the presence of the very closely related pyrrolic metabolite 2-hydroxyhemo-pyrrole-5-one (HHPO) associated with schizophrenic and porphyric symptomatology. Indeed, the evidence for this metabolite and its clinical relevance has never been stronger. Thus, the metabolite has been synthesized (6) and new analytical techniques have been devised and applied (2, 3, 5), confirming the significant associations between this metabolite and schizophrenic symptoms (7) and hereditary hepatic porphyrias (2, 3). In addition, compounds which are isomeric with the metabolite have been shown to be active inducers of excessive porphyrin formation (8, 9). While tests of the porphyrigenicity of the metabolite itself are not yet complete, such activity can be expected, and would raise the likelihood that the metabolite is involved in the pathogenesis of acute intermittent porphyria (Irvine, unpublished). Consequently, the metabolite exists, and has interesting clinical correlates and a well-defined molecular structure.

There is no longer anything puzzling about the multiple-spotting tendency of this metabolite (10); neither is there any real question about its exact chemical structure (2). Because the metabolite belongs to the hemo series of pyrrole derivatives, it is not going to arise from kryptopyrrole, should perchance any of this pyrrole be produced in the body. While it is still conceivable that HHPO might arise from hemopyrrole as such (conceivably formed at least transitorily in the body) this would be excruciatingly difficult to establish because of the well-documented extreme tendency of hemopyrrole to auto-oxidize. It is much more plausible to suggest that HHPO arises from suitably constituted end-rings of bile pigments (including the urobilinogens). This would provide a pathway to HHPO without any intermediate hemopyrrole.

In summary, then, it is important to recognize that the metabolite once known as “mauve factor” or “natural kryptopyrrole” is specifically 2-hydroxy-4-ethyl-2,3-dimethyl-Δ3-pyrroline-5-one.

References

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Higher Values for Ionized Calcium with a New Type of Electrode for Orion SS-20

To the Editor:
Since 1976 we have used the Orion SS-20 for routine determination of serum ionized calcium. Our reference range was 0.95–1.16 mmol/liter, based on serum samples from 110 inpatients and 30 outpatients without history or symptoms of disturbed calcium metabolism. This spring (1978) we had some trouble with unstable potential, which we could attribute to the electrode batch (I22). Simultaneously, the recorded ionized calcium values seemed to be under-estimated as compared with the reference range. We therefore asked Orion for a new electrode batch, which (IV1) arrived in June. The potential for this electrode was stable, but it gave considerably higher values for serum ionized calcium than I22. Because we have a great many follow-up patients whose treatment depends on results of analyses for ionized calcium, we had to establish the magnitude of this difference. We also had to change our reference range.

During three days, four series of samples were analyzed with both electrodes. The samples consisted of two series of samples sent for routine calcium analysis (n = 32) and two series (reference group, n = 30) of samples from healthy laboratory workers and outpatients with no known disturbance of calcium metabolism. All samples were taken in Vacutainer Tubes (Becton-Dickinson, Inc.) without addition, and serum was aspirated through the stopper for the analysis.

The relationship between the values for ionized calcium in serum for the two electrodes is shown in Figure 1. It is evident that the new electrode gives higher recordings than the old one. The difference between the recordings was 0.178 ± 0.014 (mean ± SEM, throughout) for the reference group and 0.184 ± 0.039 for all pairs of observations. The greater SEM for the latter group could be attributed to one of the routine series containing many sera with pathological values (14/20 beyond the reference range).

Our old reference range took into account that inpatients have a significantly higher (1.067 ± 0.0046) serum ionized calcium than do outpatients (1.039 ± 0.0074)—(t = 3.22, P < 0.005). We assumed that our reference group and our outpatient group were randomly selected:
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 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 Öhman 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 Sulfonic 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 sulfonic 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 standardized our bilirubin procedures by 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 reaction mixture, 77 nmol of azide from 90 μl of sample was enough to consume