variation of 5.7% was obtained at a level of 11 mmol/ml per hour.

The figure shows the levels of erythrocyte uroporphyrinogen I synthase detected in three generations of a family with AIP. The level of activity of the proband (IV.1) was just within the statistical reference range. Her father (III.1) and daughter (V.3) had values for activity that were 43% of the mean value of the reference range. These activities are similar to those in affected subjects in the Scandinavian family described in Peterson et al.

It is clearly important to detect the latent as well as the overt forms of this potentially disabling disease, precipitable by drug therapy, as early as possible in life. I would therefore like to recommend the use of the Peterson method for the detection of AIP, but at the lower substrate concentration of 20 mmol/liter. This lowering of the cost per test should influence its acceptability in many countries.

Reference


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Evaluation of the “Space Stat 20 Ionized Calcium Analyzer”

To the Editor:

The report of Husdan et al. (1) needs some comment concerning the difference between readings for ionized calcium in serum and plasma. We did essentially the same evaluation of the Orion SS-20 ionized calcium analyzer, but we included whole blood in the study. Blood was taken from 30 outpatients called to this laboratory for reasons other than disturbances in calcium metabolism. From each patient, blood was sampled into two Vacutainer Tubes (Becton-Dickinson), one with heparin (green stopper) and one with no additive (red stopper). The specimens were analyzed with the SS-20 within 2 h, by aspirating the sample through the stopper into 1-ml tuberculin syringes and injecting it immediately into the machine. First, whole blood was analyzed after carefully mixing the content in the heparin tube, and then the tube was centrifuged for the same determination in plasma. After centrifuging the other tube, we analyzed serum in the same way.

We can confirm the findings of Husdan et al. (1) that the reading for ionized calcium in heparinized plasma is lower than that for serum (Figure 1), a phenomenon that they attributed to a calcium-complexing action of heparin. However, we found that the reading for ionized calcium in the whole blood from which the plasma was derived was also higher than that of serum (Figure 2). Obviously, the difference cannot be explained only by a calcium-complexing action of heparin. Instead, we believe the differences between values for serum, plasma, and whole blood are caused by differences in liquid-junction potentials between the sample and the 2 mol/liter KCl solution that is pumped through the reference electrode. Such potentials appear when positively and negatively charged ions in the sample have different diffusion coefficients, such as in colloid-containing samples (2, 3). Ladenson and Bowers (4) suggested this theory for the other system for ionized calcium, the Orion 99-20, but Fuchs et al. (5) found no notable difference between plasma and whole blood for the Orion SS-20 system, although their data showed slightly (2%) higher values for whole blood than for plasma. We found this difference to be 0.09 ± 0.04 (SD) mmol/liter.

The liquid-junction potential is included in the constant term in the Nernst equation:

\[ E = \text{const.} + \frac{RTn_i}{F} \ln a_i \]

where \( E \) is the observed potential in volts, \( R \) is the gas constant, \( T \) is the absolute temperature, \( F \) the Faraday number, and \( n_i \) and \( a_i \) are the charge and activity of the analyzed ion i. Small changes (<1 mV) in the liquid-junction potential hence influence the result, especially if the ion is divalent. For example, the mean difference between readings for serum and plasma calcium with the SS-20 was 0.05 mmol/liter, corresponding to a potential shift of only 0.65 mV.

The following brief study was done to see if the whole difference between serum and plasma was attributable to blood components, or if heparin per se had any effect on the ionized calcium reading. Serum was collected in Vacutainer Tubes from six healthy laboratory workers. After clotting and centrifugation, 2-ml aliquots of each serum were transferred to other Vacutainer Tubes with and without heparin. Ionized calcium was determined with the SS-20 after wetting the whole inside of the tubes with the contents. Heparin showed a lowering effect on the ionized calcium readings (mean difference, 0.08 mmol/liter; SD, 0.04 mmol/liter) for all six paired observations. Since 2 ml is only 20–25% of the maximal volume of the tube, heparin should lower the ionized calcium readings by about 0.02 mmol/liter if the tube were completely filled. Hence about 40% of the difference found between serum and plasma can be explained by an effect of heparin. This effect is not necessarily due to a complexing of calcium; because heparin is a highly charged molecule, it may influence the liquid junction as well.

The differences in ionized calcium readings are at least partly due to the proteins and cells in the sample. We therefore suggest that the readings for whole blood and plasma should be adjusted to those obtained for serum—i.e., 0.05 mmol/liter should be added to plasma readings, and 0.05 mmol/liter subtracted from whole-blood readings—as suggested by Ladenson and Bowers (4) for the Orion 99-20 system.

These observations will explain why measurements with ion-selective electrodes (including pH measurements) in samples containing colloids and (or) cells will always show a systematic error, as compared with reference solutions composed only of electrolytes. They may also be a caution to those who intend to use the readings of ionized calcium as an "absolute" measurement of the free calcium fraction. This fact will not dis-
rupt the clinical value of the method (5, 7–9); for this purpose there is no need for “absolute” readings.

References

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