Measurement of Serum and Plasma Ionic Calcium with the "Space-Stat 20 Ionized Calcium Analyzer"

Harry Husdan,1 Mary Leung,2 Dimitrios Oreopoulos,3 and Abraham Rapoport4

Ca2+ was measured in sera and plasma from 36 normal subjects (18 men 23–57 years old, and 18 women 20–52 years old) with the Space-Stat 20 Ionized Calcium Analyzer. Within-day and between-day precision (CV) for commercial serum-based specimens varied from 0.64–0.96% and 1.1–3.8%, respectively, depending on their mean concentrations. Plasma and serum showed within-day CV's of 1.05 and 0.79%, respectively. The linear regression of serum Ca2+ (y) on plasma Ca2+ (x) was y = 0.63x + 0.45 mmol/litre with a highly significant (P < 0.001) coefficient of correlation (r) of 0.69. An approximate 5% increase in the mean Ca2+ concentration in serum over that found in plasma is probably due to heparin complexing. No significant sex-related difference was observed for either serum or plasma. The mean Ca2+ concentration of 29 refrigerated specimens tended to decrease in seven days (by 2.3%, not statistically significant). The normal Ca2+ range in serum and plasma (n = 35) was 1.08–1.18 and 1.02–1.14 mmol/litre, respectively.

In 1969 Orion Research, Inc. produced the first commercially available Ca2+ electrode system (1) to function in a biological solution under anaerobic conditions (Model 99-20 Calcium flow-through electrode system). Although Ca2+ in serum and plasma could be measured with this instrument, it was not suitable for a routine laboratory. In 1975 their "SS-20 ionized calcium analyzer" (2) appeared, with many desirable and improved features. We report here our experience with this instrument.

Materials and Methods

Apparatus

The Space-Stat 20 Ionized Calcium Analyzer (Orion Biomedical Division of Orion Research, Inc., Cambridge, Mass. 02139) consists of a calcium ion-selective electrode, which senses free Ca2+ only, contains a porous organophilic membrane saturated with a water-immiscible organic liquid ion-exchanger, and a reference electrode, both maintained thermostatically at 37 °C. Ca2+ ions are selectively exchanged between the ion-exchanger of the calcium sensor and the sample solution, resulting in an electrical potential in the sensor, the magnitude of which is related by the Nernst equation to the Ca2+ concentration of the sample. The reference electrode is a AgCl pellet, through which is pumped a 2 mol/litre KCl reference solution saturated with Ag+. The electronics section measures the potential difference across the electrode pair, and the Ca2+ concentration (in mmol/litre) equivalent to this difference is displayed on the instrument's face. The pump delivers these solutions and also supplies an internal Ca2+ standard solution (1 mmol/litre) for instrument calibration. The instrument contains a fluid pack, which carries the standard, reference solution, and a bag, which receives the waste solution. The standard solutions and reagents are supplied by the manufacturer. The Analyzer possesses controls that correct the effect of Na+ and adjust the slope of the linear calibration curve. Two electromagnetic valves are programmed to direct alternate segments of standard and air, or standard or air only, into the electrode module as required by the method. Figure 1 shows the flow-through diagram of the SS-20 Ionized Calcium Analyzer. Operator instructions regarding sample analysis, calibration, and information on the status of the system are displayed in correct sequence on the face of the instrument.

Reagents

Fluid Pack/Ionized Calcium: Reference solution, 2 mol/litre KCl saturated with AgCl, standard solution, 1 mmol of Ca2+ per litre, 150 mmol of Na+ per litre, and 3 mmol of tris(hydroxymethyl)methylamine buffer per litre (pH 7.4).

Calcium standard solutions: These contain, per litre, 0.5, 1.0, 2.0 and 5.0 mmol of Ca2+, 150 mmol of Na+, and 3 mmol of tris(hydroxymethyl)methylamine buffer (pH 7.4).

Sample Preparation

Blood from fasting subjects was collected and processed for plasma and serum according to the procedure described in the instruction manual (2) except for the differences indicated below. Blood was drawn with a "no tourniquet" technique (3), and delivered to the laboratory immediately after collection.

For plasma, we used a disposable 5-ml plastic syringe fitted with a No. 19 needle and containing 0.1 ml of an equimolar mixture of heparin solution ("Liquaem," 1000 USP units/ml; Organon, Inc., West Orange, N. J. 07052) and sterile isotonic saline. Immediately after the syringe was filled, the needle was removed, any air expelled, the syringe capped with a disposable Luer tip cap (Becton-Dickinson and Co., Rutherford, N. J. 07070), and the contents were well mixed by inversion. For serum, a 10-ml pink-stoppered "acid-washed" evacuated blood-collection tube (Vacutainer No. 4719; Becton-Dickinson & Co., Canada, Ltd.) was used (this particular one because of

Departments of Medicine1-4 and Clinical Biochemistry,5 University of Toronto; and the Metabolic-Renal Unit and Laboratory of the Toronto Western Hospital1-3,5 599 Bathurst St. Toronto, Ontario, M5T 2S8, Canada.

Received Feb. 16, 1977; accepted May 23, 1977.
earlier reports of calcium contamination from the use of Vacutainers (4). It is important to allow the Vacutainer to fill until blood ceases to flow into the tube; this prevents significant loss of CO₂ from serum into the residual air space in the tube (5), with a concomitant increase in serum pH and decrease in Ca²⁺ concentration.

When samples of blood for both plasma and serum were required, the syringe and Vacutainer were connected by a Model K-75 three-way plastic stopcock (Pharmaseal, Inc., Puerto Rico 00758). The Vacutainer and syringe were centrifuged at 2000 rpm for 30 min.

Plasma and serum were removed with 1-ml tuberculin syringes, any trapped air was expelled, and the syringes were fitted with disposable Luer tip caps and covered with Parafilm. The tuberculin syringes were then refrigerated at 4 °C until required for analysis, which was usually within 2 h.

Method

The procedure we used for the daily check out and the technique used for specimen analysis were as described in the instruction manual (2). Specimens and standards were measured in duplicate; the mean values are reported. The responsiveness of the Ca²⁺ sensor also was monitored daily by noting the reading in millivolts on the control panel for different Ca²⁺ standards. For adequate sensitivity, there should be a 6 to 9 mV difference in readings for the 1 and 2 mmol/litre standards.

Subjects

The subjects of this study were 36 healthy staff members with no history of renal or bone disorders or acid–base disturbances. Of these, 18 were men 23 to 57 years old (mean age: 34.1 years) and 18 were women 20 to 52 years old (mean age: 35.0 years). Sera was collected from 17 men and 18 women, plasma from 18 men and 17 women.

Results

Analytical Variables

Precision. Table 1 shows within-day and between-day precision that we found for samples. Except in one case, the within-day CV was smaller than the corresponding between-day value.

The precision we found with the SS-20 ionized calcium analyzer was similar to that reported for the Model 99-20 flow-through system (6).

Values for serum vs. plasma. Plasma and serum Ca²⁺ was measured concurrently for 34 normal subjects by the described technique. The calculated linear regression equation was $y = 0.63x$ (plasma) + 0.45 mmol/litre. The correlation coefficient ($r$) was 0.69, statistically highly significant ($P < 0.001$). By the paired $t$-test, the mean difference of 50 mmol/litre between corresponding serum and plasma specimens (d) was also highly significant ($P < 0.001$), the range of differences being 10–110 mmol/litre and the calculated difference range based on 95% confidence limits, 10–100 mmol/litre. These findings are consistent with the mean 5% greater value for serum than plasma found by others (5).

Specimen stability. Sera from 18 subjects gave a mean Ca²⁺ value, when fresh and after 24-h at 4 °C, of $X_1 = 1.13 ± 0.082$ (SD) mmol/litre and $X_2 = 1.12 ± 0.082$ (SD) mmol/litre. There was no significant difference in the overall mean difference ($P > 0.5$).

Various individual specimens ($n = 29$) gave initial mean Ca²⁺ concentrations that decreased by 2.3% during seven days of refrigeration, but this decrease was not significant ($P > 0.5$).

Frequency distribution. Sera and plasma Ca²⁺ values of both men and women, plotted on probability paper, indicated their distributions were nearly gaussian. No statistically significant difference was observed between the means for males and females for each serum or plasma (using the unpaired $t$-test, for serum: $t = 1.15, P > 0.05, n = 35$; for plasma: $t = 0.262, P > 0.05, n = 35$).

Normal Ranges for Serum and Plasma

Since Ca²⁺ values showed no statistically significant sex-related difference for either serum and plasma, normal ranges were based on $n = 35$ subjects. For plasma: $X = 1.08 ± 0.03$ (SD) mmol/litre; the 95% confidence limits range was thus 1.02–1.14 mmol/litre. The actual range was 1.03–1.15 mmol/
litre. For sera collected simultaneously with plasma, X = 1.13 ± 0.03 (SD) mmol/litre; and the 95% confidence limits was thus 1.07-1.19 mmol/litre. The actual range found was 1.08-1.18 mmol/litre. Our data from the paired t-test indicate that the mean values for serum and plasma are significantly different. Since their standard deviations are identical and both substances have gaussian distributions, the normal ranges for serum and for plasma are therefore different.

The serum and plasma Ca2+ values we report with the SS-20 Ionized Calcium Analyzer differ from corresponding values reported with the earlier Model 99-20 serum calcium flow-through electrode system for at least two reasons (6, 7). The triethanolamine and trypsin originally recommended as additives to the Ca2+ standards to prevent electrode coating with protein partly bind Ca2+, reduce the electrode potentials in the standards, and hence increase the apparent serum and plasma Ca2+ concentrations. Secondly, the SS-20 electrodes are thermostated at 37 °C and thus give an apparent 2-3% smaller concentration (6) than they would at room temperature (25 °C).

Discussion

According to the manufacturer, the Space-Stat 20 is suitable for measuring Ca2+ in whole blood, plasma, or serum. The decision as to which fluid to use is to some extent arbitrary (5, 8). Whole blood is sticky, and likely to interfere with the components of the flow-through system and sample injection port. Hemolysis would not be apparent and fibrin would be difficult to detect visually. A syringe containing heparin would have to be prepared for each specimen, and whole blood is not stable for more than 1 h. The preparation of both plasma and serum require centrifugation, but this can conveniently be done while the instrument is being calibrated. Both plasma and serum are appreciably more stable than whole blood when refrigerated.

A potential difficulty noted with the Model 99-20 (when low heparin concentrations are used) was the tendency of plasma to form fine threads of fibrin, which were not always visible in the tuberculin syringe but which could spontaneously gel while traversing the flow-through system. An additional advantage of serum is that its mean value is 5% greater than the corresponding mean plasma value. The explanation for this is mainly the complexing effect of Ca2+ by heparin in the amount recommended (10 USP units/ml blood), according to several investigators (6).

Because of the cost of the fluid packs, consideration was given to the mode with which the analyzer was to be set when not in use. The instrument was normally left in the STAT mode, in which case reagents were pumped through the system every 4 h. When the instrument was left in the NON-STAT mode, useful life of the calcium sensor seemed to be lessened. This mode was therefore discontinued.

One of the main advantages offered by this model is its freedom from "read out" drift after the initial daily check out procedure. This obviates the need to alternate each specimen with standards, as was required with the Model 99-20 system, and results in a much faster and reproducible set of analyses. Other useful features include a counter that tells the user the residual volume of the Fluid Pack, and hence the number of additional tests which the pack can provide. An ERROR legend lights up on the front panel of the instrument if anything in the system, such as an air bubble, results in electronic noise. A temperature exceeding 38 °C or the need to change a fluid pack are indicated by an illuminated SERVICE legend.

We find the Space-Stat 20 Ionized Calcium Analyzer to be a convenient and reproducible instrument with advantages associated with printed-board electronic circuitry. It reads out directly in mmol of Ca2+ per litre and requires no graph-calibration or involved calculations. However, careful attention to and regular maintenance of this instrument is essential for "trouble free" use. Obvious limitations include the expense of its fluid pack and the cost and relatively short useful life of the present type calcium sensor. These limitations, it is hoped, will soon be rectified.

We thank Orion Research Inc. for permission to slightly modify the flow-through diagram of the SS-20 analyzer published in their instruction manual, Professor D. B. W. Reid for statistical aid, and Miss N. Lagdamen and Mrs. L. Grass for technical assistance.

References