Simpler Flame Photometric Determination of Erythrocyte Sodium and Potassium: The Reference Range for Apparently Healthy Adults

Karl D. Fortes Mayer and Bryan J. Starkey

Separated erythrocytes are washed repeatedly with iso-osmolar magnesium chloride solution, lysed by adding saponin, and sodium and potassium measured in the diluted hemolysate by flame photometry. The coefficient of variation for the method was <4%. Reference intervals determined for a healthy population and hospitalized (elective surgery) patients without electrolyte disorders were 4.6–7.8 mmol/liter for erythrocyte sodium concentration and 94–110 mmol/liter for erythrocyte potassium concentration (2.5–97.5 percentiles).

Estimation of serum or plasma electrolyte concentrations is a well-established clinical tool for the investigation of electrolyte disorders. However, the plasma electrolyte concentration may not reflect the electrolyte status of the intracellular compartment. Whole-body isotope studies have shown that considerable total-body electrolyte deficits may exist in the presence of normal serum electrolyte concentrations (1, 2). The intracellular electrolyte composition of various cell types has been studied (3). Although the concentration in any one cell type may not reflect the absolute concentration in any other, changes in the electrolyte content within that cell type may parallel those within other cell types and provide useful clinical information.

The most accessible cells for investigation are the erythrocytes. Although these are atypical cells, having no nuclei and an internal environment that alters because of their role in oxygen and carbon dioxide transport, recent studies show that knowledge of electrolyte concentrations in erythrocytes is useful in such conditions as nonrespiratory alkalosis (4–6), uremia (7), marasmus (8), hyperthyroidism (9), and hyperaldosteronism (10), and in patients treated with thiazide diuretics (11) or digoxin (5, 12, 13). Most published methods however, have not been simple enough for routine use. This paper describes a method that is relatively simple, reliable, and suitable for use in the routine laboratory.

Materials

Unless otherwise stated, all reagents were of analytical grade and were purchased from B.D.H. Chemicals Limited, Poole, Dorset, England.

Magnesium chloride (112 mmol/liter). Dissolve 22.77 g of MgCl2·6H2O in distilled water. Dilute to 1 liter.

Lithium nitrate diluent, 15 mmol/liter. Dissolve 1.305 g of LiNO3·H2O in distilled water. Dilute to 1 liter.

Saponin. Dissolve 2 g of saponin (hemolysis grade) in 10 ml of the magnesium chloride solution.

Electrolyte standard. Dissolve 6.709 g of KCl and 0.3446 g of NaCl in distilled water. Dilute to 1 liter. This solution contains 90.0 mmol of K⁺ and 6.0 mmol of Na⁺ per liter. Use acid-washed glassware throughout, and store solutions at 4°C.

Methods

Procedure

Draw at least 4 ml of venous blood with as little stasis as possible and transfer it to lithium heparin-coated plastic tubes (Stayne Laboratories Ltd., Middlesex, England). Mix the blood by gentle inversion, centrifuge at 450 × g (1500 rpm) for 5 min, and remove plasma for estimation of urea, sodium, and potassium concentration if required. Aspirate and discard the buffy coat (the layer containing the leukocytes and platelets). Wash the erythrocytes three times at room temperature by suspension in magnesium chloride solution (112 mmol/liter), centrifugation at 450 × g for 5 min, and aspiration of the supernate. Retain the final supernate for estimation of sodium and potassium concentration; neither electrolyte should be detectable in the final wash. Mix the washed erythrocytes by gentle inversion and take a microhematocrit capillary sample for packed cell volume (PCV) determination. The centrifugation conditions described give a PCV of about 80% and avoid too viscous a lysate. Lyse the suspension of cells by adding 10 μl of saponin solution. Add 0.3 ml of the well-mixed lysate to 10 ml of lithium nitrate diluent (15 mmol/liter) with an automatic pipette (Oxford Laboratories Ltd., U.K.). The viscous nature of the lysate necessitates the use of a "wash out" pipetting technique for good precision. Dilute the standard electrolyte solution in the same manner and use it to calibrate a flame photometer. (We used a Model II 143, Instrumentation Laboratories Inc., Lexington, Mass. 02173.) The sensitivity of the instrument is increased by expanding the scale 10-fold for sodium measurement and by using the 0–200 mmol/liter scale for potassium measurement. The instrument is set to zero on a mixture of 0.3 ml of distilled water and 10 ml of lithium nitrate diluent. The diluted standard solution is then aspirated and the instrument set to read 60 mmol of
sodium and 90 mmol of potassium per liter. The diluted lysate is aspirated and the erythrocyte sodium and potassium concentrations are calculated from the formulas:

\[
\text{Erythrocyte sodium, mmol/liter} = \frac{\text{recorded Na}^+ \text{ value}}{10} \times (100/\text{PCV})
\]

\[
\text{Erythrocyte potassium, mmol/liter} = \frac{\text{recorded K}^+ \text{ value}}{10} \times (100/\text{PCV})
\]

**Precision Studies**

To assess the "within-day" precision of the method, we collected 130 ml of venous blood from a healthy volunteer (K.F.M.), mixed with 500 arb. units of calcium heparin in a flask, and analyzed 32 4-ml aliquots in the same batch on the same day. We also analyzed, with each batch of specimens, one aliquot of a bulk preparation (stored at \(-20^\circ\text{C}\)) of erythrocyte lysate.

**Stability of Heparinized Blood**

The time for which heparinized blood could be left uncentrifuged at room temperature before analysis without significantly affecting the analytical results was assessed. Venous blood (50 ml) was drawn from each of four healthy volunteers (three men, one woman). Each collection was divided into 10 aliquots and transferred to lithium heparin-coated tubes, which were left at room temperature for different time intervals before centrifugation and analysis. All estimations were performed in duplicate.

**Reference Intervals**

We studied two populations, a healthy population of 15 men and 15 women, age range 18-36 years, and a hospitalized population comprising 14 patients admitted for elective surgery with normal plasma values for sodium, potassium, and urea, and no clinical evidence of electrolyte disorder. Venous blood (10 ml) was drawn from each individual for analysis. Results for the healthy population were obtained in three different batches of analyses and those for the hospitalized group in two batches.

**Results**

Tables 1 and 2, respectively, summarize the results of the precision studies. The CV of 3.3% for erythrocyte sodium and 1.6% for potassium incorporates errors inherent in the whole procedure, whereas the corresponding figures (1.6 and 0.9%) for the second precision determination incorporate only the errors of lysate dilution and flame photometry.

Table 1 shows the results of the study to determine the stability of heparinized blood. There were minor variations in erythrocyte electrolyte concentration, but for time periods of as long as 4 h between collection and centrifugation these changes were insignificant compared to the precision of the method; longer intervals affected the results significantly.

**Discussion**

This method has several advantages over those previously reported. Washing erythrocytes free of plasma by centrifugation and resuspension in iso-osmolar magnesium chloride solution has been described previously (9, 12, 15, 16), although we have found ice-cold conditions (9) unnecessary. The use of this wash procedure coupled to packed cell volume determination obviates the estimation of the amount of trapped plasma. Many previously published methods have corrected erythrocyte sodium concentration for trapped plasma sodium by the use of such markers as hemoglobin, insulin, lactose (17), plasma proteins (18), 59Na (19), 59Fe (20), and indocyanine green (21), and this increases greatly the complexity and potential error of such methods. Other authors (22) have criticized the use of isotonic salt solutions to wash erythrocytes free of plasma on the grounds that ions migrate across the erythrocyte membrane when this is done. However, there was no detectable sodium and potassium in the final magnesium chloride wash solution in the method described here, which implies that the conditions used give rise to no significant leakage of sodium and potassium from the erythrocytes. A recent report by Astrup (12) supports this view.

The use of saponin for cell lysis is well established. It is quicker and more efficient than methods that depend on freezing and thawing or subjecting cells to osmotic shock. Lysis of the whole erythrocyte column is preferable to lysis of only a sample of the cells, because erythrocyte sodium and potassium concentrations vary at different levels of the centrifuged column (23, 24).

Flame photometry reliably measures electrolyte concentrations. Moreover, by careful adjustment of the flame photometer and the use of appropriate standard solutions it is possible to measure both erythrocyte sodium and potassium concentration in a single dilution of the lysate.

Precision studies (Tables 1 and 2) demonstrate that the method gives CV's of 3.3% and 1.6%, respectively, for erythrocyte sodium and potassium concentrations measured within-day. No directly comparable day-to-day precision is possible, because heparinized whole blood is too unstable. The precision obtained, however, by dilution and analysis of aliquots of a pooled lysate (CV 1.6% and 0.9% for sodium and potassium, respectively), indicates very good day-to-day precision in these two stages of the procedure.

The reference intervals found for erythrocyte sodium and potassium concentration in a healthy population (Table 4) did not differ significantly from gaussian and, in agreement with other reports (9, 11, 21, 24), we found no significant difference between erythrocyte sodium and potassium concentrations.

**Table 1. Within-Day Precision Data for Erythrocyte Sodium and Potassium a**

<table>
<thead>
<tr>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol/liter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>6.6-7.4</td>
<td>6.9</td>
<td>0.2</td>
</tr>
<tr>
<td>K</td>
<td>99-104</td>
<td>101.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* From 32 aliquots of the same sample of blood.

**Table 2. Precision Data for Sodium and Potassium in 12 Aliquots of the Same Erythrocyte Lysate, Analyzed on Different Days**

<table>
<thead>
<tr>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol/liter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>6.6-6.9</td>
<td>6.8</td>
<td>0.1</td>
</tr>
<tr>
<td>K</td>
<td>101-104</td>
<td>102.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Table 3. Stability of Heparinized Blood: Means for Erythrocyte Sodium and Potassium Concentration in Blood Left for Various Times before Centrifugation

<table>
<thead>
<tr>
<th>Collection no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time before centri., h</td>
<td>Na⁺</td>
<td>K⁺</td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td>0.5</td>
<td>8.6</td>
<td>107</td>
<td>5.2</td>
<td>110</td>
</tr>
<tr>
<td>1</td>
<td>8.0</td>
<td>107</td>
<td>5.2</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
<td>102</td>
<td>4.9</td>
<td>109</td>
</tr>
<tr>
<td>4</td>
<td>8.1</td>
<td>106</td>
<td>4.8</td>
<td>104</td>
</tr>
<tr>
<td>20</td>
<td>8.5</td>
<td>102</td>
<td>4.7</td>
<td>105</td>
</tr>
</tbody>
</table>

Table 4. Reference Ranges Found for Erythrocyte Sodium and Potassium

<table>
<thead>
<tr>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>For 15 healthy men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>4.8–7.3</td>
<td>6.2</td>
</tr>
<tr>
<td>K</td>
<td>96–107</td>
<td>102.3</td>
</tr>
<tr>
<td>For 15 healthy women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>5.0–7.9</td>
<td>6.2</td>
</tr>
<tr>
<td>K</td>
<td>96–109</td>
<td>102.6</td>
</tr>
<tr>
<td>For 30 healthy volunteers (15 men, 15 women)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>4.8–7.9</td>
<td>6.2</td>
</tr>
<tr>
<td>K</td>
<td>96–109</td>
<td>102.4</td>
</tr>
</tbody>
</table>

References


We acknowledge the grant we received from the Central Birmingham Health District Medical Endowment Research Fund for expenses incurred, and Dr. A. M. Bold for his help, advice, and the laboratory facilities that he made so freely available at all stages during this project.
Increased Serum Carnitine Concentration in Renal Insufficiency

Shi-Hua Chen¹ and Susan D. Lincoln

We measured serum carnitine in groups of patients with various diseases. The concentration was frequently above normal in patients with renal insufficiency. The clinical significance of such an increase in renal insufficiency may be analogous to that of increases in urea nitrogen, creatinine, or uric acid in the serum.

Additional Keyphrases: normal values • lipid metabolism • muscle necrosis • myocardial infarction • enzymatic methods • liver cirrhosis • cancer • coronary insufficiency • hemodialysis • correlation with blood urea nitrogen • kidney disease • diagnostic aids

L-Carnitine (L-3-hydroxy-4-N-trimethylaminobutyrate) plays an important role in lipid metabolism (1) and is a normal constituent of human serum. A few investigators have reported alterations in the concentration of carnitine in serum of patients with acute muscle necrosis (2), renal diseases (3), and systemic carnitine deficiency (4). Thus, such data may be clinically useful in diagnosis and management of certain diseases.

Some convenient and specific techniques have been developed for measuring serum carnitine concentration (5, 6). The enzymatic assay procedure can easily be performed in most clinical chemistry laboratories. Attempting to explore the possibility of using this as a laboratory test, we applied the procedure (6) in several groups of patients, and describe the results here.

We were particularly interested in the concentration of carnitine in the serum in cases of myocardial infarction. Carnitine is widely distributed in many mammalian tissues, the highest concentrations being found in skeletal and heart muscle (7). Carnitine increases during attacks of myoglobinuria (2). Because myocardial infarction is known to give rise to transient myoglobinuria (8), we wondered if serum carnitine might be a useful index of muscle destruction in myocardial infarction. Our results for 16 such patients, several of whom had daily carnitine determination, indicated that their serum carnitine concentration was not significantly altered.

Liver and kidney are respectively the sites of biosynthesis and excretion of carnitine. For this reason, we also measured serum carnitine in patients with liver cirrhosis or chronic renal failure, or both. Most of these patients showed an increase, which suggests that measurement of serum carnitine may be useful in evaluating kidney function.

Materials and Methods

Assay of Carnitine

We purchased acetyl coenzyme A, carnitine acetyltransferase (EC 2.3.1.7), and D.L-carnitine HCl from Sigma Chemical Co., St. Louis, Mo. 63178. Dithionitrobenzoic acid was obtained from Aldrich Chemical Co., Milwaukee, Wis. 53233.

Patients fasted overnight and venous blood was sampled early in the morning. The blood was allowed to clot, centrifuged, and serum collected. Occasionally plasma was collected from blood drawn in ethylenediaminetetraacetate-coated tubes. The anticoagulant had no apparent effect on results for carnitine. Serum or plasma was stored at −20°C for various periods. Immediately before carnitine was determined, the serum or plasma samples were thawed and deproteinized as described by DiMauro et al. (2). Carnitine was measured by the enzymatic method of Pearson et al. (6). The reaction mixture contained the following in a final volume of 1 ml: 0.15 mmol of acetyl CoA, 0.125 mmol of dithionitrobenzoic acid, 100 mmol of tris(hydroxymethyl)aminomethane HCl (pH 7.8), 1.25 mmol of ethylenediaminetetraacetate and 0.2 ml of deproteinized serum. The reaction was started by adding 10 μl of carnitine acetyltransferase (1 g/liter). A standard solution of D.L-carnitine HCl was run with each set of assays.