Comparative Evaluation of Two Calcium Ion-Selective Electrode Systems, and Their Utility for Monitoring Steady-State Changes in [Ca$$^{2+}$$]

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We compared ionized calcium concentrations ([Ca$$^{2+}$$]) as measured with two ionized-calcium analyzers: the NOVA 2 and the Orion SS-20. Samples were obtained from 43 human volunteers, 213 patients, and five dogs (106 samples). In the [Ca$$^{2+}$$] range of 0.85 to 1.8 mmol/L, [Ca$$^{2+}$$] measurements in whole blood with the NOVA 2 consistently exceeded those measured with the Orion SS-20. However, in the normal range, this difference appeared to be smaller when we compared values for plasma or serum, and was absent over the entire range when we compared aqueous solutions. The normal human [Ca$$^{2+}$$] in whole blood as measured with the NOVA 2 is 1.22 ± 0.01 mmol/L (mean ± SEM) and that with the Orion SS-20 is 1.12 ± 0.01 mmol/L ($p < 0.0001$ by paired t-test); the 95% confidence intervals were from 1.14 to 1.30 and from 1.02 to 1.22 mmol/L, respectively. Using dogs, we also tested the usefulness of the ionized-calcium electrode for monitoring [Ca$$^{2+}$$] during infusion of either citrate or calcium chloride solutions, to produce steady-state alterations in [Ca$$^{2+}$$] equilibrium. Frequent successive [Ca$$^{2+}$$] measurements were essential to appropriately adjust the infusion rates of these solutions to achieve steady-state [Ca$$^{2+}$$].

Additional Keyphrases: coronary sinus vs systemic arterial blood (canine) - matrix effects on apparent [Ca$$^{2+}$$] - reference intervals

Although the importance of the calcium ion for myocardial contraction has been recognized for almost a century (1), direct measurement of the calcium ion in blood, plasma, and serum has only become practicable during the last decade with improved electrode technology. Such measurements are important, not only for the diagnosis and management of hyperparathyroidism (2), but also for detection of derangements of calcium ion balance in critically ill patients. For example, the ionized-calcium analyzer has been used in the Acute Care Laboratory of the Massachusetts General Hospital to document severe derangements of calcium ion equilibrium in patients with compromised hemodynamic function (3), in patients with acute thermal injury (4), and after cessation of cardiopulmonary bypass in patients undergoing cardiac operations (5). Similar abnormalities have been documented in patients with sepsis (6). The abnormality in calcium ion balance is not always reflected by total-calcium measurements in such patients.

Instruments of this sort used in an acute care laboratory should have a number of important features. They should be suitable for whole-blood samples; the volume of blood required for analysis should be small; the instrument should allow for rapid and reliable calibration over the clinical range; and analysis time should be short. The ionized-calcium analyzer appears to meet these criteria, so calcium replacement therapy in patients with acute hypocalcemia can now be undertaken on the basis of frequent [Ca$$^{2+}$$] determinations.

The purpose of the present investigation was twofold. First, we wished to compare [Ca$$^{2+}$$] values obtained by two commercially available ionized-calcium analyzers. With both instruments, we analyzed [Ca$$^{2+}$$] in blood specimens from normal volunteers and patients with a various pathological conditions, and from dogs. Second, in view of the speed of [Ca$$^{2+}$$] measurement, we expected the instrument to be valuable for closely monitoring [Ca$$^{2+}$$]. Accordingly, we documented the presence of different steady-state [Ca$$^{2+}$$] alterations in the dog, and used the calcium electrode as a guide to adjust the infusion rate of drugs known to alter blood [Ca$$^{2+}$$].

Materials and Methods

Methods

For all [Ca$$^{2+}$$] analyses, we used two calcium ion-selective electrode systems: the NOVA 2 (Nova Biomedical, Newton, MA 02164) and the Orion SS-20 (Orion Research, Cambridge, MA 02139). Both instruments include a flow-through design and their operating temperature is 37°C. We calibrated both instruments with standard solutions supplied by the respective manufacturer, and confirmed the calibrations by analyzing aqueous solutions containing 0.5, 1.5, and 2.5 mmol of ionized calcium per liter. We calibrated each instrument at the start of each experiment and after analysis of about five samples. In addition, we analyzed the aqueous standard solutions of one instrument as unknowns on the other instrument. We analyzed all specimens in duplicate on each instrument, accepting duplicate measurements when they agreed within 3%.

Protocol and Sample Preparation

Human volunteers. We established a reference [Ca$$^{2+}$$] interval in man by analyzing whole blood and corresponding plasma and serum sampled from 43 human volunteers. For each volunteer, two 12-mL plastic syringes, one of which contained 50 μL of a heparin solution (Liquaemin, Organon, 1000 int. units/mL), were each fitted to a three-way stopcock. Both syringes were filled with venous blood by venipuncture, promptly capped, and placed on ice. We divided the heparinized whole-blood specimen into two portions (one, whole blood; the other, to be centrifuged with the tip in the upward position to obtain plasma from 39 of the 43 blood specimens). We prepared serum from the blood specimen that had been collected without heparin. We measured the pH of all specimens (whole blood, plasma, and serum); the greatest difference in pH among related specimens was 0.03 pH unit. We then determined [Ca$$^{2+}$$] in duplicate with both calcium instruments concurrently.

Ionized calcium in patients. We measured [Ca$$^{2+}$$] with both instruments in specimens of whole blood from 213 patients,

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1 By "blood [Ca$$^{2+}$$]" we mean [Ca$$^{2+}$$] measured in plasma of a sample of whole blood.
who were in either the operating room or a postoperative intensive-care unit.

Human whole blood experiments. We collected specimens of venous whole blood (about 60 mL) from healthy volunteers into heparinized syringes (heparin solution, 5 int. units/mL), and divided them anaerobically into smaller portions. We then added increasing amounts of calcium chloride anaerobically to these portions, and measured $[Ca^{2+}]$ in duplicate in whole blood and the corresponding plasma with both instruments concurrently.

Human erythrocyte suspension. We prepared a human erythrocyte suspension in isotonic NaCl (150 mmol/L) to give hematocrit of 0.35. The total-protein concentration was 1 g/L. We then added increasing amounts of calcium chloride to different portions of this erythrocyte suspension, adjusted the pH to 7.40, and measured $[Ca^{2+}]$ in duplicate with both instruments concurrently.

Human protein solution. To compare the performance of the two instruments for samples containing protein but no erythrocytes, we diluted commercial human salt-poor albumin (250 g/L) in isotonic sodium chloride solution (9 g/L) to give a total-protein concentration of 65 g/L as measured by refractometry. We adjusted the pH to about 7.40 by adding some Tris buffer, then added increasing amounts of calcium chloride to portions of this protein solution and measured $[Ca^{2+}]$ in duplicate with both instruments concurrently.

Processed vs native albumin solutions. The clinical observation (7) of important hemodynamic consequences of rapid infusion of plasma protein fractions prompted further in vitro studies. We diluted commercial human salt-poor albumin (250 g/L) in a buffered salt solution (Na = 135 mmol/L, bicarbonate = 25 mmol/L, pH = 7.40) to make solutions with total-protein concentrations ranging from 15 to 80 g/L, as determined by refractometry. We added calcium chloride to each of these protein solutions to yield total calcium concentrations of 2 mmol/L. We then measured $[Ca^{2+}]$ in duplicate on both instruments concurrently.

For comparison, we prepared a native protein solution from venous blood from a normal volunteer. After venipuncture, the blood was allowed to flow through a cation-exchange resin (Dowex-50) to remove the calcium, thereby providing anticoagulation (8). The erythrocytes were separated from the plasma, which was then concentrated in an ultracentrifuge. We diluted the protein solution so obtained with the buffered salt solution described above to yield different protein concentrations ranging up to 120 g/L. The albumin concentration was calculated from these total protein values by assuming an albumin/globulin ratio of 1.8. We added calcium chloride to each solution to make the total calcium concentration 2 mmol/L, and measured $[Ca^{2+}]$ with the Orion instrument.

Steady-state ionized-calcium alterations in the dog. We tested the usefulness of the calcium ion analyzer in monitoring for the presence of steady-state ionized hypocalcemia and hypercalcemia, which were established in random order in five anesthetized dogs on right heart bypass.

Ionized hypocalcemia was achieved by infusion of about 25 mL of trisodium citrate–citric acid buffer (ACD, formula A, USP), the pH of which had been adjusted to 7.4 by addition of Tris buffer. The $[Ca^{2+}]$ concentration so obtained was then maintained by further infusion of the ACD solution by means of a variable-speed constant-infusion pump (Harvard Apparatus, Millis, MA 02054). The rate of citrate infusion was adjusted as required, according to the frequent $[Ca^{2+}]$ measurements, the results of which were available within 3 min after withdrawal of the coronary venous blood sample.

Ionized hypercalcemia was achieved by infusion of calcium chloride (approximately 400 mg), and the $[Ca^{2+}]$ value so obtained was maintained by further calcium infusion with the constant-infusion pump. Again, the infusion rate of calcium chloride was adjusted frequently, as required, according to the frequent $[Ca^{2+}]$ measurements obtained.

We determined $[Ca^{2+}]$ in coronary venous blood with both instruments concurrently. In the case of some of the dogs, we sampled coronary venous and aortic blood in pairs, to look for any difference in $[Ca^{2+}]$ across the coronary vascular bed.

For statistical evaluation of all data we used a programmable HP 67 computer (Hewlett-Packard Co., Lexington, MA 02173). To compare $[Ca^{2+}]$ data obtained with the two calcium ion instruments, we used Student's t-test for paired observations.

### Results

Reference interval. Comparative $[Ca^{2+}]$ results for samples from human volunteers are shown in Table 1. The mean for $[Ca^{2+}]$ in whole blood as determined with the Orion SS-20 was $1.12 \pm 0.01$ mmol/L; that obtained for the same blood specimens with the NOVA-2 was $1.22 \pm 0.01$ mmol/L. The difference between these values was significant ($p < 0.0001$, paired t-test). The 95% confidence interval (mean ± 2 SD) for $[Ca^{2+}]$ as measured with the Orion SS-20 was from 1.02 to 1.22 mmol/L; that for $[Ca^{2+}]$ as measured with the NOVA-2 was from 1.14 to 1.30 mmol/L. Mean $[Ca^{2+}]$ as measured in plasma with either instrument was similar ($p = 0.60$). Furthermore, $[Ca^{2+}]$ in plasma as measured with NOVA-2 was identical to that for whole blood, as well as for plasma, as measured with the Orion SS-20.

### Patients

Comparative results obtained for 213 whole-blood patients. Each patient was studied three times with the two instruments, using the same venous blood sample. The results are shown in Table 2. The mean $[Ca^{2+}]$ with the NOVA-2 was $1.20 \pm 0.20$ mmol/L; with the Orion SS-20 it was $1.22 \pm 0.20$ mmol/L. The difference between these values was significant ($p < 0.01$). We found no significant difference in the variability of results obtained with the two instruments.

### Table 1. $[Ca^{2+}]$ in Whole Blood and the Corresponding Plasma and Serum Compared *

<table>
<thead>
<tr>
<th></th>
<th>NOVA 2</th>
<th>Orion SS-20</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% confidence interval</td>
</tr>
<tr>
<td>$[Ca^{2+}]$, mmol/L</td>
<td>± SEM</td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>1.22 ± 0.01</td>
<td>1.14 - 1.30</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.13 ± 0.01</td>
<td>1.03 - 1.23</td>
</tr>
<tr>
<td>Serum</td>
<td>1.22 ± 0.01</td>
<td>1.14 - 1.30</td>
</tr>
</tbody>
</table>

* Samples from 43 ostensibly normal persons
samples from patients (Figure 1) demonstrate that in the [Ca\textsuperscript{2+}] interval from 0.80 to 1.60 mmol/L, higher values were consistently obtained with the NOVA instrument, although linear regression analysis showed a good correlation (r = 0.90).

Experiments with human whole blood, protein, and erythrocyte suspension. The results for human whole blood (Figure 2) again show that [Ca\textsuperscript{2+}] in whole blood as measured with the NOVA 2 consistently exceeded that measured with the Orion instrument, but that the difference may be smaller for plasma. To examine a possible cause of this difference, we measured [Ca\textsuperscript{2+}] in a human erythrocyte suspension. The results (Figure 3) show a discrepancy between results obtained with the two instruments, as observed for whole blood. In contrast, when [Ca\textsuperscript{2+}] was varied in a human protein solution (total protein = 65 g/L), results obtained with both instruments were similar and the data fall close to the line of perfect fit (Figure 4). As shown in Table 2, [Ca\textsuperscript{2+}] in aqueous standard solutions measured by both instruments was nearly identical.

Processed human vs native human-protein solutions. As shown in Figure 5, [Ca\textsuperscript{2+}] increased with decreasing protein concentration in the native human protein solutions. In the processed human albumin solution such progressive increase in [Ca\textsuperscript{2+}] was only seen below a protein value of 30 g/L. Although the total calcium concentration in all specimens of both types of albumin solutions was 2 mmol/L, apparent

**Discussion**

There are two major findings in this study. First, in the [Ca\textsuperscript{2+}] interval from moderately below normal to well above normal, apparent [Ca\textsuperscript{2+}] in a whole blood sample

![Fig. 2. [Ca\textsuperscript{2+}] in human whole blood and corresponding plasma as measured with the two instruments](image)

![Fig. 3. [Ca\textsuperscript{2+}] as measured with the two instruments in a human erythrocyte suspension in which total protein was reduced to 1 g/L](image)

![Fig. 4. [Ca\textsuperscript{2+}] as measured in a matrix of processed human albumin](image)

![Fig. 5. [Ca\textsuperscript{2+}] as measured with the two instruments in a human erythrocyte suspension in which total protein was reduced to 1 g/L](image)

**Table 2. [Ca\textsuperscript{2+}] in Aqueous Standard Solutions**

<table>
<thead>
<tr>
<th>Mfr's. std. soln.</th>
<th>NOVA 2</th>
<th>Orion SS-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca\textsuperscript{2+}], mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.50</td>
<td>2.52 ± 0.02 *</td>
<td>2.49 ± 0.02</td>
</tr>
<tr>
<td>1.50</td>
<td>1.51 ± 0.01</td>
<td>1.47 ± 0.01</td>
</tr>
<tr>
<td>1.00</td>
<td>0.99 ± 0.01</td>
<td>1.01 ± 0.01</td>
</tr>
<tr>
<td>0.50</td>
<td>0.49 ± 0.01</td>
<td>0.53 ± 0.01</td>
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</tbody>
</table>

\* ± values are SEM.

![Human Whole Blood](image)

![Human Plasma](image)

![Protein Solution](image)
measured with the NOVA 2 is consistently higher than when measured with the Orion SS-20, and this difference increases with increasing [Ca\(^{2+}\)]. The observed discrepancy is smaller when [Ca\(^{2+}\)] is measured in plasma or serum, and it is absent when aqueous solutions are so compared.

Second, we found that the ionized calcium analyzer, because it allowed rapid analysis of sequential blood samples at short time intervals, provided us with the means of monitoring changes in circulating ionized calcium, and thus was essential in establishing and maintaining steady-state alterations in [Ca\(^{2+}\)] equilibrium in the experimental animal.

The observed difference in [Ca\(^{2+}\)] in whole blood as measured with the two instruments is of interest for two principal reasons. First, to assess the significance of a [Ca\(^{2+}\)] value in a patient, it must be compared with an established normal value. However, the use of this normal value itself as a standard against which presumed pathological values are compared has presented difficulties in the past, because a relatively wide range of such normal values has been previously reported by various authors, the difference between extremes of mean values being as great as 0.28 mmol/L (9). In the studies forming the basis for these values, the different authors used an instrument of one manufacturer. In a previous study (9), which we initiated to clarify such wide variations in normal values, we demonstrated that [Ca\(^{2+}\)] in 100 normal human volunteers was maintained within a very narrow range (1.12 ± 0.01 mmol/L). The present [Ca\(^{2+}\)] data obtained in 43 human volunteers with the Orion instrument are in complete agreement with these previous results; further, they establish the 95% confidence intervals for both the Orion SS-20 and NOVA 2 (Table 1). In addition, whereas in the normal [Ca\(^{2+}\)] range the difference between mean [Ca\(^{2+}\)] in whole blood obtained by both instruments was about 9%, this difference was greater for moderate hypercalcemia and greater still at the upper limit of the clinical hypercalcemia range. Clearly, a normal value obtained with one instrument can only be used for appropriate interpretation of results with another after careful comparison over the entire range in which [Ca\(^{2+}\)] may be measured in patients. Hence, in communications on the magnitude of presumed pathological [Ca\(^{2+}\)] values, the calcium ion analyzer used to obtain such values should always be specified and the reference interval for that instrument should be known.

Our findings of a discrepancy in results obtained with the two instruments are in substantial agreement with those of Larsson and Ohman (10), who tested the two instruments for [Ca\(^{2+}\)] measurements in serum. However, in the present study we present data on [Ca\(^{2+}\)] in whole blood. Such whole-blood analyses, obviously more attractive, are now routinely performed in the Acute Care Laboratory of the Massachusetts General Hospital. The discrepancy between the two instruments, detected in human whole blood from normal volunteers (Table 1), was confirmed in patients (Figure 1) and in dogs (Figure 6). In contrast, such a discrepancy was not seen in the aqueous standard solutions supplied by the manufacturers (Table 2). Because whole blood can be viewed as an electrolyte solution containing erythrocytes and protein, we collected data in which increments of calcium chloride were added to a human erythrocyte suspension in which total protein was only 1 g/L. The discrepancy between [Ca\(^{2+}\)] as measured with the two instruments was noted (Figure 3) as it had been for whole blood (Figures 1, 2, and 6). In the normal [Ca\(^{2+}\)] interval, this discrepancy was smaller for plasma (Table 1) but similar to that for whole blood over the [Ca\(^{2+}\)] range studied (Figures 2 and 6); it was virtually absent in the case of a human albumin solution (Figure 4) or aqueous standard solutions (Table 2). The slopes of the regression equations describing the data points in human whole blood, plasma, and erythrocyte suspensions (Figures 1, 2, 3) are similar, so we can exclude the erythrocytes as a major contributor to the observed discrepancy, even though they are known to affect the junction potential of the electrode (11). Further, the discrepancy between the two instruments is unlikely to be due to interference by proteins per se, as demonstrated in Figure 4.

The discrepancy between the two instruments does not mean that [Ca\(^{2+}\)] is measured "correctly" by one instrument and "incorrectly" by the other. Rather, our findings emphasize the need for specification of the instrument used in reports on presumed abnormal [Ca\(^{2+}\)] values, and our findings are useful in the assessment of the normal [Ca\(^{2+}\)] interval.

In the presence of albumin (Figure 5) at a given concentration of total calcium (2.0 mmol/L), apparent [Ca\(^{2+}\)] was
generally lower than in a human native protein solution with the same total calcium concentration. Evidently the processed human albumin has a higher affinity for calcium ion than does native albumin. The clinical implication of this finding is that rapid intravenous infusion of such processed albumin solutions may transiently lower [Ca\(^2+\)] in the recipient and thereby may produce a transient decrease in blood pressure (12, 13) and cardiac pump performance (14, 15).

In our dog experiments, monitoring [Ca\(^2+\)] allowed us to make adjustments that resulted in [Ca\(^2+\)] plateaus that were maintained within 0.10 mmol/L of the desired [Ca\(^2+\)] value. Such steady-state [Ca\(^2+\)] alterations are of considerable physiological interest, because their presence allows study of physiological events that are not instantaneous. For example, to establish the relation between [Ca\(^2+\)] and cardiac pump performance, as defined by left ventricular function curves, [Ca\(^2+\)] should be maintained while ventricular function curves are generated. Thus, in our experiments, all cardiac outputs comprising a given left ventricular function curve were obtained at one [Ca\(^2+\)].

Finally, we found that, in the [Ca\(^2+\)] interval encountered clinically, differences between [Ca\(^2+\)] in coronary venous and systemic arterial blood were insignificant (Figure 7). This finding agrees with previous data (9) showing that [Ca\(^2+\)] measured in systemic venous and arterial blood was similar. These present findings also suggest that the myocardial uptake or release of [Ca\(^2+\)] is not measurable in terms of millimolar concentration.

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