sampled (in 1976) from the healthy population, and if this assumption was true, the mean value (1.000 ± 0.0064) of the former group was significantly lower (t = 4.0, P < 0.001) than that of the latter. This finding strengthened our suggestion, that the IZ2 electrode underestimated the serum ionized calcium, compared with the electrode used in 1976. Hence the reference range for this electrode should be 0.92–1.12 mmol/liter instead of 0.96–1.16 mmol/liter. When these limits were introduced in the regression equation for Figure 1, the limits for the IV1 electrode were calculated to 1.10–1.31 mmol/liter. The mean ± 2 SD limits for the reference group was calculated to be 1.10–1.25 mmol/liter on the same electrode. The lower limit agreed well for these two calculations, but the upper limit should be 1.30 rather than 1.25 mmol/liter because of the higher values for inpatients. One of the persons in our reference group had an ionized calcium value (1.09 mmol/liter) outside this reference range.

Recently three evaluations (1–3) of the Orion SS-20 ionized calcium analyzer have appeared. The dates they were received (October 1976, February 1977, and September 1977) make it likely that their reference range was established with electrodes manufactured in 1976. These reported results agreed well with our findings in 1976. Electrodes produced later may give lower recordings for serum ionized calcium. The composition of the most recent electrodes (expiration date May 1979 or later) is changed (4), resulting in higher recordings for serum ionized calcium.

The electrode potential depends not only on calcium ion activity in the sample, but on other components that partly interfere with the measurements (5), and it is likely that the differences in ionized calcium readings are due to some component(s) in serum. Proteins were suggested to have this effect (5) on early calcium-selective electrodes. A slight correlation between the difference of the ionized calcium readings and albumin could be seen for the reference group, but not for the whole material. Probably the difference is due to several factors in serum.

Because serum ionized calcium measurements are no longer in the research stage and an increasing number of clinical reports will include this variable, the influence of an electrode change should be kept in mind when results from different times are being compared.

References

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The vendor offers the following response:

To the Editor:
Orion agrees with authors Ohman and Larsson that higher ionized calcium values will be observed with calcium sensors received since May 1978. This shift has been documented by in-house and field studies and is of the magnitude they report. Notification to expect higher values has been sent with all ionized calcium sensors shipped since May 1978. In addition, Orion has recommended that the normal range for each laboratory be redetermined. This increase in the observed ionized calcium value is the result of a modification in the sensor, which has increased the precision and sensitivity of the ionized calcium measurement. Reliability of the modified sensors has also been improved. There is no reason to suspect that there will be any further changes in the values obtained with future lots of calcium sensors.

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Azide Interference with Bilirubin Procedures Using Diazotized Sulfanilic Acid (Ehrlich’s Reagent)

To the Editor:
Two episodes in the past 18 months have emphasized to us the seriousness of azide as an interfering substance in bilirubin methods in which diazotized sulfanilic acid (Ehrlich’s reagent) is used. Michaelsson (1) pointed out that azide can be used to convert excess Ehrlich’s reagent to a colorless addition product, but more recent texts (2, 3) fail to discuss the use of azide for this purpose or its potential interference with the method. We use three procedures to quantitate total serum bilirubin in our laboratory: Cross et al.’s (4) modifications of the Jendrassik-Grof method for use with a GEMSAEC centrifugal analyzer for routine samples, the DuPont aca for urgent samples, and Ichida and Nobuoka’s (5) ultramicro modification of Michaelsson’s (1) alkaline azobilirubin blue method for pediatric samples.

We first encountered azide interference about 18 months ago when one day no color would develop in the GEMSAEC bilirubin procedure. The problem was traced to the use of sodium azide-preserved normal saline for washing the samples into the transfer discs on the automatic sample loader. The azide-containing saline, delivered to the laboratory in error, came in containers essentially identical to the non-preserved saline except for some small print at the bottom saying “contains 0.1% sodium azide as preservative.”

In our more recent encounter sodium azide worked its way into our bilirubin assay even more surreptitiously. We standardize our bilirubin procedures using National Bureau of Standards (NBS) bilirubin dissolved in buffered 50 g/liter bovine serum albumin (BSA) as recommended by Cross et al. (4). Each new batch of standard solution is checked against the former batch. Generally there is very good agreement between the weight of bilirubin added and the values determined by all three of our methods. However, a recent batch of standard solution containing 201 mg of NBS bilirubin per liter read 204 by the DuPont aca and 205 by the pediatric procedure, but read variably between 120 and 180 by the GEMSAEC procedure. After considerable consternation, we recalled that in making all former batches of standard solution, crystalline BSA was used, while for the batch in question a 300 g/liter solution of BSA was used as suggested by Cross et al. (4). However, we discovered that the BSA solution we used also contained 1 g of sodium azide per liter, which resulted in 0.17 g of sodium azide per liter in the final standard solution. In the final reagent mixture, 77 nmol of azide from 50 µl of sample was enough to consume