticoagulated with small amounts of calcium-free heparin (Liquemine-Roche) showed significantly lower [Ca\(^{2+}\)] than those obtained in serum; the significance of the difference was more marked with increasing concentrations of heparin (Table 1).

**[Ca\(^{2+}\)] in whole blood** mmol/L

![Graph](image)

**Fig. 1. [Ca\(^{2+}\)] in 50 paired samples of arterial blood obtained from patients in intensive-care: results for whole-blood samples anticoagulated with S-4500 Heparin (Radiometer) compared with those for serum.**

The dotted line is the line of identity; the regression equation for the data points is y = 0.9x + 0.13 (r = 0.95). Note that the heparinized samples tend to show slightly higher values than serum for low [Ca\(^{2+}\)] and slightly lower values than serum for high [Ca\(^{2+}\)].

**Table 1. Effect of Different Heparin Preparations on Values for [Ca\(^{2+}\)]**

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Ca(^{2+})], mmol/L, mean and SD (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1.15 ± 0.09</td>
</tr>
<tr>
<td>Plasma with</td>
<td></td>
</tr>
<tr>
<td>Liquemine-Roche</td>
<td>0.99 ± 0.07(^d)</td>
</tr>
<tr>
<td>Kabi-Vitrum Heparin</td>
<td>0.90 ± 0.07(^d)</td>
</tr>
<tr>
<td>Novo Heparin</td>
<td>0.92 ± 0.07(^d)</td>
</tr>
<tr>
<td>Liquemin Organon</td>
<td>0.90 ± 0.07(^d)</td>
</tr>
<tr>
<td>Elkins-Sinn Heparin</td>
<td>0.89 ± 0.06(^d)</td>
</tr>
<tr>
<td>Whole blood, Liquemine-Roche</td>
<td>1.13 ± 0.11(^*)</td>
</tr>
<tr>
<td>Whole blood, Liquemine-Roche</td>
<td>1.10 ± 0.11(^*)</td>
</tr>
</tbody>
</table>

\(^*\) Final concentration of heparin in each sample: 125 int.units/mL. \(^d\) Final concentration of heparin 10 int.units/mL. \(^f\) Final concentration of heparin 25 int.units/mL. \(^\text{SD}\) Significantly different from serum by Student’s t test: p < 0.001. \(^\text{SD}\) Significantly different from serum by Student’s t test: p < 0.01. \(^\text{SD}\) Significantly different from results with other heparin preparations by a two-way analysis of variance: p < 0.001.

**Effects of storage.** The value for [Ca\(^{2+}\)] in the whole-blood samples left at room temperature in capped plastic syringes for 2 h was slightly but significantly (p < 0.001, Student’s t test) greater than that in the samples analyzed immediately: 1.18 (SD 0.06) vs 1.17 (SD 0.06) mmol/L (n = 14). Storage on ice for 2 h did not significantly alter the [Ca\(^{2+}\)] (1.16, SD 0.06, mmol/L; n = 14).

**Discussion**

The wide range of normal reference intervals for [Ca\(^{2+}\)] reported in the literature has a number of causes and makes it necessary to define precisely the population being studied, the nature and handling of the samples, the electrode system used, and, for whole-blood and plasma samples, the type of anticoagulant used (1).

For practical reasons, the use of whole-blood samples is best suited to intensive-care units and operating theaters: by permitting rapid and repetitive measurements, they facilitate the implementation of corrective measures for unstable patients. The two major drawbacks of measuring [Ca\(^{2+}\)] in whole-blood samples are the positive bias introduced by erythrocytes (6) and the negative bias from the use of heparin (caused in part by simple dilution and in part by complexing of Ca\(^{2+}\) by the heparin molecules) (6–8).

The problems related to the use of heparin can be partly overcome in two different ways.

The use of very low concentrations of sodium heparin in whole-blood samples (final concentration < 5 int. units/mL) appears to yield [Ca\(^{2+}\)] values similar to those obtained for serum (6, 9). However, the frequent clogging of the electrode system and the need to prepare the proper heparin dilution make this solution less attractive for daily use in intensive-care units.

Alternatively, one can partly correct for the effect of heparin by using a calcium-containing heparin preparation, as we chose to do in the present study. The correlation between values for serum and whole-blood when such a preparation is used is excellent (Figure 1), and at a concentration of 20 int. units/mL the electrode is rarely clogged with the sample constituents. The tendency of heparin-treated blood samples to show slightly higher values than serum for low [Ca\(^{2+}\)] and slightly lower values than serum for high [Ca\(^{2+}\)] is certainly of no clinical consequence for an individual patient, but has to be taken into account when the effects of therapeutic interventions on [Ca\(^{2+}\)] for any group of patients are compared.
Our values (1.23 ± 0.04) agree closely with those obtained by others who used the same electrode and low doses (4 int. units/mL) of sodium heparin as an anticoagulating agent on whole-blood samples (1.22 ± 0.01 mmol/L) (9), and with some of the more recent results for [Ca²⁺] measurements in serum with various electrodes (10).

The use of serum instead of whole-blood as the sample avoids biases from erythrocytes and heparin, but larger samples and a more complex handling procedure are required. This delays results, and thus it is less appropriate for clinical use with acutely ill patients.

Some authors (11, 12) advocate the use of serum samples treated aerobically with CO₂ before measurement, to restore pH values. This technique allows for prolonged periods of storage but is also time-consuming and could even be occasionally inadequate for certain patients in severe acid-base imbalance if the actual [Ca²⁺] is to be precisely determined.

Whole-blood samples anticoagulated with sodium heparin (10 or 25 int. units/mL) showed significantly lower [Ca²⁺] than paired serum samples (Table 1). Various calcium-free heparin preparations, when used in relatively high concentration (125 int. units per milliliter of sample), all induced a marked decrease in [Ca²⁺], as compared with serum results. This constitutes a pointed reminder that the use of conventionally anticoagulated syringes, such as those used for blood gas analysis, to determine [Ca²⁺] is inappropriate.

The importance of defining the population under study before referring to "normal" values is illustrated by the differences between [Ca²⁺] from venous blood in healthy volunteers and that from arterial blood of randomly selected intensive-care patients. These differences most probably reflect effects of the supine position, intravenous infusions, altered renal or hemodynamic state, pH fluctuations, etc. Whether the sample is of venous or arterial origin does not significantly influence [Ca²⁺] (9).

We stored samples anaerobically on ice when determining our normal values, having found that such sample handling does not significantly alter [Ca²⁺] for up to 2 h as compared with results obtained with immediate processing (13). Storing whole blood samples at room temperature for 2 h slightly but significantly increased [Ca²⁺], but this effect was too slight to be clinically relevant for any individual patient.

We thank Nova Biomedical for the loan of the electrode used in this study.

The design of this study was approved by the Ethical Committee of our hospital.

References