OBSERVATIONS ON THE USE OF THE FLAME PHOTOMETER FOR ANALYSES OF BLOOD*

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Barnes et al. (1) described a flame photometer and various authors have since analyzed the sources of errors in the technique and made various suggestions for eliminating them (2-12). These investigators have been primarily concerned with the design and operation of the instrument, the interference of one substance with another, and similar problems. The purpose of the present investigation was to determine the most accurate and most convenient method of preparing blood for flame photometric analyses of Na and K and to demonstrate what corrections should be applied to compensate for errors introduced in preparing the blood.

Materials and Methods

Two general procedures have been followed by various workers. (1) Analyses have been made on whole blood and on the serum and the values of Na and K in the cells calculated, or (2) the blood has been centrifuged immediately and analyses have been made on the cells and on the serum. With either procedure, at least three methods are then possible: (a) the erythrocytes can be hemolyzed in water, the ghosts centrifuged, and analyses run on this hemolysate and on the serum; (b) the cells can be hemolyzed as above, but the protein in both hemolysate and serum can be precipitated and removed by centrifugation; or (c) the serum Na and K and cell K can be determined as in (a), but the blood can be ashed and the iron removed and whole blood and cell Na then determined. Method 1, a was used by Hunter (13), Methods 1, b and 2, b by Overman and Davis (3) with trichloroacetic acid as the precipitating agent, and Method 2, c by Hald (2).

Before checking on these various methods, the question of preventing coagulation of the blood was considered. In several of the preceding investigations (3, 13, 14), it was specifically stated that heparin was the

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anticoagulant used. Although it may not be true of all commercial hepa-
rin, the products of several companies are Na salts. Analysis of one
preparation showed that 4 mg. contained 1.74 m.eq. of Na. Although
this introduces only a small error when 4 mg. of heparin are added to 20
cc. of blood, it seemed wiser to defibrinate the blood (cf. Mullins et al. (15)
p. 95).

By making analyses on whole blood, erythrocytes, and serum, Methods
1 and 2 were checked by Methods 1, a, b, and c. Defibrinated, sterile,
chicken blood obtained by cardiac puncture was used. In procedure (a)
1 cc. of blood, on which hematocrits had been taken, was added to 20 cc.
of quadruply glass-distilled water containing 105 p.p.m. of Li. The re-
mainder of the blood sample was centrifuged for 30 to 45 minutes at 2670
× g. After this, the hemolyzed blood was centrifuged for 5 to 10 minutes
at 2670 × g to remove the ghosts. 4 cc. of this centrifuged hemolysate
were added to 20 cc. of water containing 100 p.p.m. of Li. Na and K
were determined on this sample in the manner to be described later. This
gave a value for Na and K in whole blood.

1 cc. of the serum from the centrifuged whole blood was added to 20 cc.
of water containing 105 p.p.m. of Li. Serum Na and K were determined
on this. The remainder of the serum and theuffy layer of cells were
removed by aspiration, hematocrits were run on the packed cells, and 0.5
cc. of the remaining erythrocytes was added to 10 cc. of water. After
standing for 30 minutes, this hemolysate was centrifuged as before to
remove the ghosts. 2 cc. of the supernatant fluid were added to 20 cc. of
water containing 110 p.p.m. of Li. Cell Na and K were read on this last
solution. An alternative procedure was to add 0.5 cc. of erythrocytes to
20 cc. of water containing 102.5 p.p.m. of Li. After standing, this was
centrifuged and readings were made on the supernatant fluid.

Procedure (b) follows. 1 cc. of blood was added to 20 cc. of water con-
taining 125 p.p.m. of Li. After hemolysis, 4 cc. of 20 per cent trichloro-
acetic acid were added and the precipitate was removed by centrifugation.
Na and K determinations on this and subsequent solutions were made as
before. 2 cc. of serum were added to 20 cc. of water containing 125 p.p.m.
of Li and the protein was precipitated with 3 cc. of trichloroacetic acid.
0.5 cc. of packed cells was added to 5 cc. of water and then 5 cc. of tri-
chloroacetic acid were added. Following centrifugation, 5 cc. of this
supernatant fluid were added to 20 cc. of water containing 125 p.p.m. of
Li. This had to be diluted (2 cc. + 20 cc. of H₂O + 100 p.p.m. of Li)
to obtain K readings.

In procedure (c), serum Na and K, whole blood K, and cell K were de-

1 Manufactured by Hynson, Westcott and Dunning, Inc.
2 These were spun in an air turbine at 25,000 × g.
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For whole blood Na, 1 cc. of whole blood plus 1 cc. of 4 N \( \text{H}_2\text{SO}_4 \) was evaporated and charred in a porcelain crucible over a steam bath. The crucible was then transferred to a muffle oven and heated at 430–450° until a white ash remained. This ash was eluted with ten 2 cc. portions of water containing 100 p.p.m. of Li. The ash-water mixture was centrifuged 5 to 10 minutes at 2670 \( \times g \), and whole blood Na was determined on the supernatant fluid. For cell Na, 0.5 cc. of cells was hemolyzed in 4 cc. of water. 0.5 cc. of \( \text{H}_2\text{SO}_4 \) (density 1.84) was added. This was dried and ashy as above and then eluted with seven 2 cc. portions of water containing 100 p.p.m. of Li. After centrifugation, Na was read.

A Perkin-Elmer flame photometer, model No. 52-A, with a red-sensitive phototube and an acetylene flame was used to measure the amounts of Na and K in the solutions described above. The internal standard method was followed. In most instances appropriate dilutions were made so that K values were read against a 0 to 50 p.p.m. K + 100 p.p.m. Li standard curve; cell Na, against a 0 to 40 p.p.m. Na + 100 p.p.m. Li standard curve; and serum or whole blood Na, against a 0 to 400 p.p.m. Na + 100 p.p.m. Li standard curve.

Sources of Error

Regardless of which of the methods of preparation was used, it was extremely difficult to obtain reproducible values for cell Na and serum K. This resulted in part from the fact that both of these were small quantities and routine errors made accurate determinations difficult. When cells and serum were separated by centrifugation as previously described, 85 to 95 per cent packing was achieved. This means that a small amount of serum, which contained a relatively high concentration of Na, was added to the hemolysate of the cells and appeared as cell Na when a direct analysis was made on the cells. A correction could be applied, based on hematocrits run on the packed cells, which reduced the cell Na to an almost negligible quantity (cf. Sheppard and Martin (16)). As another possible method to eliminate this error, the packed cells were washed with KCl or sucrose. The former solution was unsatisfactory, since it resulted in progressive hemolysis with each washing. The sucrose was more satisfactory in this regard. However, it was impossible to determine when one began to wash K from the cells. Consequently it was decided to correct the Na in the hemolysate on the basis of the packed cell hematocrit. The values of Na in chicken erythrocytes previously published (13) are high because they were not corrected for the presence of small amounts of serum.

The other routine correction which must be applied compensates for
the relatively large amounts of K which appeared in the serum as a consequence of the hemolyses of a small number of cells. Unless one exercises great care, such as using coated glassware and drawing blood under oil, some of the cells will usually hemolyze during the drawing of the blood and the initial centrifugation. The amount of this hemolysis can be determined spectrophotometrically and the K contributed by that many cells can be subtracted from the value obtained on the serum.

Calculations—To clarify the details of the corrections and calculations, a typical experiment follows.

<table>
<thead>
<tr>
<th>per cent</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood hematocrit</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>Packed cell hematocrit</td>
<td>96.9</td>
<td></td>
</tr>
<tr>
<td>Hemolysis in serum</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Flame photometer determination

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>12.8</td>
<td>111.0</td>
</tr>
<tr>
<td>Serum</td>
<td>175.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Correction for cell Na, 0.031 cc. serum × 175.0 = 5.4; (12.8 - 5.4)/0.969 = 7.6 m.eq. per liter cells

Cell K, 111.0/0.969 = 114.6 m.eq. per liter cells

Serum Na (no corrections), 175.0 m.eq. per liter serum

" K, 114.6 × 0.21 × 0.01 = 0.2; 3.9 - 0.2 = 3.7 m.eq. per liter serum

If one is interested in trying to relate cation shifts to changes in osmotic behavior of erythrocytes, volume changes of the cells must be taken into account. One way to do this is to calculate all cation values in terms of the volume of erythrocytes as determined by hematocrit measurement on the initial sample analyzed.

Results

Table I shows (1) a series of values for cell Na before and after correction is made for serum Na, based on packed cell hematocrits, and (2) serum K before and after correction for K added by hemolyzed cells.

A comparison of the values obtained with the various methods of preparing the blood is given in Table II. In general, there is so close an agreement among the three methods, that, from the standpoint of accuracy, there is no choice among them. Since procedure (a) is the simplest, it would be recommended were it not for the fact that the serum sometimes becomes milky on standing, which tends to clog the aspirator of the flame photometer. For this reason, procedure (b) is recommended. Spectrographic analyses3 of the trichloroacetic acid precipitates by this

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3 The authors are indebted to Mr. R. G. Y. Wycoff of the Department of Physics, University of Oklahoma, for these measurements.
method show that traces of Na but no K or Li is carried down with the protein. The amounts, however, appear to be negligible. Because it involves direct analysis of both cells and serum, Method 2 is to be preferred.

**Table I**

*Influence of Small Amount of Serum on Cell Na and of Small Amount of Hemolysis on Serum K in Chicken Blood*

<table>
<thead>
<tr>
<th>Hematocrit (per cent)</th>
<th>Cell Na, m.eq. per liter cells</th>
<th>Hemolysis</th>
<th>Serum K, m.eq. per liter serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncorrected</td>
<td>Corrected</td>
<td>Uncorrected</td>
</tr>
<tr>
<td>85.0</td>
<td>29.5</td>
<td>7.8</td>
<td>2.5</td>
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<tr>
<td>87.8</td>
<td>17.3</td>
<td>1.4</td>
<td>3.7</td>
</tr>
<tr>
<td>87.9</td>
<td>16.3</td>
<td>0</td>
<td>3.7</td>
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<tr>
<td>90.2</td>
<td>15.9</td>
<td>0.3</td>
<td>4.0</td>
</tr>
<tr>
<td>90.9</td>
<td>23.1</td>
<td>9.9</td>
<td>4.0</td>
</tr>
<tr>
<td>91.1</td>
<td>13.7</td>
<td>0.4</td>
<td>6.6</td>
</tr>
<tr>
<td>91.1</td>
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<tr>
<td>91.4</td>
<td>11.4</td>
<td>3.6</td>
<td>7.3</td>
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<tr>
<td>92.0</td>
<td>15.8</td>
<td>3.4</td>
<td>7.3</td>
</tr>
<tr>
<td>92.6</td>
<td>14.7</td>
<td>0</td>
<td>7.3</td>
</tr>
<tr>
<td>93.0</td>
<td>18.4</td>
<td>9.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

**Table II**

*Comparison of Results on Chicken Blood by Various Methods of Preparation*

Values in milliequivalents.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Procedure</th>
<th>Per liter serum</th>
<th>Per liter cells</th>
<th>Per liter cells calculated (Method 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na</td>
<td>K</td>
<td>Na</td>
</tr>
<tr>
<td>1</td>
<td>(a)</td>
<td>141.5</td>
<td>4.7</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>(b)</td>
<td>141.3</td>
<td>4.6</td>
<td>6.7</td>
</tr>
<tr>
<td>2</td>
<td>(a)</td>
<td>159.2</td>
<td>2.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>159.2</td>
<td>2.6</td>
<td>7.0</td>
</tr>
<tr>
<td>3</td>
<td>(a)</td>
<td>141.8</td>
<td>3.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(b)</td>
<td>141.8</td>
<td>4.4</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>141.8</td>
<td>3.9</td>
<td>7.4</td>
</tr>
</tbody>
</table>

over Method 1. With a somewhat different apparatus and more dilute solutions for analysis, Willebrands (17) reported low values when trichloroacetic acid was used. It may be that with the internal standard method the difficulties which he encountered are eliminated.
SUMMARY

1. In determining the amount of K in blood serum, a correction should be applied, based on the K contributed by hemolyzed erythrocytes.

2. In determining the amount of Na in erythrocytes, a correction should be applied, based on the amount of Na in the serum which is included with the cells.

3. A comparison of three different methods of treating blood and erythrocytes prior to analyzing them for Na and K showed that similar results could be obtained with each method.

BIBLIOGRAPHY

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