(Figure 2) or normal plasma (Figure 3) seems to eliminate this deviation.

The Heptest facilitates easy and rapid measurement of heparin concentration in plasma. In cases of concomitant administration of oral anticoagulant, the Heptest often exaggerates the plasma heparin concentration. Optimal reliability for the whole population of patients is not achieved by following the manufacturer's current procedure. For this reason we advocate the addition of either prothrombin (1 int. unit/mL, final concentration) or 40 volumes of normal plasma per 100 volumes to the test specimen before the Heptest assay.

We gratefully acknowledge the technical assistance of Robert Polenewen.

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Effect of Hematocrit and Added Heparin on Ionized Calcium in Capillary Blood Samples from Neonates

Nina Nelson,¹ Sten Ohman,² and Lasse Larsson²

Sampling of capillary blood for determination of ionized calcium (Ca²⁺) in neonates requires that extra heparin be added to prevent clotting in the sampling tube and (or) in the Ca²⁺ analyzer. Because the additive dissolves in the plasma compartment, different hematocrit (erythrocyte volume fraction, EVF) values may cause different results for Ca²⁺. To study the effect of EVF and heparin additive, we repeatedly removed plasma, thereby increasing the EVF. These samples with different EVF's were aspirated into commercial capillary tubes containing heparin and, according to our routine procedure, an additional 10 μL (~0.9 int. unit) of sodium heparin. We found a negative bias of 0.05–0.09 mmol/L in Ca²⁺, depending on the EVF. Adding saline instead of heparin gave the same effect, indicating that this bias was entirely due to dilution. We suggest compensating for this by adding 0.09 mmol/L to the actual value for ionized calcium when EVF exceeds 70%. The increase in Ca²⁺ in neonates on days 1 to 5 postpartum is physiological and not an effect of change in EVF.

Additional Keyphrases: erythrocyte volume fraction · age-related effects · sample handling

To avoid time-consuming and unnecessary resampling owing to coagulation in the tube or in the Ca²⁺ analyzer, additional heparin is to be added to capillary tubes used in determination of ionized calcium (Ca²⁺) in neonates (1). However, the heparin may alter Ca²⁺ values (2–5), because of (a) formation of a complex with calcium, (b) a direct effect on components in the analytical equipment such as the electrode, or (c) dilution of the sample. Of these, only the first has been thoroughly investigated (6).

Because heparin dissolves in the plasma compartment, the dilution effect, if any, should depend on the hematocrit (erythrocyte volume fraction, EVF). Newborn infants have high EVF's, which decrease during the first nine weeks postpartum (7), so such a dilution effect may vary during this period. We reported earlier an increase in Ca²⁺ in neonates during the first five days postpartum (8). To investigate if this was a true physiological phenomenon or simply a result of sample handling, we performed experiments to determine the effect of added heparin and EVF on Ca²⁺. We also examined the relationship between Ca²⁺ and EVF in a group of newborn infants.

Materials and Methods

Experimental study. Venous blood from 10 adult volunteers was collected into 10-mL evacuated tubes (Becton Dickinson Co., Rutherford, NJ 07070) containing enough dry sodium heparin to give a final concentration of about 16 int. units/mL in full tubes.

We prepared samples with successively higher values of EVF by centrifugation of the evacuated collection tubes (4200 rpm, 5 min) twice. After each centrifugation we removed 0.7 to 2 mL of the supernatant plasma anaerobically. The tube was then rotated to mix the blood components again before analysis.

Before (stage A) and after (stages B and C, respectively) each centrifugation we determined Ca²⁺ and EVF in samples taken directly from the evacuated collection tube. The same analytes were also determined in blood from the evacuated collection tube aspirated into two 140–160 μL capillary tubes. These capillary tubes (Radiometer, Copenhagen DK-2400, Denmark) contained ~53 int. units of dry sodium heparin per milliliter (~8 int. units per capillary tube) plus an additional 10 μL of sodium heparin (87.5 int. units/mL, i.e., ~0.9 int. unit of extra heparin per tube) or sodium chloride solution (0.15 mmol/mL). The initial as well as the extra heparin in the capillary tubes was titrated with calcium chloride.

References


In all sample handling we used anaerobic technique, to minimize loss of CO₂.

To evaluate the effect of additional sodium heparin or sodium chloride on Ca²⁺ as compared with the heparin content of the Radiometer capillary tubes, we performed a separate study, using samples from seven healthy adults. Serum from venous blood and capillary whole-blood samples from these individuals were treated identically. Ca²⁺ in serum was determined before transferring the serum into the capillary tubes. We determined Ca²⁺ in the pre-heparinized Radiometer capillary tubes (140–160-μL tube = sample volume) containing ~53 int. units of sodium heparinate per milliliter (~8 int. units per tube) and also in the Radiometer tubes after we added either 10 μL of sodium heparin (87.5 int. units/mL heparin solution, i.e., ~0.9 int. unit of extra heparin per tube) or 10 μL of isotonic saline (sodium chloride 0.15 mmol/mL). The dry sodium heparinate as well as the sodium heparinate solution were titrated with an amount of calcium chloride such that a sample with Ca²⁺ of 1.25 mmol/L was not affected by heparin binding of calcium, whereas samples with lower Ca²⁺ values would have a small (<1%) positive bias, and, vice versa, samples with higher Ca²⁺ values would have a similar negative bias (3).

Infant study. Capillary blood samples from 11 healthy, full-term babies were taken on days 1, 2, and 5 postpartum and handled as previously reported (8).

Analytical methods. The pH of each sample was measured and Ca²⁺ was measured with a semiautomatic ion-selective method (ICA 1, Radiometer). We determined EVF by a standard manual method (9).

Statistics. Results were compared by the Wilcoxon signed rank test. We studied the correlation (linear regression analysis) between Ca²⁺ and EVF, both for changes between stages or sampling occasions and for the separate values observed at different times.

Results

Experimental Study

Table 1 lists the mean values and standard deviations for Ca²⁺ and EVF. Control values of Ca²⁺ and pH did not change significantly between the stages. Thus there was no bias attributable to inappropriate handling of the sample (e.g., CO₂ loss) or to the EVF change alone.

Additional heparin, as well as sodium chloride, lowered Ca²⁺ significantly (P < 0.01) at all stages (A, B, and C). At mean EVF values of 47%, 62%, and 79% this systematic error was 0.05, 0.06, and 0.09 mmol/L, respectively (Figure 1). Ca²⁺ decreased significantly (P < 0.01) in the sodium heparin- and sodium chloride-treated samples between each analytical stage (A–B–C). The increases in mean EVF between A–B (15%) and B–C (17%) corresponded to changes in mean Ca²⁺ of 0.01 and 0.04 mmol/L, respectively. The coefficient of correlation between the EVF change and the Ca²⁺ change was significant only between stages A and B (P < 0.01) in treated samples. No other correlations between EVF and Ca²⁺ values were statistically significant. For the adults’ serum, Ca²⁺ values from untreated venous blood samples agreed well with Ca²⁺ in whole blood obtained from the preheparinized Radiometer tubes (Table 2). The additional 10 μL of sodium heparin or sodium chloride resulted in a similar decrease in Ca²⁺.

Infant Study

Table 3 lists mean values and standard deviations for Ca²⁺ and EVF. Mean EVF decreased from day 1 to day 2 but did not change significantly from day 2 to day 5. Ca²⁺ increased from day 2 to day 5. The change in Ca²⁺ from day 1 to day 2 was not significant.

The decrease in mean EVF was 3.7% from day 1 to day 2 (P < 0.01) and 1.5% from day 2 to day 5 (not statistically significant). There were no significant correlations between changes in EVF and Ca²⁺ or between Ca²⁺ and EVF on the separate days.

Table 1. Effect of Plasma Removal on Ca²⁺ and EVF in Blood Samples from 10 Adults

<table>
<thead>
<tr>
<th>Ca²⁺, mmol/L</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control samples</td>
<td>1.24 (0.02)</td>
<td>1.24 (0.02)</td>
<td>1.23 (0.03)</td>
</tr>
<tr>
<td>Sodium heparin treated</td>
<td>1.19 (0.03)</td>
<td>1.18 (0.03)</td>
<td>1.14 (0.03)</td>
</tr>
<tr>
<td>Sodium chloride treated</td>
<td>1.19 (0.03)</td>
<td>1.17 (0.04)</td>
<td>1.12 (0.03)</td>
</tr>
<tr>
<td>EVF, %</td>
<td>47 (2.7)</td>
<td>62 (3.3)</td>
<td>79 (3.9)</td>
</tr>
</tbody>
</table>

Table 2. Effect of Addition of Heparin or NaCl on Ca²⁺ Measured in Serum and Capillary Blood Samples from Seven Adults

<table>
<thead>
<tr>
<th>Mean (and SD) Ca²⁺, mmol/L</th>
<th>Serum</th>
<th>Capillary</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additive</td>
<td>1.26 (0.01)</td>
<td>1.20 (0.02)</td>
</tr>
<tr>
<td>Capillary tube alone*</td>
<td>1.27 (0.03)</td>
<td>1.20 (0.02)</td>
</tr>
<tr>
<td>+ heparin, ~0.9 int. unit</td>
<td>1.22 (0.03)</td>
<td>1.21 (0.03)</td>
</tr>
<tr>
<td>+ NaCl, 1.5 μmol</td>
<td>1.23 (0.03)</td>
<td>1.21 (0.03)</td>
</tr>
</tbody>
</table>

*Original heparin content was about 8 int. units of sodium heparinate per tube.

Table 3. Mean (and SD) Changes in Ca²⁺ and EVF in 11 Infants on Days 1, 2, and 5 Postpartum

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺, mmol/L</td>
<td>1.16 (0.05)</td>
<td>1.18 (0.04)</td>
</tr>
<tr>
<td>EVF, %</td>
<td>86.5 (7.8)</td>
<td>64.8 (8.6)</td>
</tr>
</tbody>
</table>

*Samples for calcium assay were collected into Radiometer tubes containing 53 int. units of sodium heparin per milliliter final volume + 10 μL sodium heparin (87.5 int. units/mL).
Discussion

Many factors influence measurement of Ca²⁺, e.g., sampling conditions, sample type, and sample handling. If plasma or whole blood is to be analyzed, effects on the Ca²⁺ caused by the anticoagulant (which is usually heparin) must be considered. For adults, Ca²⁺ is preferentially determined in venous serum; thus an anticoagulant is obviated. However, in the neonate, capillary blood is generally preferable, and thus heparin is needed to prevent clotting after sampling. When we used commercially available pre-heparinized capillary tubes, clotting still occurred. Therefore, we routinely add 0.9 int. unit of extra heparin to the tubes before sampling (1).

To clarify the effect of this additional heparin on Ca²⁺, we compared values for Ca²⁺ obtained with the above-described sample handling with those after adding the same volume of sodium chloride instead of the extra heparin. The added sodium chloride produced the same lowering effect on Ca²⁺ as did heparin. Evidently the effect of our heparin addition was entirely ascribable to dilution. Consequently, this effect was EVF-dependent and more pronounced at high EVF values. It could be noted, however, that Ca²⁺ in blood is a part of a calcium equilibrium system in which more than half of the calcium is complex-bound. Therefore, the dilution effect was not as large as expected if only the volumes were taken into account. For example, addition of 10 μL to a 150-μL sample volume, where 53% is plasma, should result in a dilution effect of Ca²⁺ from 1.24 to 1.09 mmol/L, but in our study this addition resulted in a measured Ca²⁺ of 1.19 mmol/L (Table 1).

The systematic error caused by dilution can be compensated for (see Figure 1). At normal values for EVF and Ca²⁺, however, this error is hardly of clinical importance, but for infants with very high EVF (>70%) and (or) Ca²⁺ near the lower normal reference limit it is more notable and should be considered. Ca²⁺ and EVF were not correlated in our study, and the changes in Ca²⁺ during the first five postnatal days were larger than any systematic error caused by the additive or by changes in EVF. Hence we propose that the increase in Ca²⁺ during this period (2) is of physiological origin and not an artifact of the sampling method.

We conclude that heparin added to capillary samples causes an EVF-dependent systematic error in Ca²⁺ by dilution. This should be considered when EVF values are high and (or) Ca²⁺ is near the lower reference limit. We recommend a compensatory adding of 0.09 mmol/L to the measured actual Ca²⁺ value at EVF >70%. The increase in Ca²⁺ seen in normal infants during the first postnatal week evidently is of physiological origin and not an effect of a decreasing hematocrit in combination with heparin additive.

References

Micro-Quantity Tissue Digestion for Metal Measurements by Use of a Microwave Acid-Digestion Bomb

James R. P. Nicholson,1 M. Geraldine Savory,1 John Savory,1,2 and Michael R. Wills1,3

We describe a simple and convenient method for processing small amounts of tissue samples for trace-metal measurements by atomic absorption spectrometry, by use of a modified Parr microwave digestion bomb. Digestion proceeds rapidly (≤90 s) in a sealed Teflon-lined vessel that eliminates contamination or loss from volatilization. Small quantities of tissue (5–100 mg dry weight) are digested in high-purity nitric acid, yielding concentrations of analyte that can be measured directly without further sample manipulation. We analyzed National Institute of Standards and Technology bovine liver Standard Reference Material to verify the accuracy of the technique. We assessed the applicability of the technique to analysis for aluminum in bone by comparison with a dry ashing procedure.

Additional Keyphrases: sample preparation • atomic absorption spectrometry • aluminum • bone • trace elements

Trace-metal measurements in tissues by atomic spectroscopic techniques require preliminary digestion of the sample, most often by heating in strong acids. However, conventional open-vessel techniques require considerable time (4–

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