Factitiously High Sodium Activities on the Ektachem 400 Owing to Interferences by High Gamma-Globulin Concentrations

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Sera from patients with above-normal gamma-globulin concentrations give sodium values as much as 10% higher when measured in the Kodak Ektachem 400 analyzer than results obtained with the Beckman Astra-8 analyzer. Results obtained with the Astra were in agreement with flame photometry. A similar relative bias was observed for potassium measurement in the Ektachem. This discrepancy was corrected when we used a new generation of reference fluid provided by Kodak, which is currently available for general use.

Additional Keyphrases: ion-selective electrodes • potentiometry • potassium • albumin • multilayer film analysis • analytical error

Ion-selective electrodes are increasingly used to measure sodium in the clinical laboratory. Indirect potentiometry and flame photometry give similar results for sodium, because both techniques involve dilution of the sample. In contrast, direct potentiometry measures sodium activity directly in plasma or serum without dilution; therefore, in serum with altered water content owing to hyperlipidemia or hyperproteineemia, direct potentiometry is considered a more physiologically accurate indicator of sodium concentration and of the patient's clinical condition (2, 3). In the present case, we report on the effect of increased gammaglobulin concentrations in serum on the direct potentiometric measurement of sodium in the Ektachem 400 analyzer.

Case Report

Patient F. H., a 38-year-old woman with a history of ethanol abuse, was brought to the emergency room by ambulance. She was noted by the pastor of a local mission to have increasing jaundice and pain in the right upper quadrant of her abdomen, with nausea, vomiting, and decreasing mental status. History and physical and laboratory examination supported a diagnosis of ethanol-induced hepatitis, gastritis, and encephalopathy. At admission, laboratory personnel noted that the patient's serum gave widely divergent values for sodium when measured in the Ektachem 400 and the Astra-8: 149 and 138 mmol/L, respectively. Her serum was not hyperlipidemic or hyperproteineemic.

Checking other sera revealed a group of several patients whose serum also gave such discrepant results, some as much as 15 mmol/L higher in the Ektachem. A similar 10% higher result was observed for potassium measurements. However, because of the low concentration of potassium in the extracellular space, the numerical value of the difference was not as noticeable.

Review of these patients' charts revealed that all had ethanol-induced liver disease, with low values for albumin and high values for globulin, common changes in alcoholic liver disease (4). Patient F. H. had a serum albumin concentration of 16 g/L, a total protein concentration of 49 g/L, and a calculated total globulin concentration of 33 g/L. Serum electrophoresis revealed beta-gamma bridging and a gamma-globulin value of 20 g/L. To test the hypothesis that either low albumin or high gamma-globulin concentrations were affecting the sodium activity measured in the Ektachem analyzer, we devised an experiment involving patients' sera with added gamma-globulin and albumin solutions, as described below.

Materials and Methods

Serum sodium was measured in an Astra-8 analyzer (CV 0.6%; Beckman Instruments, Fullerton, CA), an Ektachem 400 multilayer film analyzer (CV 0.8%; Eastman Kodak, Rochester, NY), and an IL 643 flame photometer (CV 0.9%; Instrumentation Laboratory, Lexington, MA), according to the guidelines of the manufacturers. A 220 g/L solution of bovine serum albumin (Immunor, Inc., Norcross, GA) and a 50 g/L solution of gamma-globulin ("Gammagard"; Travalen Laboratories, Lessines, Belgium) were prepared for the experiment. Specimen 1 was serum from patient F. H. Specimen 2 was an aliquot of specimen 1 (865 μL) plus 136 μL of the albumin solution, to bring the concentration of albumin in the specimen to 44 g/L. Specimen 3 was serum from a patient with low concentrations of albumin (31 g/L) and globulin (23 g/L). Specimen 4 was an aliquot of specimen 3 (865 μL) plus 136 μL of the albumin solution, to give a final concentration of albumin in the specimen of 57 g/L. Specimen 5 was an aliquot of specimen 3 (600 μL) plus 400 μL of the gamma-globulin solution, to bring the concentration of globulin in the specimen to 34 g/L. Specimen 6 was serum from a patient with normal concentrations of albumin (41 g/L) and globulin (33 g/L). Specimen 7 was an aliquot of specimen 6 (600 μL) plus 400 μL of the gamma-globulin solution, to bring the globulin concentration in the specimen to 40 g/L. To avoid interference from hydrogen ion, we adjusted the pH of the gamma-globulin and albumin solutions to 7.0. Test solutions (including the gamma-globulin and albumin solutions) were measured with the Ektachem and Astra analyzers and the IL flame photometer. We also remeasured all specimens with the Ektachem, using a new generation of reference fluid currently being developed (generation number 04, provided by Eastman Kodak).
Results

Results of our experiment are shown in Table 1. Addition of albumin to hypoalbuminemic sera (specimens 1, 2, 3, and 4) increased the measured sodium concentration in all methods, because of the high sodium concentration of our albumin solution. However, addition of albumin did not correct the relative positive bias of the Ektachem value seen in specimen 1. Addition of gamma-globulin to hypogammaglobulinemic (specimens 3 and 5) and normal (specimens 6 and 7) sera increased sodium values on the Ektachem, whereas values on the Astra and flame photometer remained essentially unchanged. Use of a reformulated reference fluid in the Ektachem largely corrected the bias seen in the hypogammaglobulinemic specimens.

Discussion

In this study, the measurement of sodium by different analyzers indicated that in sera with high concentrations of gamma-globulin, measured sodium concentrations were as much as 10% higher on the Ektachem than on the Astra and the flame photometer. This discrepancy, initially seen in a patient with high gamma-globulins secondary to alcoholic liver disease, was reproduced in experiments involving added gamma-globulins. Subsequently, serum from a patient with multiple myeloma and monoclonal IgG >4 g/L was found to show a similar bias in sodium measurement. The observed discrepancies do not appear to be due to hypoalbuminemia, because the relative bias was unchanged by the addition of albumin to these specimens.

Although the mechanism for the observed interference remains unknown, several possibilities exist. First, the Ektachem uses direct potentiometry, whereas both the Astra and the flame photometer use dilution methods. Indirect methods are known to be sensitive to protein and lipid content, and theoretically may give values ~7% less than direct methods do (1–3). In practice, however, direct methods have traditionally been adjusted to give values similar to indirect methods in the normal range. Furthermore, the total protein content in many of our specimens was normal.

The second possible mechanism, an interferent solely affecting the Ektachem method, was also unlikely. There are few sodium interferents in ion-specific direct potentiometry, and most result in only small biases. The only recognized interferent causing a significant positive bias in the Ektachem was benzalkonium chloride, a surfactant used in some topical disinfectants (5), but this was not used in obtaining the specimens for the present study.

The most likely mechanism involves an abnormal junction potential generated between hyperglobulinemic specimens and the reference fluid in the Ektachem. Although the junction potential is minimized by proper formulation of the reference fluid, samples with abnormal ionic composition can produce significant junction potentials (6). Specifically, specimens with unusually large anion gaps reportedly give negatively biased sodium results. In our experience, positive sodium bias is seen in specimens with low anion gaps, including those from the patients in this study. Perhaps the altered ionic composition in the hypogammaglobulinemic specimens created a significant junction potential leading to factiously high sodium values in these specimens. This possibility was supported by the observation of a similar bias in potassium values in the Ektachem (results not shown). Using the same reference fluid, we found that potassium values were high by the same percentage as sodium values were in the hypogammaglobulinemic specimens. The correction of both sodium and potassium discrepancies by use of a reformulated reference fluid strongly suggests an analytic variable related to the reference fluid, such as the junction potential.

Because the Ektachem analyzer is now in widespread use in clinical laboratories, especially in situations where laboratories utilize more than one type of analyzer for sodium determinations, unusual serum samples may show confusing variations in sodium concentrations when assayed with different analyzers. It was reassuring that the new generation of reference fluid appeared to correct this problem. We believe this case report reinforces the need for quality control between different methodologies for the same analyte.

References