A Modified Indirect Method for Determining Erythrocyte Sodium and Potassium Concentrations

Larry L. Small¹ and Dwight B. Coulter²

An automated method was adapted to measure the concentration of Na and K in plasma, nonhemolyzed whole blood, and hemolyzed whole blood, and thus allow the calculation of erythrocyte electrolyte concentrations by a modified indirect method. The Na concentrations of nonhemolyzed whole blood and plasma were used to calculate the percent cell volume (hematocrit) of a blood sample. The percent cell volume and concentrations of Na and K in nonhemolyzed whole blood were used to modify the indirect method of calculating erythrocyte Na and K concentrations in canine, porcine, and human blood samples. Significant differences were found between the two indirect methods (indirect and modified indirect) used to calculate erythrocyte Na and K concentrations of human blood samples.

Additional Keyphrases: packed cell volume • hematocrit • erythrocyte electrolytes • trapped plasma • dialysis • AutoAnalyzer • data from pigs, dogs • Na cell volume

Determination of PCV³ produces a major error when calculating erythrocyte (RBC) electrolyte concentration by the indirect method (1). McCance and Widdowson (2) stated that because plasma becomes trapped among the RBC’s, it is impossible complete-ly to separate the cells from the plasma by one single operation of centrifugation. Beilin et al. (3) demonstrated that an error of 1% in the PCV estimation in a human blood sample may create a 20% error when the RBC Na concentration is calculated by the indirect method. The volume of plasma trapped among the RBC’s of a centrifuged blood sample depends on the amount (4, 5) and such physical properties of the RBC’s as size (4) and flexibility of the RBC membranes (6). Therefore, a common correction factor for trapped plasma would be inappropriate for individual animals within a species. The percentage of trapped plasma should be determined for each sample being analyzed (7). Many different plasma markers have been used to measure the percentage of trapped plasma, but discrepancies exist among the different markers being used (3, 7, 8–10).

Our objective was to eliminate the error associated with the PCV determinations, and thus increase the accuracy of the indirect method of calculating RBC Na and K concentrations. Assuming that the RBC Na and K ions are bound to or contained within the RBC and not readily diffusible, we dialyzed nonhemolyzed whole-blood samples diluted in an isotonic stream of LiCl and measured the diffusible Na and K (that amount of diffusing out of plasma that contained cells). The ratio of Na concentration in nonhemolyzed whole blood to Na concentration in plasma was used to calculate the percent cell volume of each blood sample, designated here as the “Na cell volume” (analogous to the micro-hematocrit). The appropriate electrolyte concentrations (Na or K) in hemolyzed whole blood and nonhemolyzed whole blood were used with the Na cell volume to modify the indirect method of calculating RBC electrolyte concentration.

Methods

Apparatus

Na and K were analyzed in duplicate by automated⁴ flame photometry (11). The Na and K manifold

⁴Technicon’s AutoAnalyzer method file N-20b; Technicon Corp., Tarrytown, N.Y. 10591.
was modified to accommodate the measurement of Na and K concentrations in plasma, nonhemolyzed whole blood, and hemolyzed whole blood. Two double mixing coils were added to the manifold as shown (Figure 1) to ensure proper mixing.

Reagents

Isotonic lithium chloride (LiCl, 6.4 g/liter) was used as an internal standard. Isotonic calcium chloride (CaCl₂, 11.2 g/liter) was used as the between-sample wash solution to prevent hemolysis of whole blood samples. The Na and K standards (Table 1) were prepared from NaCl and KCl solutions and treated in the same manner as the sample being analyzed.

Procedures

Venous blood samples (12 ml) were drawn from 30 mongrel dogs (4.5–18.2 kg body weight), 32 Landrace-Yorkshire-Poland China crossbred pigs (6.8–14.3 kg), and 21 adult white humans (46.6–98.3 kg) of both sexes. Each blood sample was placed in a test tube containing 0.1 ml of a solution of sodium heparin (1000 units per milliliter), and thoroughly mixed. Each blood sample was then divided equally into two smaller test tubes, one being centrifuged and the clear plasma removed while the other was gently mixed at room temperature until the whole blood analyses could be performed (1 h). Nonhemolyzed whole blood samples were placed in the sample tray one at a time just before sampling (during wash) to ensure that the cells were in suspension.

To determine Na and K concentrations in plasma and nonhemolyzed whole blood, we pumped isotonic LiCl through reagent lines 2 and 4 (Figure 1). Na and K concentrations in hemolyzed whole blood were determined differently, depending on the species being sampled. Canine whole blood samples were hemolyzed by pumping de-ionized water through reagent line 2 (Figure 1). Isotonicity was restored before dialysis by pumping hypertonic LiCl (12.8 g/liter) through reagent line 4. Canine whole blood samples were hemolyzed in this manner to minimize release of the high amounts of K present in leukocytes (10, 12). Because the RBC’s of pig and man have relatively high K and low Na concentrations (the opposite of canine RBC’s), their Na and K concentrations in hemolyzed whole blood were measured from a protein-free filtrate to ensure release of total RBC Na and K (8). The protein-free samples were prepared by diluting whole blood (10-fold) with trichloroacetic acid (50 g/liter) and collecting the supernatant fluid after centrifugation.

PCV’s were determined in triplicate by the microhematocrit method and the two closest values recorded. Blood-filled capillary tubes were plugged with clay, centrifuged in a micro-hematocrit centrifuge (Micro-hematocrit centrifuge Model MB; International Equipment Co., Needham Heights, Mass. 02192) for 6 min, and then the hematocrit was measured over a fluorescent light. The packed volume of erythrocytes and the total PCV (includes the white blood cells) were calculated. The packed white cell volume was calculated by subtracting the packed red cell volume from the total PCV.

We used the following equations (see footnote 3) to calculate the Na cell volume and RBC Na and K concentrations by both the indirect and modified indirect methods:

\[
\text{sodium cell volume, } \% \\
\text{NaCV} = 100 - \left[ (\text{NHWBNa} \div \text{PINa}) \times 100 \right]
\]

indirect method, mmol/liter of RBC’s

\[
\text{RBCe} = \left( \text{HWBe} - \left[ (100 - \text{tPCV}) \div \text{PrCV} \right] \times 100 \right) \div \text{PrCV}
\]

modified indirect method, mmol/liter of RBC’s

\[
\text{RBCe} = \left( \text{HWBe} - \text{HWBe} \div \text{NaCV} \div \text{PrCV} \right)
\]

When nonhemolyzed whole blood Na and K concentration is measured, it is readily evident that erroneous results may be obtained from the cellular efflux of Na or K. To determine whether RBC Na and

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**Table 1. Na and K Standards**

<table>
<thead>
<tr>
<th>Blood component analyzed</th>
<th>Na ( \text{mmol/liter} )</th>
<th>K ( \text{mmol/liter} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>130–160</td>
<td>2–8</td>
</tr>
<tr>
<td>NHWB</td>
<td>70–100</td>
<td>2–5</td>
</tr>
<tr>
<td>HWB</td>
<td>100–130</td>
<td>2–8</td>
</tr>
<tr>
<td>Porcine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>130–160</td>
<td>2–8</td>
</tr>
<tr>
<td>NHWB</td>
<td>70–100</td>
<td>2–5</td>
</tr>
<tr>
<td>HWB</td>
<td>70–100</td>
<td>40–70</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>130–160</td>
<td>2–8</td>
</tr>
<tr>
<td>NHWB</td>
<td>70–100</td>
<td>2–5</td>
</tr>
<tr>
<td>HWB</td>
<td>70–100</td>
<td>40–70</td>
</tr>
</tbody>
</table>

*Four standards for each blood variable measured.

Pl, NHWB, and HWB indicate plasma, nonhemolyzed whole blood, and hemolyzed whole blood, respectively.*
K efflux could cause a significant error, we measured NHWB K concentrations by flame photometry and calculated by the following equation:

\[
\text{NHWB} K = \text{PI}(\text{NHWB}\text{Na} / \text{PI}\text{Na})
\]

**Results**

We determined means and standard deviations for the measured and calculated blood variable concentrations used to determine RBC Na and K concentrations, and made statistical comparisons (Table 2).

The reproducibilities of the two methods (sodium cell volume and micro-hematocrit) used to estimate the percent cell volume (hematocrit) of a blood sample were determined, and means and standard deviation calculated for the differences between duplicate Na cell volume (0.4 ± 0.3) and micro-hematocrit (0.3 ± 0.2) determinations. No significant differences, by Student's t-test, were found when reproducibilities for sodium cell volume and micro-hematocrit were compared.

Linear regressions calculated for Na cell volume vs. micro-hematocrit determinations are shown in Table 3.

### Table 2. Measured and Calculated Blood Variable Concentrations (Means ±SD) Used to Calculate RBC Na and K Concentrations

<table>
<thead>
<tr>
<th>Blood component</th>
<th>Species</th>
<th>Dog (30)</th>
<th>Pig (32)</th>
<th>Human (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmol/liter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI Na</td>
<td></td>
<td>146.4 ± 2.5</td>
<td>142.7 ± 2.6</td>
<td>141.5 ± 1.4</td>
</tr>
<tr>
<td>NHWB Na</td>
<td></td>
<td>63.9 ± 7.9</td>
<td>93.2 ± 3.8</td>
<td>83.0 ± 5.0</td>
</tr>
<tr>
<td>HBO Na</td>
<td></td>
<td>130.2 ± 3.4</td>
<td>95.2 ± 4.2</td>
<td>85.2 ± 5.0</td>
</tr>
<tr>
<td>RBC Na</td>
<td></td>
<td>112.6 ± 5.8</td>
<td>7.8 ± 5.1</td>
<td>16.0 ± 3.6</td>
</tr>
<tr>
<td>RBC K</td>
<td></td>
<td>110.6 ± 6.1</td>
<td>5.9 ± 4.1</td>
<td>5.5 ± 3.0</td>
</tr>
<tr>
<td>PI K</td>
<td></td>
<td>4.1 ± 0.3</td>
<td>5.0 ± 0.5</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>NHWB K</td>
<td></td>
<td>2.4 ± 0.3</td>
<td>3.6 ± 0.5</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>HBO K</td>
<td></td>
<td>5.3 ± 0.4</td>
<td>45.6 ± 2.8</td>
<td>45.0 ± 4.3</td>
</tr>
<tr>
<td>RBC K'</td>
<td></td>
<td>6.9 ± 1.0</td>
<td>124.2 ± 4.6</td>
<td>97.4 ± 4.2</td>
</tr>
<tr>
<td>RBC K&quot;</td>
<td></td>
<td>6.9 ± 1.0</td>
<td>125.9 ± 4.1</td>
<td>104.0 ± 4.6</td>
</tr>
</tbody>
</table>

#### Percent

<table>
<thead>
<tr>
<th>Species vs.</th>
<th>Dog (30)</th>
<th>Pig (32)</th>
<th>Human (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCV vs. tPCV</td>
<td>0.001</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>RBC Na vs. RBC Na</td>
<td>NS</td>
<td>NS</td>
<td>0.001</td>
</tr>
<tr>
<td>RBC K' vs. RBC K&quot;</td>
<td>NS</td>
<td>NS</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Significant differences (probabilities less than 0.001)

<table>
<thead>
<tr>
<th>PI, NHWB, and HBO indicate plasma, nonhemolyzed whole blood and hemolyzed whole blood, respectively. NaCV, tPCV, PrCV, and PWCV indicate sodium cell volume, total packed cell volume, packed red cell volume, and packed white cell volume, respectively. Differences between paired analyses were used in Student's t-test for NaCV vs. tPCV. The remaining comparisons were computed on the differences between concentration means ±SD.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Indirect method.</td>
</tr>
<tr>
<td>b Modified indirect method.</td>
</tr>
<tr>
<td>c No. animals sampled shown in parentheses.</td>
</tr>
</tbody>
</table>

### Table 3. Linear Regression Equations for the Sodium Cell Volume (NaCV) vs. the Total Packed Cell Volume (tPCV) for Each Species

<table>
<thead>
<tr>
<th>Species</th>
<th>NaCV Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog (30)</td>
<td>NaCV = −0.71 + 0.969 (tPCV)</td>
</tr>
<tr>
<td>Pig (32)</td>
<td>NaCV = 3.23 + 0.896 (tPCV)</td>
</tr>
<tr>
<td>Human (21)</td>
<td>NaCV = −2.01 + 0.968 (tPCV)</td>
</tr>
</tbody>
</table>

The reproducibility of the modified indirect method for determining the concentration of Na and K in RBC's of dog, pig, and man were determined for each species (Table 4). For each species, we found no significant differences by Student's t-test, between calculated and observed concentrations of K in nonhemolyzed whole blood.

**Discussion**

The concept of calculating the percent cell volume of a blood sample from the Na concentrations of nonhemolyzed whole blood and plasma is based on the fact that in whole blood the Na is in two compartments, cells and plasma. Cellular Na is bound to or contained within the cells and does not readily permeate the cell membrane in an isotonic solution, whereas plasma Na is in the ionized form and is readily diffusible. Therefore, when nonhemolyzed whole blood is carried through the dialyzer by an isotonic solution of LiCl and the diffusible Na measured, one is actually measuring the plasma Na concentration, uncorrected for dilution with cells. Studies in which cation-exchange resin was used with canine, porcine, and human RBC's (13-16) support the assumption that efflux of either Na or K through the RBC membrane (while in an isotonic solution for less than 3 min) would not significantly alter the accuracy of Na or K measurements in nonhemolyzed whole blood. To ensure that such efflux was insignificant for our purpose, we both measured and calculated nonhemolyzed whole blood K concentrations. The accuracy of the calculated K concentration in nonhemolyzed whole blood depends on the exactness with which Na and K are measured in plasma and nonhemolyzed whole blood. No significant differences (t-test) were found between the means (±SD) of the

### Table 4. Reproducibility of the Modified Indirect Method for Determining Na and K Concentrations in Erythrocytes

<table>
<thead>
<tr>
<th>Species</th>
<th>Na ±SD of RBC's</th>
<th>K ±SD of RBC's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog (30)</td>
<td>2.0 ± 1.6</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Pig (32)</td>
<td>1.2 ± 1.1</td>
<td>2.1 ± 1.4</td>
</tr>
<tr>
<td>Human (21)</td>
<td>2.0 ± 1.8</td>
<td>1.3 ± 1.2</td>
</tr>
</tbody>
</table>

No. animals sampled shown in parentheses.

Means ± SD's of the difference between duplicate electrolyte determinations.
measured and calculated K concentrations in nonhemolyzed whole blood. Evidently, no significant error results from the exchange of either Na or K between cells and plasma during the measurement of Na and K concentrations in nonhemolyzed whole blood of dog, pig, or man. Some error could have resulted from ionic exchange between cells and plasma during the 1-h mixing. Cation-exchange studies (6, 17) imply that this would not be a considerable factor. However, it is advisable to cool the sample and minimize the mixing time.

Beilin et al. (3) stated that when calculating RBC Na and K concentrations by the indirect method, a small error in the estimation of the PCV may cause a large error in the calculated RBC electrolyte concentration. When the PCV of a blood sample is determined, plasma becomes trapped among the cells, which results in an overestimate of PCV. The volume of trapped plasma present in the PCV largely depends on the percentage of cells present: the greater the PCV, the greater the volume of trapped plasma (6, 18). Because the estimate of Na cell volume is not affected by the trapped plasma present, one would expect the difference between the total PCV and the sodium cell volume to increase as the total PCV increases. This phenomenon was demonstrated by calculating the linear regression equation for the total PCV (x-axis) vs. Na cell volume (y-axis) for each species (Table 3). Values for regression slope were less than 1.000 in all three species, indicating that as total PCV increases, the difference between Na cell volume and total PCV also increases. Occasionally, especially when swine blood was being analyzed, Na cell volume would be greater than total PCV. This may be explained by previous investigations (6, 18), which illustrate that fresh blood cells are extremely flexible and can be distorted by a weak centrifugal force, which in turn decreases the amount of plasma trapped by the cells. To obtain a Na cell volume greater than the total PCV, the cells must decrease in volume; consequently, the error associated with the force and time of centrifugation must be greater than the error associated with trapped plasma. Anderson (19), using radio-iodinated human serum albumin as a plasma marker, found little correlation between the volume of trapped plasma and PCV of swine blood.

The Na cell volume is an estimation of the total percent cell volume of a blood sample. To estimate the percent red cell volume of a blood sample, one must correct for the percent white cells present in a blood sample. The packed white cell volume is estimated by subtracting the packed red cell volume from the total PCV. Because packed white cell volume is usually small (less than 1%, Table 2) the error resulting from the volume of trapped plasma in the "buffy-coat" layer is insignificant.

By Student's t-test analysis, results for the two methods (indirect method vs. modified indirect method) used to calculate the RBC Na and K concentrations of canine, porcine, and human blood samples (Table 2) were significantly different only for RBC Na and K concentrations in the human blood samples. The RBC Na concentrations determined by the modified indirect method, which suffers no error caused by trapped plasma, were lower than those determined by the indirect method (Table 2). Beilin et al. (3) reported that (with human blood samples) correcting for the volume of trapped plasma causes a decrease in the RBC Na concentration as determined by the indirect method. These findings indicated that the error associated with the indirect method of determining RBC Na and K concentrations (Table 2) in dog and pig only slightly decreases the accuracy of the results. Although there were no statistically significant differences between the two methods used to calculate the RBC Na and K concentrations in dog and pig, it may be advisable to use the modified indirect method for all species.

A significant error may result from individual variations in the rigidity of RBC's within a species, causing results for RBC electrolyte to be erroneous by the indirect method. Although errors caused by centrifugation were eliminated by the modified indirect method, an extreme value for plasma osmotic pressure could possibly result in errors because of the dialysis step. This type of error was minimized by the dilution with isotonic LiCl before dialysis.

Errors in the calculated RBC Na and K concentrations resulting from Na and K from platelets and leukocytes in hemolyzed whole blood were not evaluated. The errors would be the same in the indirect and modified indirect methods.

The RBC Na and K concentrations found by the modified indirect method (Table 2) are similar to those reported by Coulter et al. (20), Cividalli and Loker (21), Duggan et al. (22), McCance and Widowson (2), Spurr and Barlow (17), and Valberg et al. (23). The Na and K concentrations reported here for canine and porcine RBC's differ somewhat from those values reported by Coldman and Good (9), who measured RBC Na and K concentrations by the indirect method and used the macro-hematocrit method to estimate the PCV of the blood samples. Chien et al. (4) reported that the error caused by the trapped plasma volume in the macro-hematocrit method of determining PCV was greater than the error caused by trapped plasma present in the micro-hematocrit method. Whether differences among normal RBC electrolyte values from different investigators are the result of methodology or genetics is not known.

From this study we conclude that the modified indirect method of determining RBC Na and K concentrations minimizes the major errors associated with the indirect method. We found significant differences when we compared the two methods used to determine Na and K concentrations in human RBC's. Because of the speed, accuracy, and simplicity of the modified indirect method—e.g., extraneous materials such as radionuclides and dyes are not required to
eliminate trapped plasma errors associated with PCV
determination—the modified indirect method can be
adapted for use by most research and clinical lab-
oration.

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the National Heart Institute, NIH, Bethesda, Md.

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