Activity Measurements of Calcium, Sodium, Potassium, and Chloride after Equilibrium Dialysis Used to Show Lack of Evidence for Protein Interference with Calcium Electrodes

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We measured the activity of Ca2+, Na+, K+, and Cl− with ion-selective electrodes after equilibrium dialysis of solutions with different albumin concentrations. The calculated Donnan ratio was the same for all ions in the same solution and increased with the albumin concentration, as predicted by the Donnan theory. The Donnan distribution ratio for Ca2+ was similar, as determined with instruments from three different manufacturers. For healthy subjects and patients with renal stone disease, we did not find any correlation between serum concentrations of ionized calcium and albumin. The discordance between measured ionized calcium and albumin-corrected total calcium depended on the correction algorithm we utilized. The difficulties of absolutely proving or disproving a protein error in these measurements are discussed, but our data are not consistent with protein being a source of error in measurements of ionized calcium.

Additional Keyphrases: electrolytes - Donnan potential - ion-selective electrodes - liquid-junction potential - potentiometry - ultrafiltrate - analytical error

Recent suggestions that albumin may interfere with direct potentiometric measurements of ionized calcium are based on three observations. First, the concentration of measured ionized (free, ionic) calcium (cCa2+) in a serum sample is greater than in an ultrafiltrate of the same sample (1−3). Second, a positive correlation between ionized calcium and albumin in serum has been recently reported (2, 4, 5); and lastly, in identifying hypocalcemia, a discordance between the concentration of total calcium corrected for albumin (cCa2+ Alb) and cCa2+ measurements has been noted in some patients (4). As suggested elsewhere, the first of these observations regarding the lower values for cCa2+ in an ultrafiltrate may be due to the effects of the Donnan potential during ultrafiltration (6–8); i.e., in the presence of a nondiffusible macro-ion (e.g., albumin), the diffusible ions (Ca2+, K+, Cl−) distribute themselves unequally, creating an electrical potential. Because measurement of ionized calcium is now a routine analysis in many laboratories and has been recommended as a replacement for total calcium (9), its diagnostic value will be compromised to some degree if such a protein interference exists. We therefore investigated the influence of albumin on the response of several commercially available calcium electrodes. We evaluated the Donnan potential by direct measurement of the activity of Ca2+, Na+, K+, and Cl− after equilibrium dialysis. We also assessed the relation between ionized calcium and albumin concentrations in sera from healthy subjects and from a group of patients, and investigated the agreement between different algorithms for correcting total-calcium measurements for the presence of protein-bound calcium.

Materials and Methods

Analytical Studies

Preparation of solutions. Human albumin (Cohn Fraction V; Sigma Chemical Co., St. Louis, MO) was dissolved (120 g/L) in a buffer solution containing 2.5 mmol of CaCl2, 150 mmol of NaCl, 5 mmol of KCl, and 5 mmol of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4 at 25°C) per liter. We dialyzed this albumin solution (Spectra/Per tubing 4 (M, cutoff 12 000→14 000, i.d. = 286 mm; Fisher Scientific, Pittsburgh, PA; cat. no. 132-680) against 2 L of the same buffer solution for 24 h, changing the buffer after 12 h. Albumin solutions of various concentrations (14–120 g/L) were prepared from this dialyzed solution by mixing aliquots of it with the buffer solution.

Equilibrium dialysis. We dialyzed 5 mL of the albumin solutions against the buffer solution (same ionic composition) in rigid acrylic plastic cells (Fisher Scientific; cat. no. 08 666-16), using dialysis membranes suitable for the cells (10×16 cm sheets, M, cutoff 6000, cat. no. 08-666-19; Fisher Scientific), with constant rotation at room temperature for 24 h.

Analytical procedures. Using ion-selective electrodes (NOVA 1, 3, and 7; NOVA Biomedical Inc., Waltham MA), Orion SS 20 (Orion Research Corp., Boston, MA 02129), and an ICA1 (Radiometer, Westlake, OH 44145), we measured Ca2+, Na+, K+, and Cl− in the protein solutions and in the aqueous solutions (protein-free) after the equilibrium dialysis. Alternatively, we also measured Na+ and K+ by flame photometry (IL 643; Instrumentation Laboratory Inc., Lexington, MA 02173). The analyzers were calibrated with calibrators supplied from the manufacturers.

Calculations. We calculated the Donnan distribution ratio after equilibrium dialysis as follows. At equilibrium, the electrochemical potential difference across the dialysis membrane is zero, and the permeable ions are distributed according to the Donnan potential (10). This potential arises from the presence of negatively charged albumin, which cannot cross the dialysis membrane. The distribution of the ions in the albumin solution (P) and aqueous solution (A) is expressed via the Donnan ratio as:

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\frac{[\text{Ca}^{2+}\text{(P)}]}{[\text{Ca}^{2+}\text{(A)}]}^{1/2} = \frac{[\text{Na}^+\text{(P)}]}{[\text{Na}^+\text{(A)}]} = \frac{[\text{K}^+\text{(P)}]}{[\text{K}^+\text{(A)}]} = \frac{[\text{Cl}^-\text{(P)}]}{[\text{Cl}^-\text{(A)}]}
\]
Correlations between ionized calcium and albumin were studied in 48 healthy fasting subjects (24 of each sex, ages 22–68 years), and in 80 patients with renal stones but without known calcium metabolic disease (38 single-stone formers and 42 recurrent stone formers, ages 22–90 years). We measured ionized calcium with the ICA1 and albumin by the brom cresol green method in a GMAC continuous-flow analyzer (Technicon Corp., Tarrytown, NY).

Comparison between algorithms. The patients studied and the analytical procedures have been previously described (11). Briefly, the data assessed were from 375 patients in whom anaerobically prepared serum was assessed for ionized calcium (with a modified Orion 99/20, Orion Research Corp., Cambridge, MA), total calcium (by atomic absorption spectrophotometry), protein (biuret reaction), albumin [2-(4'-hydroxyazobenzene)benzoic acid dye method], and pH (Radiometer Model 5021A microcapillary electrode). Patients with creatinine values \(>20\) mg/L were excluded, which left 325 patients in the study. We then identified patients whose ctCa\(^{2+}\) value (reference range 1.18–1.38 mmol/L) was discordant with their ctCa(alb)corr by the algorithm of Payne and coworkers (4, 12): ctCa(alb)corr = 0.025 ctCa + 0.025 (40 – albumin). Their reference range was 2.20–2.60 mmol/L. Discordance was identified when the two procedures were either under- or overestimation of hypocalcemia, normocalcemia, or hypercalcemia. We identified 23 (group 1) and 18 (group 2), respectively, with low serum ctCa\(^{2+}\) and normal serum ctCa(alb)corr and vice versa. Mean (and range) values in group 1 were serum ctCa\(^{2+}\) 1.15 mmol/L (1.09–1.18), albumin 41 g/L (28–55), and protein 69 g/L (61–82), respectively. For group 2 mean (and range) values were serum ctCa\(^{2+}\) 1.21 mmol/L (1.19–1.29), albumin 35 g/L (16–47), and protein 65 g/L (49–78), respectively. We further assessed the discordant values by utilizing ctCa(alb)corr based on multiple regression of the entire data base from Ladenson et al. (11), or based on the algorithm of Parfitt (13): ctCa(alb)corr = 0.025 cCa/0.55 + [(protein)/160], or that of Sigggaard-Andersen et al. (14): ctCa(alb)corr = 0.025 cCa × 189119 + 1.417 [albumin] + 0.15 [protein]. The units of measure for these algorithms are ctCa(alb)corr = mmol/L; cCa = mg/L, albumin = g/L, and protein = g/L.

Results

Table 1 shows the Donnan distribution ratio for Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) after equilibrium dialysis of solutions with different albumin concentrations. As expected, we found a greater activity in the protein solution than in the aqueous solution for the cations Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\), but less activity for the anion Cl\(^{-}\). The Donnan distribution ratio is the same for all ions in each solution and increases with the albumin concentration. A similar but higher Donnan distribution ratio was found for Na\(^{+}\) and K\(^{+}\) measured by flame photometry, recalculated to molality (albumin concentrations in parentheses): 1.18, 1.13 (120 g/L); 1.11, 1.10 (75 g/L); 1.09, 1.08, (29 g/L); and 1.07, 1.05 (14 g/L) respectively. The Donnan distribution ratio for Ca\(^{2+}\) after equilibrium dialysis against an albumin concentration of 106 g/L, as determined with three different instruments, was similar: 1.09 (Nova 7), 1.12 (Orion SS 20), and 1.09 (Radiometer ICA1).

No significant correlation was observed between the concentration of serum albumin and cCa\(^{2+}\) (7.4) either in the control group (\(r = 0.13, P > 0.1\)) or in the stone patients (\(r = 0.12, P > 0.1\); albumin concentrations 38.0 to 51.2 g/L).

When we used the algorithm of Butler et al. (4, 12), 23 patients had low cCa\(^{2+}\) and normal cCa(alb)corr (Group 1), and 18 patients had normal cCa\(^{2+}\) and low cCa(alb)corr (Group 2). When the other three algorithms were applied to the 23 patients in Group 1, the number of discordant patients decreased to 19, 21, and 15 for the algorithms of Ladenson et al., Parfitt, and Sigggaard-Andersen et al., respectively. Likewise, the 18 patients in Group 2 decreased to 11, 8, and 8, respectively.

Discussion

By direct measurement of the activity of Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) after equilibrium dialysis of solutions with different albumin concentrations, we found the same distribution ratios for all ions for each albumin solution, in agreement with the Donnan theory. Importantly, we found the same Donnan distribution ratio for chloride, the calculation for the Donnan distribution ratio for anions being the reciprocal of that for cations; i.e., the activity of chloride in protein solution was less than that in aqueous solution. Heretofore, the findings of a higher activity of Ca\(^{2+}\) and Na\(^{+}\) with increased protein concentrations has been explained as protein interference on the calcium electrode, either directly on the calcium-sensitive membrane or as an effect of protein on the liquid-junction potential (4, 5). The influence of a liquid-junction potential would be the same as the Donnan potential, e.g., high values for cations, low values for anions. Our results make it unlikely that there is a common effect on the sensing electrodes, which were of different types and composition: bis(diocylpheryl)phosphate for Ca\(^{2+}\), valinomycin for K\(^{+}\), glass for Na\(^{+}\). An influence of the liquid-junction potential is also unlikely because the calcium analyzers use different salt bridge compositions: concentrated KCl (Nova, Orion) or concentrated sodium formate (Radiometer). They also utilize different systems for forming the liquid junction, flowing (Nova, Orion) and static (Radiometer). That a true Donnan potential exists is furthermore suggested by the similar but higher Donnan ratio we found for the nonelectrochemical measurement (flame photometry) of Na\(^{+}\), K\(^{+}\).

We failed to show a significant correlation between ionized calcium and albumin in this study. While others have also not found a correlation (15), several investigators have identified a small but statistically significant correlation between albumin or total protein and ionized calcium (4, 5, 16, 18). The importance of the small correlation reported in these other studies remains unclear. From a physiological point of view, a weak positive correlation between ionized calcium and albumin should be expected in serum of healthy subjects, given that their concentrations are known to decrease and that of parathyroid to increase with age (19, 20). The positive correlation shown in small populations be-

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Table 1. The Donnan Distribution Ratio for Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) after Equilibrium Dialysis of Solutions with Different Albumin Concentrations

<table>
<thead>
<tr>
<th>Albumin concn, g/L</th>
<th>([\text{Ca}^{2+}]<em>0/\text{Ca}^{2+}</em>{(A)})</th>
<th>([\text{Na}^{+}]<em>0/\text{Na}^{+}</em>{(A)})</th>
<th>([\text{K}^{+}]<em>0/\text{K}^{+}</em>{(A)})</th>
<th>([\text{Cl}^{-}]<em>0/\text{Cl}^{-}</em>{(A)})</th>
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<tr>
<td>120</td>
<td>1.11</td>
<td>1.10</td>
<td>1.09</td>
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<td>75</td>
<td>1.04</td>
<td>1.05</td>
<td>1.05</td>
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<td>29</td>
<td>1.03</td>
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<td>1.02</td>
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<tr>
<td>14</td>
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The activity of the ions was measured potentiometrically with two instruments after equilibrium dialysis in the aqueous solution (A) and in the protein-containing solution (P).
between ionized calcium and albumin could also be explained by an in vivo Donnan phenomenon (4, 5).

Additional empirical information appears relevant but difficult to interpret. There is a small change in ionized calcium after changes in posture and its concomitant change in protein concentration. An increase of ~2% in ionized calcium is observed in subjects going from the upright to supine positions, while the albumin increases by ~12%. However, the change in ionized calcium did not correlate with the change in albumin during 15 experiments involving 11 healthy men (21). Moreover, a study of the influence of protein on sodium values determined with six different direct potentiometric instruments showed a correlation of protein concentration with the difference between direct-potentiometric and flame-potentiometric sodium values for four such instruments, including the instruments from Nova and Radiometer, but not for two instruments from other manufacturers (22).

We observed that the discordance in identifying hypocalcemia between $c_{Ca}^{1+}(meas)$ and $c_{Ca}^{1+}$, depended on which algorithm was utilized (mean discrepancies between algorithms were 35–40%). These findings are in keeping with the poor agreement between various algorithms used to calculate ionized calcium and the measured ionized calcium in previous reports (11, 23–25). Moreover, there was an a priori assumption in the study of Butler et al. (4) that the calcium status of the patients from the gastroenterology service with low albumin was normal. This is extremely difficult to validate.

The published study that comes closest to addressing this issue was that of Bouillon et al. (26), who studied several factors related to calcium metabolism in 32 patients with biopsy-proven cirrhosis of varying etiology. They concluded that (a) the observed abnormalities of calcium metabolism in patients with cirrhosis were mainly due to decreased protein synthesis, and (b) in patients with severe cirrhosis the ionized calcium was maintained within reference limits by a compensatory secondary hyperparathyroidism. The implications of their study would be that patients with liver disease could have low or normal ionized calcium, depending on the degree and possibly the duration of the liver disease. Such variables would make it difficult if not impossible to directly assess the validity of the ionized calcium measurements by studying patients with low albumin values from a gastroenterology service.

In conclusion, our data do not support an in vitro influence of albumin on $Ca^{2+}$ measurements. However, the reports of correlation between albumin and $Ca^{2+}$ warrant further investigation. Such studies will probably be difficult to design, given the problem of independently assessing calcium metabolism in patients with low albumin concentrations.

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References


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