Direct Potentiometric Measurement of Sodium and Potassium in Whole Blood

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I compared results for sodium and potassium in whole blood and plasma as measured with a newly available potentiometric analyzer, the "Orion SS-30." No significant difference was found for either sodium or potassium in 207 such comparisons. With use of the flowing, high mixing-velocity liquid junction of the Orion SS-30, the residual liquid junction potential due to blood cells was found to be less than 0.1 mV and to be independent of the hematocrit. This is in contrast to the hematocrit-dependent residual liquid junction potential of ~0.6 mV noted by others at normal hematocrit values with the open capillary liquid junctions now commonly used in pH instruments. I also found that the potassium concentration can increase significantly during the mixing of whole blood, and such samples should be mixed gently, if at all. Evidently sodium and potassium can be accurately and easily measured directly in heparinized blood.

Additional Keyphrases: electrolytes · Orion SS-30 analyzer · effects of sample handling · effect of blood cells on liquid-junction potentials · whole-blood/plasma comparisons

Rapid analysis for sodium and potassium has become important for proper patient management in many situations, but currently is slowed because the plasma must be separated from the cells before analysis by flame photometry. An expected advantage of using ion-sensitive electrodes directly, without sample dilution, is that whole blood might be used directly to measure these electrolytes in plasma water.

There are no extensive published data on possible differences in sodium or potassium values between whole blood and plasma. For data on blood from fewer than 10 subjects, no difference was found between whole blood and plasma for either sodium (1–3) or potassium (2, 3). Indirect comparison of results by direct potentiometry for whole blood and flame photometry for plasma also suggested that results for whole blood and plasma were essentially the same for sodium or potassium (4).

Instruments based on direct potentiometry are commonly used for measuring the pH of whole blood, and the results reportedly are ~0.01 unit lower than those for plasma pH (5–7). This difference has been attributed to a change in liquid junction potential when cells are present. The effects of blood cells on the liquid junction potential are a function of the composition and temperature of the salt bridge and the hematocrit of the sample when an open capillary liquid junction is used (6, 7). These effects of blood cells may depend on instrument design, since they can be minimized by using a restricted flow liquid junction (8).

Recently, the first commercial instrument capable of the routine determination of sodium and potassium by direct potentiometry has been introduced. The relationship of results for plasma by direct potentiometry and flame photometry are reported elsewhere (9). Here, I describe the results of comparing whole blood and plasma in order to assess the applicability of direct potentiometry for whole blood analysis.

Materials and Methods

Direct potentiometric analysis (without sample dilution) for sodium and potassium were performed with a Model SS-30 sodium/potassium analyzer (Orion Biomedical, Cambridge, Mass. 02139). Heparinized whole blood or plasma was used for all determinations. Hematocrits were measured with a "Readacrit" centrifuge (Clay-Adams, Parsippany, N.J. 07054). The samples used for the comparison of whole blood and plasma were all samples for which first-priority ("stat") blood-gas or electrolyte analyses were requested at Barnes Hospital. These samples were stored at room temperature before the comparison studies, to prevent large changes in potassium concentration in the cells.

Four different methods were used to prepare samples for the comparison of values in whole blood or plasma:

Method 1A: Plasma obtained by sedimentation after removal of whole blood. Heparinized arterial samples in a syringe were mixed by hand rotation and sodium and potassium measured on the whole blood sample. The samples were then allowed to sediment with the
plunger down. After 30 min, a plasma sample was removed by means of a 1-ml syringe with a blunt needle and analyzed for sodium and potassium. The plasma sample had a hematocrit of less than 1% in all cases.

**Method 1B: Plasma removed by centrifugation after removal of whole blood.** Venous blood samples were remixed by removing after removing the plasma separator (“Sure-Sep”; General Diagnostics, Morris Plains, N.J. 07590) and transferred to a new plain evacuated blood-collection tube. A specimen of whole blood was removed via a needle-less 1-ml syringe and then the remaining sample was centrifuged at 2000 × g for 10 min and a plasma sample removed. The plasma and whole blood samples were then analyzed in random order.

**Method 2A: Plasma obtained by sedimentation before removal of whole blood.** This method was similar to method 1A except that the sample was mixed, allowed to settle for 30 min, the plasma removed, and then the syringe contents were remixed for the whole-blood specimen. The order of analysis of the plasma and whole blood samples was randomized.

**Method 2B: Plasma obtained by centrifugation before removal of whole blood.** This was similar to method 1B except that the whole blood sample was obtained by remixing the sample after removal of the plasma.

Methods 1A and 1B, therefore, represent plasma separated by sedimentation or centrifugation, respectively, after the whole-blood sample was removed. Methods 2A and 2B represent similar procedures, except that the samples were remixed and the whole-blood sample obtained after, rather than before, the plasma sample. Hematocrits were measured for the whole blood samples with methods 1B, 2A, and 2B. The effects of blood cells were also assessed by repeatedly processing specimens by method 1B in order to have progressively increasing hematocrit values.

The data were evaluated in terms of the difference in measured potential between whole blood and plasma as well as the actual values obtained. By the Nernst equation, the measured potential for either sodium or potassium is given by the equation:

$$ E = E^0 - \frac{RT}{F} \ln A $$

where $E =$ the measured potential, $E^0 =$ the standard potential, $R =$ the gas constant, $F =$ Faraday's constant, $T =$ the temperature (K), and $A =$ the activity of the monovalent cation.

On converting from ln to log, using the measurement temperature of 37 °C (310 K), and substituting for the constants, the difference in potential between whole blood and plasma is given by the equation:

$$ \Delta E \text{ (volts)} = 0.0615 \log \left( \frac{\text{whole blood value}}{\text{plasma value}} \right) $$

The difference in measured potential between whole blood and plasma is therefore a direct function of the logarithm of the result for whole blood/result for plasma. This ratio was used to investigate the residual liquid junction potential attributable to the presence of blood cells.

**Results**

The results for sodium in whole blood and plasma are shown in Figure 1, for potassium in Figure 2. The results for these in whole blood were not significantly different.
than those for plasma; the correlation between the two samples was 0.958 for sodium and 0.984 for potassium.

Table 1 shows the ratio of values for whole blood/plasma (W/P) for samples in which the plasma was separated after the whole blood (methods 1A and 1B), as compared to samples in which the plasma was separated before the whole blood (methods 2A and 2B). The results indicate that when the sample is remixed to obtain the whole blood sample, the W/P for potassium is significantly higher than the W/P for sodium. When the plasma is separated after removal of the whole blood sample, the W/P for sodium and potassium are not significantly different. In addition the W/P for sodium and potassium are significantly correlated (r = 0.380, P = .001) when the plasma was obtained after the whole blood but were not correlated when the plasma was obtained before the whole blood. Thus, the method of preparing whole blood and plasma samples influences the results for potassium when whole blood and plasma are compared.

The differences in potassium results depending on the method used to prepare the whole blood and plasma samples could be due to several factors. The most likely are: (a) an increase in the measured potassium of the whole blood sample due to potassium release from cells during analysis, (b) a change in the residual liquid junction potential due to the presence of blood cells, or (c) an increase in potassium due to the technique used to mix the blood, or some combination of these. A difference in the patient populations studied with the different sample preparation techniques is another but very doubtful possibility, since the patients were randomly selected and many of the samples examined by methods 1B and 2B were from the same patients.

The first two possibilities were shown to be unlikely on the basis of additional experiments. Samples were repeatedly processed by method 1B in order to obtain higher hematocrit values. As seen in Figure 3, there was no effect of an increase in blood cells on the W/P for sodium. For potassium (Figure 4) only the three samples with hematocrits greater than 70 had a W/P that significantly differed from those with hematocrits of less than 40. These results make unlikely an effect of blood cells on the residual liquid junction potential when the flowing, high-velocity, liquid junction of the Orion SS-30 is used, and are in contrast to the effect of blood cells on pH measured with open capillary liquid junctions (6, 7).

I obtained evidence that values for potassium in whole blood sample due to potassium release from cells during analysis, (b) a change in the residual liquid junction potential due to the presence of blood cells, or (c) an increase in potassium due to the technique used to mix the blood, or some combination of these. A difference in the patient populations studied with the different sample preparation techniques is another but very doubtful possibility, since the patients were randomly selected and many of the samples examined by methods 1B and 2B were from the same patients.

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whole blood depend on the manner in which the blood is mixed. Nineteen samples were repeatedly centrifuged, remixed, the whole blood and plasma values were measured, and the W/P ratio was calculated for each whole blood–plasma pair. When the whole blood samples were measured before centrifugation (method 1B), I found no significant difference in W/P for sodium or potassium in the samples with higher hematocrits as compared to the first sample. Also, the W/P ratios for sodium and potassium were not significantly different. However, when the whole blood sample was obtained by remixing after centrifugation (method 2B) the samples with higher hematocrits showed an increase in W/P for potassium (1.060 ± 0.0467) as compared to the first sample (1.01 ± 0.0155). This effect of remixing was not observed for sodium. The plasma potassium values were also found to increase progressively as the samples were repeatedly processed.

The effects of mixing were also directly assessed. A full tube of blood was gently mixed, and a whole blood sample removed and analyzed. The tube was then vigorously inverted 10 times, and another whole blood sample removed and analyzed. This was then repeated until no sample remained. In the four specimens tested, the potassium values progressively increased with mean respective changes of +0.4, +0.9, and +2.1% of the initial value. For sodium the corresponding changes were only +0.4, +0.1, and +0.2%. In another experiment, whole blood was removed, the samples were centrifuged, and then the separated plasma was remixed with the cells. In 10 specimens, the potassium was 2.7% higher in the remixed whole-blood specimens, while the sodium results were only 0.3% higher. Finally, nine samples of blood in syringes were gently mixed and the whole blood sodium and potassium measured. The syringe was then one-third filled with air, mixed by inversion for 45 s, and the sample remeasured. The potassium increased by 1.9%, the sodium by only 0.5%. Evidently the sample handling used in obtaining whole blood can significantly influence the results for whole blood potassium and their comparison with results for plasma. The data indicate the need for gentle mixing of blood in order to avoid spuriously elevated potassium values.

Discussion

No significant differences between sodium and potassium measured in whole blood or plasma were observed (Figures 1 and 2). However, when the data were expressed as the ratio of the value obtained with whole blood to that obtained with plasma (W/P), it is apparent that the method used in preparing the whole blood and plasma samples can influence the results. The W/P for potassium increased when the samples were remixed after the plasma was separated and this increase was greater when the plasma was separated by centrifugation than when it was separated by sedimentation. The increase in W/P for potassium was associated with a small decrease in the W/P for sodium (Table 1). This could be due to actual cellular damage or to a break-down in sodium–potassium exchange. The samples were not visibly hemolyzed. These observations suggest that samples should be gently, not vigorously, mixed—if indeed they are mixed at all—before analyzing whole blood for potassium.

Evidently the presence of blood cells has little effect on the results when the flowing, high-velocity liquid junction of the Orion SS-30 is used. The W/P ratios for sodium and potassium were identical, although only that for sodium was significantly less than 1 (0.9971 ± 0.0097). The W/P for potassium (0.9976 ± 0.0220) was probably not significantly less than 1 due to the larger standard deviation observed. With use of equation 2, the W/P for sodium indicates a residual liquid junction potential of less than 0.1 mV. These results are in keeping with the lack of difference in free calcium concentration between whole blood and plasma found by Fuchs et al. (10), who used a liquid junction of the same design. In earlier work comparing pH values in whole blood and plasma a lower pH value was noted for whole blood than for plasma (5–7). The difference between whole blood and plasma pH was greater at higher hematocrit values. The 0.01pH difference at a pH of 7.40 and a normal hematocrit reported by Maas (7) corresponds to a W/P of 1.023 and a residual liquid junction potential of 0.6 mV. DeRaedt et al. (8) showed that the junction design could greatly influence the relationship of whole blood and plasma pH. They found that an open liquid junction resulted in a lower pH value for whole blood than did a restricted liquid junction. They also observed that the difference between whole blood pH measured with an open liquid junction compared to a restricted liquid junction was larger with increasing hematocrits. Apparently the design of the liquid junction can significantly affect the residual liquid junction potential, which presumably arises due to creation of cells and (or) precipitation of proteins at the junction of sample and KCl. The flowing, high mixing-velocity liquid junction I used in these studies is only minimally affected by the presence of blood cells and so presumably would be superior to the commonly used open capillary liquid junctions for whole blood pH measurements.

Another possible source of error in the measurement of whole blood potassium could be release of potassium from cells during analysis before the sample reaches the potassium electrode. The data shown here indicate that such potassium release is quite small, if it occurs at all, since increasing the hematocrit had little effect on the results. I have not knowingly compared potassium values in whole blood and plasma from patients with very fragile peripheral blood cells, such as in cases of hereditary spherocytosis or in leukemia after chemotherapy, and cannot exclude a problem with whole-blood analysis of such patients, but such a problem is unlikely, because the stress of the analysis cycle is probably not greater than that of centrifugation. Samples with leucocyte counts exceeding 50 000/mm³ did not show any differences from other samples, so high proportions of leucocytes per se have no effect.
It appears that the expected advantages of direct potentiometry for the measurement of electrolytes in whole blood can be realized in an instrument designed for the routine laboratory. The lack of effect of protein and lipids on direct potentiometric measurements of sodium and potassium (9), and the capability for directly measuring these in whole blood make this approach faster and probably more accurate than flame photometry. Potentiometric analysis of sodium and potassium performed directly on whole blood specimens appears to be more rapid and accurate than potentiometric or flame photometric analysis on diluted plasma specimens.

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References