Thiocyanate in smokers interferes with the Nova magnesium ion-selective electrode

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Thiocyanate found in serum ordinarily is the metabolite of cyanide that is inhaled with tobacco smoke and ingested with cyanogenic foods. We investigated the effect of the thiocyanate ion (SCN⁻) on the ionized magnesium (iMg) and ionized calcium (iCa) results determined with the AVL and Nova magnesium and calcium ion-selective electrodes (ISEs). We analyzed saline and pooled serum with added SCN⁻, and serum from apparently healthy nonsmokers (n = 20) and smokers (n = 20). The mean (and range) of the measured serum SCN⁻ concentration was 0.019 (0.008–0.046) mmol/L for nonsmokers and 0.077 (0.020–0.138) mmol/L for smokers. Only the Nova iMg results decreased with increasing SCN⁻ concentration, and the change was dependent on the baseline iMg concentration. In the absence of Mg, SCN⁻ decreased the voltage response of the Nova Mg ISE to calcium ions. At apparently normal serum iMg and iCa concentrations, the interference by SCN⁻ appeared to be equimolar (iMg = −1.04 × SCN⁻ + 0.52). Thus, the serum SCN⁻ commonly found in smokers causes a significant (P < 0.0001) decrease in the Nova iMg results.

The magnesium ion-selective electrodes (Mg ISEs) manufactured by AVL [1], Kone [2], and Nova [3] were specifically designed for determining the ionized magnesium (iMg) concentration in blood samples. The goal was to provide clinical laboratories with a test that would reflect the extracellular activity of the physiologically active Mg fraction better than the traditionally measured concentration of the total Mg concentration (tMg). The recent incorporation of these ISEs in commercial analyzers offers a fast, convenient method for routine laboratory determination of iMg.

In the US, only the AVL and Nova Mg ISEs are approved by the Food and Drug Administration for clinical application. However, the accuracy of each method has not yet been determined, because of the lack of a reference material. Further, the interpretation of iMg results is not clearly established, especially in relation to tMg. Most of the studies with the Nova Mg ISE were reported by Altura et al., who found a decreased iMg associated with “normal” tMg (values within the reference range) in patients with several different pathological abnormalities (reviewed in ref. 4) and in healthy male adults [5]. Wu et al. [6] also reported low Nova iMg results associated with normal tMg in alcoholic patients admitted to the emergency department of a trauma hospital. In our studies of patients undergoing treatment with IL-2 [7] and of patients with chronic alcohol consumption [8], many patients also had a normal serum tMg and an abnormally low Nova iMg result. However, of 56 samples with this combination of results, only 2 had an abnormally low AVL iMg result. When we performed a comparison study between the AVL and Nova iMg methods with serum samples from randomly selected patients for which Nova or AVL iMg results were abnormal [9], all samples with normal tMg and abnormally low Nova iMg results (n = 25) again had normal AVL iMg results, and we suggested the possibility of interfering substances as a cause of the intermethod discrepancies. Because we also found significant intermethod differences with samples from apparently healthy individuals [10], we reviewed, retrospectively, the demographic information for each donor included in that study. We found that the cigarette smoking habit of a donor correlated with the observed intermethod differences.

Two major components of tobacco smoke are found to be increased in serum of smokers, nicotine and thiocyanate (SCN). Nicotine appears in the blood shortly after smoking and is metabolized to cotinine [11], the half-life of nicotine being ~2 h and of cotinine 10–20 h [12]. SCN is the metabolite of hydrogen cyanide and organic cyanides; ~40% of the circulating SCN is bound to albumin [13], and the half-life is relatively long (~14 days) [14]. Because of this relatively long half-life, the serum concen-
Concentration of SCN does not change as rapidly as that of nicotine or cotinine. Further, SCN has been considered a useful marker for the assessment of exposure to tobacco smoke [15–17]. However, SCN is also present in the serum of nonsmokers, given that cyanide is produced by the metabolism of vitamin B12 and of foods that contain cyanide or cyanogenic glucosides (e.g., cabbage, broccoli, almonds) [18].

In this study, we have investigated the effect of SCN on the AVL and Nova Mg ISEs. Because the albumin-bound SCN would be less likely to interact with the sensor of an ISE, we limited our investigation to the free, ionic form (SCN–).

**Materials and Methods**

**Aqueous solutions.** Solutions with different concentrations of MgCl₂ and CaCl₂ were prepared in unbuffered saline (NaCl 145 mmol/L, pH 7.4). Stock thiocyanate solutions (KSCN 1.5 mmol/L) were prepared in deionized water and in unbuffered saline. The stock KSCN in water was used to prepare the calibration solutions (KSCN concentrations 0.300, 0.200, 0.100, 0.050, and 0.020 mmol/L). The ferric nitrate solution, Fe(NO₃)₃·9H₂O (12.5 mmol/L), was prepared in nitric acid (HNO₃ 1.5 mol/L).

**Analyzers.** The Nova Biomedical CRT and the AVL (Graz, Austria) 988–4 analyzers, previously described [10], were used to determine iMg and ionized calcium (iCa). For the aqueous samples, the voltage responses of the Nova Mg and Ca ISEs were also recorded. The Hitachi 917 (Boehringer Mannheim) analyzer was used to determine serum concentrations of tMg and total calcium (tCa). The Cobas Fara (Roche) was used to determine the concentration of SCN– using the thiocyanate method. The results of the thiocyanate method were linear up to 0.30 mmol/L, the concentration of the highest calibration solution. The interassay imprecision (CV) was acceptable: 8.6% at a mean of 0.020 mmol/L, 1.2% at 0.300 mmol/L. The recovery of SCN– from aqueous solutions was between 91% and 105%. Recovery from the pooled serum decreased with increasing amounts of added KSCN. For samples with KSCN ≥0.20 mmol/L, the recovery remained constant (63–67%). This recovery indicates that 33–37% of SCN– in serum was bound, which is similar to the ~40% binding of SCN– to serum albumin reported previously [13].

The effect of SCN– on the Nova and AVL iMg and iCa results for a pooled serum with added MgCl₂ is shown in Fig. 1. The baseline SCN– concentration was 0.020 mmol/L. The AVL results for both iCa and iMg and the Nova iCa results were not affected, but the Nova iMg results decreased with increasing concentrations of SCN–. The relationship between iMg results and SCN– concentration was best characterized by a second-order polynomial regression.

Blood samples from apparently healthy smokers (n = 20) and nonsmokers (n = 20) were collected anaerobically in Vacutainer Tubes with glycerin as a stopper lubricant (Becton-Dickinson) after informed consent. All procedures were in accordance with the ethical standards laid down in the Helsinki Declaration of 1975, as revised in 1983. Serum SCN– was determined by assaying the serum ultrafiltrates, obtained with a Centricon-30 (30 000 M, cutoff; Amicon). All SCN– results reported in this study are the mean values of duplicate determinations.

**Statistics.** Linear regression was used to investigate association between the iMg, iCa, and SCN– results. P < 0.05 was considered statistically significant.

**Results**

The effect of SCN– on the Nova and AVL iMg and iCa results for a pooled serum with added MgCl₂ is shown in Fig. 1. The baseline SCN– concentration was 0.020 mmol/L. The AVL results for both iCa and iMg and the Nova iCa results were not affected, but the Nova iMg results decreased with increasing concentrations of SCN–. The relationship between iMg results and SCN– concentration was best characterized by a second-order polynomial regression. A similar nonlinear relationship between the Nova iMg results and the SCN– concentrations was observed with the saline solutions (Fig. 2). As with the pooled serum, the AVL results and the Nova iCa results were not affected. The Nova iMg results decreased, the extent of change depending on the initial iMg value [iMg(init)] determined
in the absence of SCN\textsuperscript{2-}. This dependence was also observed with the serum samples. The change for the pooled serum with iMg\textsuperscript{init} = 0.94 mmol/L (constant iCa = 1.23 mmol/L) is similar to the change for saline with iMg\textsuperscript{init} = 0.99 mmol/L (constant iCa = 1.22 mmol/L), whereas the change for sera (n = 5) with iMg\textsuperscript{init} between 0.39 and 0.48 mmol/L (iCa 0.87–1.01 mmol/L) was similar to the change for saline with iMg\textsuperscript{init} = 0.43 mmol/L (constant iCa = 1.0 mmol/L; see Fig. 2). We found that in saline, in the absence of Mg ions, and at a constant concentration of Ca ions (1, 2, or 3 mmol/L), the mV response of the Mg ISE was affected by SCN\textsuperscript{2-}. A. An increase in the SCN\textsuperscript{2-} concentration from 0 (baseline) to 0.20 mmol/L caused a decrease in the mV response of 1.90 (iCa = 1 mmol/L), 2.11 (iCa = 2 mmol/L), and 2.78 (iCa = 3 mmol/L) from the baseline. This decrease was associated with a decrease in the iMg result from 0.17 to 0.11, from 0.26 to 0.15, and from 0.35 to 0.17 mmol/L, respectively.

The serum tCa, tMg, iCa, and iMg concentrations for the apparently healthy nonsmokers (n = 20) and smokers (n = 20) were within the reference intervals established in our laboratory for each method. The mean (range) serum SCN\textsuperscript{2-} concentration was 0.019 (0.008–0.046) mmol/L for the nonsmokers and 0.077 (0.020–0.138) mmol/L for the smokers. The iCa results were not affected by SCN\textsuperscript{2-} (y = -0.16x + 1.20, r\textsuperscript{2} = 0.035, P >0.1 for Nova; y = -0.117x + 1.29, r\textsuperscript{2} = 0.02, P >0.3 for AVL). The relation between the serum AVL and Nova iMg results and the measured SCN\textsuperscript{2-} concentration is shown in Fig. 3A. The AVL results did not change, but the Nova results decreased as the SCN\textsuperscript{2-} concentration increased and, therefore, the difference between the iMg results determined by the two methods increased (Fig. 3B).

In addition, we determined the SCN\textsuperscript{2-} concentration in seven serum samples that were used in our original study of healthy individuals [10] and in serial samples of one patient treated with a high-dose IL-2 therapy [7]. All of these samples had been stored in tightly capped vials at -20 °C for as long as 36 months. For the IL-2-treated patient, the SCN\textsuperscript{2-} ranged from 0.093 (day 1, just before administration of therapy) to 0.055 (3 days after administration of therapy) mmol/L. The corresponding Nova/AVL iMg results determined at the respective times of blood collection were 0.26/0.49 (tMg = 0.70) and 0.32/0.45 (tMg = 0.54) mmol/L. For the samples from healthy individuals, in six of the seven samples for which the intermethod difference for iMg was < -0.1 mmol/L,
SCN$^-$ was between 0.040 and 0.077 mmol/L. Three of these six samples were from self-reported cigarette smokers.

**Discussion**

The introduction of the Mg ISEs moved the determination of blood iMg from the research laboratory to the clinical laboratory and revived the interest of clinicians in the diagnosis of abnormalities of magnesium metabolism. To investigate both of these issues, our studies with healthy individuals and patients were performed with the AVL and Nova Mg ISEs that became available for routine testing in this country. In all of our studies [7–10], we reported significant differences between the iMg results determined with the two ISEs, regardless of the tested individual’s health status.

Generally, the Nova iMg results were lower than the AVL results, which was reflected by a difference in our reported reference intervals for each method (0.39–0.64 mmol/L for Nova, 0.44–0.60 for AVL), even though the mean for both methods was the same (0.52 mmol/L) [10]. The largest intermethod difference [iMg(diff) = Nova − AVL] observed for a healthy donor was −0.13 mmol/L. We also found significantly lower Nova results for patients undergoing IL-2 therapy [7] and individuals with chronic alcohol consumption [8]. However, in both of these studies, the intermethod difference for individual samples varied greatly (from 0.10 to −0.47 mmol/L) and the abnormally low Nova results were often associated with a normal tMg concentration.

Interestingly, a close inspection of the Nova results for acute alcoholics reported by Wu et al. [6] revealed the same pattern: For individual samples with tMg between 0.8 and 1.0 mmol/L, the iMg results ranged from ~0.21 (abnormally low) to ~0.55 mmol/L (within the reference interval). This prompted us to compare the abnormally low and high results for serum samples (excluding those for alcoholics and IL-2 patients) that we determined with both the AVL or Nova method. All 25 samples with Nova iMg ≤ 0.39 mmol/L (the lower limit of the Nova reference interval) and normal tMg had normal AVL iMg results [9]. The intermethod difference (mean − 0.19, range 0.04 to −0.37 mmol/L) was not associated with the diagnosis of the patient; however, many were outpatients, i.e., exposed to a nonhospital environment. When we evaluated the health habits of presumably healthy volunteers and of some patients in our studies, we noted that cigarette smokers tended to have a low Nova iMg result. These observations led us to investigate the iMg results in individuals who were smokers of cigarettes. We found that nicotine and its metabolite, cotinine, had no effect on the measurements by either of the Mg ISEs [21]. However, we did see a significant inverse correlation between the Nova iMg results and the leukocyte count, which has previously been reported to be increased in smokers [22]. The observed intermethod difference for smokers (mean −0.11, range −0.01 to −0.24 mmol/L) correlated directly with the leukocyte count [21].

This study identifies SCN$^-$, at concentrations found in smokers, as an interferent with the Nova Mg ISE that causes underestimation of the results. At physiologically normal iMg and iCa concentrations, SCN$^-$ appears to cause an equimolar decrease in the concentration of Mg ions (slope = −1.04, see Fig. 3). However, from the results that we obtained with saline solutions and pooled sera (see Fig. 2), the SCN$^-$ concentration alone cannot be used to accurately predict the actual decrease in the iMg result. The most likely reason for this is the selectivity of the Mg ISE to both the Mg and Ca ions. The chemometric correction for the effect of Ca ions on the Mg ISE is based on the iCa result determined with the Ca ISE. This correction would be erroneous if the response of Mg ISE to Ca ions were affected by SCN$^-$ while, as we have shown, the iCa result was not affected. The net effect would be chemometric underestimation of the iMg concentration because of overestimation of the Mg ISE response to Ca ions. We found that, in the absence of Mg ions, the SCN$^-$ decreases the voltage response of Mg ISE to Ca ions and, as a consequence, decreases the iMg results. However, the significance of the observed change in the iMg results attributable to this interference for Ca ions is difficult to interpret. The results are generated by the chemometric calculation and actually, if that calculation were proper, all iMg results in the absence of SCN$^-$ should have been close to zero and reported as “<0.1” (the linearity limit of the method). In light of the observed decrease in the mV response of the Nova Mg ISE, we suggest that SCN$^-$, a lipophilic anion, decreases the slope of the electrode response (mV/log [ion]) to both Mg and Ca ions. Such an effect by SCN$^-$ has been reported for potassium and calcium neutral carrier electrodes that did not have negatively charged sites in the membrane [23]. The addition of lipophilic tetrathylborate anions into these membranes reduced the effect by SCN$^-$ (and by other lipophilic anions) and also enhanced the selectivity of membranes for the primary cations [24]. A similar effect of borate anions on the membrane selectivity was reported for Mg electrodes with derivatized malondiamide subunits (ETH compounds) as neutral carriers [25,26]. The membrane of the AVL Mg ISE is based on ETH 7025 as the neutral carrier with potassium tetrakis(2-pyridyl)borate as anionic sites [1]; this electrode was not affected by the presence of SCN$^-$ in the sample. The composition of the Nova membrane remains proprietary. However, we speculate that the observed interference by SCN$^-$ may result from the absence of anionic sites in the membrane of the Nova Mg ISE.

The intermethod differences we previously reported
were most likely caused by increased concentrations of serum SCN\(^-\). Certainly, most of the alcoholic patients in our study [8] were known to be cigarette smokers. We do not have a complete demographic profile for each of the patients included in our study of abnormally low iMg results [9]. However, the intermethod difference (mean \(-0.19, \) range 0.04 to \(-0.37\) mmol/L) was similar to that observed in our study of smokers. In addition, the change in the serum SCN\(^-\) for the patient treated with IL-2 (from 0.093 to 0.055 mmol/L) most likely reflects the cessation of smoking during hospitalization. For this patient, the tMg and the AVL iMg results decreased, but the Nova iMg results increased—a combination we have observed in several patients in our IL-2 study [7]. Retrospectively, we can now also explain the much lower reference interval of the Nova iMg method (0.39 – 0.64 for Nova vs 0.44 – 60 for AVL) that we determined previously with the results for both smokers and nonsmokers [10]. We found that the SCN\(^-\) concentration was increased in six of the seven samples from healthy donors for which the intermethod difference was \(-0.10\) to \(-0.13\) mmol/L, and three of these samples were collected from known cigarette smokers. This also explains why, in the present study, all Nova iMg results for the smokers are within the reference limits of that method.

We must point out that SCN\(^-\) at serum concentrations that can lead to negative interference with the Nova Mg ISE can be attained not only from exposure to cigarette smoke but also from dietary sources. Even with the small number of individuals in this study, there was an overlap between the SCN\(^-\) concentrations for the nonsmokers and smokers, and the lowest SCN\(^-\) concentration for a non-smoker was 0.008 mmol/L (see Fig. 3). Increased concentrations of total SCN were reported in two nonsmokers who daily ingested cabbage (0.090 mmol/L) and raw brussel sprouts (0.080 mmol/L) [16]. In both of these cases, the concentration of the free SCN\(^-\) (estimated as \(-0.050\) – \(-0.060\) mmol/L) would be sufficient to cause an interference with the Nova Mg ISE. Moreover, the number of cigarettes smoked/day and the dietary habits can vary, as well as the serum SCN\(^-\) concentration. The fluctuation in the SCN\(^-\) concentration is unpredictable because of its long half-life. Therefore we suggest that all Nova iMg results, especially the low ones, reported by us [7–10, 21] and by others [4–6] are questionable. The findings by Altura et al. [5] that low serum Nova iMg, but not tMg, concentrations for five healthy men were a possible indicator of Mg deficit and a potential predictor of future coronary vascular disease must be reexamined. Were these low iMg results possibly caused by SCN\(^-\) interference attributable to smoking or dietary habits? Although the configuration of the instrument used by Altura et al. (Nova Stat Profile) differed from that used in the current study, the ionophore and membrane may be similar and possibly also susceptible to the interference by SCN\(^-\). The results of the present study lead us to suggest that the Nova Mg ISE cannot provide reliable results suitable for clinical interpretation. Thus, this method should not be used in a routine patient care setting until the electrode is redesigned to eliminate the observed interference by SCN\(^-\).

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References


