Sodium and Potassium Concentration and Transmembrane Fluxes in Leukocytes

By Gabriel Cividalli and David G. Nathan

Reported values for the potassium to sodium ratio in white blood cells range from less than 1 to about 4. This marked variability might be explained by in vitro cation shifts induced by extraordinarily high cation permeability. To test this hypothesis, human leukocytes from 5 ml of blood were maintained in plasma at 37°C and separated by differential centrifugation in specially devised microtubes, within less than 30 min after sampling. Intracellular water content was determined with tritiated water, and trapped plasma with 14C-sucrose. Under these conditions, intracellular concentration of potassium in human leukocytes was 120 SD ± 7 meq/liter cell water and of sodium 16 SD ± 5 meq/liter cell water, and the resultant ratio was higher than 7. Values in the same range were obtained in rabbit leukocytes and in polymorphonuclear granulocytes isolated from peritoneal exudate in guinea pigs. To determine the cation permeability of these white cells, unidirectional fluxes of potassium and sodium were measured with 42K and 22Na. All of these fluxes were in a range from 1.2 to 2.7 meq/liter/min, values more than 50 times faster than those observed in erythrocytes. When active fluxes were inhibited by ouabain, potassium loss and sodium gain occurred at an initial rate of about 0.7 meq/liter/min. Marked changes also occurred upon incubation at low temperature. These very rapid fluxes demand rigid control of environmental conditions for accurate measurement of intracellular cations in leukocytes.

While the intracellular content of monovalent cations in red blood cells (RBC), and their movements across the RBC membrane, have been widely investigated, relatively little study has been directed towards similar studies in white blood cells (WBC). Reported values for the potassium to sodium ratio in WBC are extremely variable, ranging from less than 1 to about 4, with a higher value of 7.2 found only in leukemic lymphoblasts which had been grown in tissue culture (Table 1). Cation concentration in leukocytes appears to be very sensitive to changes in environmental temperature, the chemical composition of suspending media, and to external manipulation during cell isolation. All previously reported studies have involved washing of leukocytes in hypotonic media (to induce RBC hemolysis) and subsequent resuspension in artificial media, sometimes followed by incubation in order to...
Table 1. Published Values for Intracellular Content of Sodium and Potassium in Leukocytes

<table>
<thead>
<tr>
<th>Source of Leukocytes</th>
<th>Unit of Measurement</th>
<th>(Na)</th>
<th>(K)</th>
<th>(K)/(Na)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit polymorphonuclear leukocytes</td>
<td>meq/liter water</td>
<td>98 ± 2</td>
<td>95 ± 18</td>
<td>0.97</td>
<td>17.</td>
</tr>
<tr>
<td>Human, chronic granulocytic leukemia</td>
<td>meq/10^12 cells</td>
<td>28 ± 2</td>
<td>49 ± 2</td>
<td>1.8</td>
<td>18.</td>
</tr>
<tr>
<td>Human, chronic lymphocytic leukemia</td>
<td>meq/10^12 cells</td>
<td>18 ± 2</td>
<td>23 ± 3</td>
<td>1.3</td>
<td>18.</td>
</tr>
<tr>
<td>Human, mixed normal leukocytes</td>
<td>meq/liter water</td>
<td>60 ± 6</td>
<td>100 ± 6</td>
<td>1.7</td>
<td>19.</td>
</tr>
<tr>
<td>Human, chronic myeloid leukemia</td>
<td>meq/kg dry weight</td>
<td>162 ± 7</td>
<td>418 ± 18</td>
<td>2.6</td>
<td>7.</td>
</tr>
<tr>
<td>Human, acute leukemia</td>
<td>meq/kg dry weight</td>
<td>256 ± 7</td>
<td>581 ± 37</td>
<td>2.3</td>
<td>7.</td>
</tr>
<tr>
<td>Lymphoblasts in tissue culture</td>
<td>mM</td>
<td>19 ± 4</td>
<td>136 ± 8</td>
<td>7.2</td>
<td>15.</td>
</tr>
<tr>
<td>Human, acute leukemia</td>
<td>meq/kg water</td>
<td>53 ± 8</td>
<td>84 ± 7</td>
<td>1.6</td>
<td>20.</td>
</tr>
<tr>
<td>Human, normal lymphocytes</td>
<td>mmoles/kg water</td>
<td>34 ± 3</td>
<td>120 ± 3</td>
<td>3.5</td>
<td>8.</td>
</tr>
<tr>
<td>Human, normal polymorphonuclear leuk.</td>
<td>mmoles/kg water</td>
<td>30 ± 4</td>
<td>118 ± 3</td>
<td>3.9</td>
<td>8.</td>
</tr>
<tr>
<td>Human, mixed normal leukocytes</td>
<td>meq/liter water</td>
<td>34 ± 9</td>
<td>137 ± 11</td>
<td>4.0</td>
<td>14.</td>
</tr>
</tbody>
</table>

restore a presumed “normal” cation composition. In this study we have re-examined the monovalent cation composition of leukocytes without removing them from their natural plasma environment and with as little external manipulation as possible, in order to achieve closer representation of in vivo cation composition. Unidirectional cation fluxes measured with their radioactive isotopes and shifts in cation composition during incubation with ouabain and ethacrynic acid were also studied to determine the relative magnitude of cation transport in leukocytes compared to red cells.

MATERIALS AND METHODS

Procedures for Determination of Intracellular Cations

Heparinized blood was obtained by venipuncture of normal human volunteers or by ear arterial puncture in rabbits. All containers were plastic or siliconized, and all manipulations were performed in an ambient temperature of 37°C. Five-milliliter blood samples were immediately centrifuged for 5 min in a swinging bucket rotor at 185 g, and the supernatant plasma, containing mostly leukocytes, was carefully transferred to another tube containing 5 μl of Krebs-Ringer phosphate buffer to which had been added 5 μCi of freeze-dried 14C-sucrose (New England Nuclear, Boston, Mass., 0.394 mCi/mM) and 20 μCi 3H2O (New England Nuclear, 0.25 Ci/g). If erythrocyte sedimentation was found insufficient, a second 5-mm centrifugation under similar conditions was performed. The resulting leukocyte suspension contained less than 5% erythrocytes. It was thoroughly mixed and then transferred to specially devised microtubes (Fig. 1). After centrifugation in a swinging bucket rotor at 1350 g for 5 min, the leukocytes were collected in the capillary end of the microtube. The supernatant plasma was removed, and the capillary end was cut with a file and further centrifuged in a microhematocrit centrifuge for 30 min. Centrifugation with erythrocytes, when present, was seen as a red button at the bottom of the capillary tube, which could be easily be cut off. The upper third of the WBC column was also discarded because it was sometimes contaminated with thrombocytes and RBC membranes. The capillary containing the lower two-thirds of the leukocyte column was then cut with a file, inverted, and its content transferred by centrifugation into vials containing 3.98 ml of 0.015 M lithium carbonate (Becton Dickinson, Rutherford, N.J.). The leukocyte pellet, now transferred to the lithium-containing vials, was resuspended by stirring on a vortex mixer. Twenty microliters concentrated nitric acid was added to samples and standards and at least 30 min allowed for the disruption of cells. The content of the vials was then analyzed for sodium and potassium content (by flame photometry) and for 3H and 14C radioactivities. The latter were determined by counting 2 ml WBC lysate in 15 ml of a scintillator (Aquasol, New England Nuclear) in a Packard Tri Carb Liquid Scintillation Spectrometer. Twenty microliters plasma, collected after centrifugation of the microtubes, were similarly transferred into vials containing 3.98 ml lithium carbonate and examined for sodium, potassium, 3H and 14C radioactivities.
**Fig. 1.** Schematic representation of equipment. Separation of WBC was obtained by centrifugation of leukocyte-rich plasma in microtube A inside plastic holder B. After packing of leukocytes, the capillary end of the microtube (I.D. approximately 0.4 mm) was cut off, inverted, and passed through the cap of the lithium-containing vial C. WBC were transferred into this vial by further centrifugation.

**Computations.** The following symbols are used in the calculations:

- $v$, volume of plasma and of sodium and potassium standard put in the lithium-containing vials ($20 \times 10^{-6}$ liters).
- $^{3}\text{H}_{\text{WBC}}$ and $^{14}\text{C}_{\text{WBC}}$, activity of each isotope in an aliquot (2 ml) of WBC lysate in the lithium-containing vials.
- $^{3}\text{H}_{\text{PL}}$ and $^{14}\text{C}_{\text{PL}}$ activities in an equal aliquot (2 ml) of plasma diluted in the lithium-containing vials.
- $(\text{H}_2\text{O})_{\text{WBC}}$, water content of analyzed WBC column (liters):

\[
(\text{H}_2\text{O})_{\text{WBC}} = v \cdot ^{3}\text{H}_{\text{WBC}} / ^{3}\text{H}_{\text{PL}}.
\]

- Reading, flame photometer reading calibrated to correspond to concentration of standard (meq/liter).
- $C$, cation content (mEq) in $(\text{H}_2\text{O})_{\text{WBC}}$:

\[
C = \text{reading} \cdot v
\]

- $(\text{C})_{\text{WBC}}$, cation concentration in analyzed WBC column (meq/liter):

\[
(\text{C})_{\text{WBC}} = C / (\text{H}_2\text{O})_{\text{WBC}} = \text{reading} \cdot ^{3}\text{H}_{\text{PL}} / ^{3}\text{H}_{\text{WBC}}.
\]

- $q$, plasma trapped in WBC column as a fraction of its total $\text{H}_2\text{O}$ content:

\[
q = ^{14}\text{C}_{\text{WBC}} \cdot ^{3}\text{H}_{\text{PL}} / ^{14}\text{C}_{\text{PL}} \cdot ^{3}\text{H}_{\text{WBC}}
\]

- $(\text{C})_{\text{PL}}$, cation concentration in plasma (meq/liter).
- $(\text{C})_{\text{WBC}}^{\text{corr}}$, cation concentration in WBC, corrected for the cation content in trapped plasma (meq/liter):

\[
(\text{C})_{\text{WBC}}^{\text{corr}} = [(\text{C})_{\text{WBC}} - (\text{C})_{\text{PL}}] \cdot q / (1 - q)
\]

**Measurements of Cation Fluxes**

Most measurements were performed with suspensions of human leukocytes in plasma obtained after centrifugation of blood at 185 g. In some instances leukocytes were resuspended in plasma which was cleared of thrombocytes and erythrocyte membranes by prior centrifugation at
27,000 g. Guinea pig polymorphonuclear granulocytes (PMN), isolated from peritoneal exudate after intraperitoneal injection of casein and resuspended in autologous plasma, were also studied. These suspensions were incubated while being shaken in a water bath kept at 37°C or 0°C. 10^-4 M ouabain (Sigma Chemical Company, St. Louis, Mo.) or 10^-3 M ethacrynic acid (Merck, Sharp & Dohme Research Lab., West Point, Pa., freshly dissolved at x100 concentration in 0.1 N NaOH and adjusted to pH 7.0 with HCl) were added in some experiments.

Potassium influx was measured by adding 20 μCi 42K (Union Carbide Corp., Sterling Forest Research Center, Tuxedo, N.Y.) to a suspension of leukocytes. One-milliliter aliquots removed at various time intervals were filtered through glass-fiber filters (Reeve Angel, Clifton, N.J.). These filters were first shown to be practically free of potassium. The filters were then washed five times with cold isotonic NaCl. The dried filters were transferred to counting vials and analyzed for 42K radioactivity in a Packard Gamma Spectrometer and for total potassium content by flame photometry.

To measure potassium efflux, a concentrated (40,000 cu mm) suspension of leukocytes in plasma was incubated with 20 μCi 42K for nearly 1 hr. At a given time the suspension was diluted 1:20 with unlabeled autologous plasma which had previously been centrifuged at 27,000 g. Two-milliliter aliquots were removed at various time intervals, filtered, washed, and analyzed for 42K radioactivity and total potassium content as in the measurement of influx. Efflux studies were performed by dilution of the cells and resultant decrease in specific activity of the labeling isotope in the medium (rather than by washings as is customary in studies of RBC fluxes) because of our endeavor to avoid loss of intracellular isotope and clumping of cells during centrifugation.

Attempts to apply the same techniques to the measurement of sodium fluxes revealed very rapid flux coefficients which could not be measured accurately. In addition, the filters were variably contaminated with sodium. Therefore, a double-isotope technique was applied. Here, the specific activity of 24Na in the medium and in the cells remained unchanged, while 22Na was used to measure sodium fluxes. To measure sodium influx, a suspension of leukocytes was equilibrated for about 30 min with 150 μCi 24Na. Thirty microcuries 22Na were then added, and aliquots were removed at rapid intervals. These were quickly washed five times with cold isotonic MgCl₂. To measure sodium efflux, 800 μCi 24Na (Union Carbide Corp.) was added to a suspension of leukocytes in plasma. The leukocytes were spun down and concentrated by resuspension in 1 ml of supernatant plasma. Fifteen microcuries 22Na was now added, and the leukocytes were further incubated at 37°C to allow equilibration of both sodium isotopes. After about 30 min, the WBC suspension was diluted 1:20 with the previously separated plasma, containing 24Na but no 22Na, which had meanwhile been centrifuged at 27,000 g. Two-milliliter aliquots were filtered and washed as in previously described studies. 22Na activity was counted after most of the short-lived 24Na had decayed.

Computations. The fluxes of tracer cations in these experiments, where the cells were in a steady state with respect to their intracellular concentration of sodium and potassium, behaved as in a two-compartment system. In our computations (aWBC), represents the specific activity of the intracellular compartment at time t, and (aWBC)₀ the specific activity of the same compartment at isotopic equilibration. The rate coefficient for inward flux was therefore represented by the slope (k₁) of the graph of log₂ [1 - (aWBC)₀/(aWBC)] versus time (t). Since the volume of the intracellular compartment constituted in these experiments is less than a thousandth of the extracellular compartment, the fraction of both cations inside the latter was extremely small. It was therefore assumed that the specific activity in the extracellular compartment did not change and that (aWBC)₀ was equal to the specific activity in plasma (aPL). Therefore, k₁ would be represented by the slope of the graph of log₂ [1 - (aWBC)/(aPL)]. The rate coefficient for outward flux is represented by the slope (k₀) of the graph of:

\[
\frac{\log\left[\frac{(a_{WBC}) - (a_{PL})}{(a_{WBC}) = 0 - (a_{PL}) = 0}\right]}{\log\left[\frac{(a_{WBC}) - (a_{PL})}{(a_{WBC}) = 0 - (a_{PL}) = 0}\right]}
\]

versus time. This slope was approximated by the product of the initial slope of log₂ (aWBC)₀ versus time and 1.05 since (aPL), 0.05 (aWBC)₀, a constant, and

\[
\frac{\log\left[\frac{(a_{WBC}) - (a_{PL})}{(a_{WBC}) = 0 - (a_{PL}) = 0}\right]}{\log\left[\frac{(a_{WBC}) - (a_{PL})}{(a_{WBC}) = 0 - (a_{PL}) = 0}\right]}
\]

versus time. This slope was approximated by the product of the initial slope of log₂ (aWBC)₀ versus time and 1.05 since (aPL), 0.05 (aWBC)₀, a constant, and

\[
\frac{\log\left[\frac{(a_{WBC}) - (a_{PL})}{(a_{WBC}) = 0 - (a_{PL}) = 0}\right]}{\log\left[\frac{(a_{WBC}) - (a_{PL})}{(a_{WBC}) = 0 - (a_{PL}) = 0}\right]}
\]
TRANSMEMBRANE FLUXES IN LEUKOCYTES

In studies of potassium fluxes the specific activity of $^{42}\text{K}$ was proportional to the ratio of counts to potassium content ($S = \text{cpm } ^{42}\text{K}/[\text{K}]$). In studies of sodium fluxes the specific activity of $^{24}\text{Na}$ was maintained constant, and the specific activity of $^{22}\text{Na}$ was therefore proportional to the ratio $^{22}\text{Na}/^{24}\text{Na}$. While some tracer isotopes were lost when WBC were washed with MgCl$_2$, these ratios were assumed to remain constant. Rate coefficients for influx ($k_i$) and efflux ($k_o$) of potassium were obtained from the slopes of the following lines calculated by linear regression:

$$\log_e \left( \frac{1 - S_{WBC}}{S_{PL}} \right) = k_i \cdot t$$

and

$$\log_e S_{WBC} = k_o \cdot 0.95 \cdot t.$$

Those for sodium fluxes were obtained from the slope of the graphs for

$$\log_e \left( \frac{1 - ^{22}\text{Na}_{WBC} \cdot ^{24}\text{Na}_{PL}}{^{24}\text{Na}_{WBC} \cdot ^{22}\text{Na}_{PL}} \right) = k_i \cdot t$$

and

$$\log_e \left( \frac{^{22}\text{Na}_{WBC} \cdot ^{24}\text{Na}_{PL}}{^{24}\text{Na}_{WBC} \cdot ^{22}\text{Na}_{PL}} \right) = k_o \cdot 0.95 \cdot t.$$

Cation fluxes were obtained multiplying rate coefficients by the intracellular concentrations per liter cell water of the respective cation. This computation assumed identity between the cation concentration of total leukocytes and the leukocyte trapped on glass-fiber filters.

RESULTS

The corrected concentration of monovalent cations in leukocytes, calculated as outlined in the previous section, was independent of the percentage of trapped plasma. Trapped plasma did affect adversely, however, the difference between duplicate determinations. With a trapped plasma of about 20%, which was usually obtained, the SD of the difference between 20 consecutive duplicate determinations was 5.4 meq/liter for sodium and 4.9 meq/liter for potassium. The mean concentrations of sodium and potassium in leukocytes obtained from human and rabbit peripheral blood, and in PMN from guinea pig peritoneal exudate, are shown in Table 2. The ratio of their concentrations in human cells approaches 8. The change in intracellular cation concentration was small when leukocytes were incubated for 2 hr at 37°C in their own plasma, but when the temperature was lowered, a marked potassium loss and sodium gain took place (Fig. 2). Marked cation shifts were also observed in WBC washed with various chilled buffers. When 10$^{-4}$ $M$ ouabain was added to the medium, exchange of intracellular potassium for extracellular sodium took place even at 37°C at an

<p>| Table 2. Sodium and Potassium Concentration and Unidirectional Fluxes in Leukocytes* |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|</p>
<table>
<thead>
<tr>
<th>(Na)</th>
<th>(K)</th>
<th>No influx</th>
<th>No efflux</th>
<th>K influx</th>
<th>K efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>meq/liter H$_2$O</td>
<td>meq/liter cell H$_2$O/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed human leukocytes</td>
<td>15.7 ± 5.0 (9)</td>
<td>120 ± 7 (9)</td>
<td>1.8 (1)</td>
<td>1.4-2.7 (2)</td>
<td>1.3-1.6 (3)</td>
</tr>
<tr>
<td>Mixed rabbit leukocytes</td>
<td>18.9 ± 5.6 (5)</td>
<td>115 ± 8 (5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Guinea pig peritoneal PMN</td>
<td>27.4 ± 5.6 (4)</td>
<td>108 ± 5 (4)</td>
<td>—</td>
<td>4.1 (1)</td>
<td>2.7 (1)</td>
</tr>
</tbody>
</table>

*Mean ± SD (number of determinations).
initial rate of about 0.7 meq/liter cell water/min. By contrast, $10^{-3}$ M ethacrynic acid did not seem to affect leukocyte cations (Fig. 2). Similar results were obtained in rabbit-mixed leukocytes and in guinea pig PMN. Unidirectional fluxes of sodium and potassium derived from the cation concentrations shown in Table 2 and isotope studies of the type shown in Figs. 3-6, are set out in Table 2. A rapid movement of sodium and potassium across the membranes.
Fig. 4. Semilogarithmic plot of $^{42}$K activity ($S_{WBC}$) during measurement of potassium efflux.

Fig. 5. Semilogarithmic plot of changing $^{22}$Na activity during measurement of sodium influx.

Fig. 6. Semilogarithmic plot of decreasing $^{22}$Na activity during measurement of sodium efflux.

Fig. 7. Effect of $10^{-4}$ ouabain on the rate of equilibration of $^{42}$K in WBC and plasma. The magnitude of the effect was more marked when ouabain was added immediately before the study (•) than when it had been allowed to remain in previous contact with the leukocytes for 30 min (○). The respective control experiments are represented by filled symbols.
of the cells was observed. An inhibitory effect of ouabain on the rate of equilibration of cation isotopes* was evident whenever studied (Fig. 7). Its degree, however, varied from one experiment to another.

DISCUSSION

Our results indicate very rapid cation fluxes in leukocytes and resultant inadequacy of standard methods, used to study RBC cations, for their determination in WBC. With the use of microtubes specially devised for separation and packing of small quantities of cells, we were able to minimize external manipulation of leukocytes and avoid changes in environmental conditions during their separation. The water content of this small quantity of analyzed leukocytes was determined with tritiated water, which achieves nearly instant equilibration in RBC11 and, presumably, also in WBC. The main potential source of error seems to lie with the large correction necessary because of the high percentage of plasma trapped in packed leukocytes. 14C-sucrose was chosen to measure trapped plasma because it has been widely used for this purpose with RBC,12,13 does not penetrate into WBC, and does not liberate 14CO2 when WBC are disrupted with nitric acid.8 The percentage of trapped plasma and the resultant correction necessary for determination of intracellular cations was even higher in other recent studies of WBC cations.5,14

Reported data on unidirectional cation fluxes in leukocytes are restricted to leukemic lymphoblasts grown in tissue culture.15 Our measurements of sodium and potassium fluxes in normal leukocytes show values of a similar magnitude. The flux rates were approximately fifty times faster than those observed in RBC. While measurements of potassium fluxes were fairly linear (Figs. 3 and 4), measurements of sodium fluxes (Figs. 5 and 6) appear to be less accurate, probably because of the lower intracellular concentration and more rapid turnover of this cation. Measurements of sodium influx (Fig. 5) could actually suggest the presence of two rate coefficients, since the curve had a \( t = 0 \) intercept much lower than one, thus indicating very rapid loss of a large portion of the exchangeable sodium in leukocytes. However, the \( t = 0 \) intercept of this curve could also be affected by continuation of some 22Na influx during the initial stage of filtration and washing of leukocytes. These processes, even when performed quickly, took considerable time in relation to the extremely rapid turnover of sodium in leukocytes. The slope in Fig. 5 and the calculation of the rate coefficient for sodium influx would not be affected if that happened, providing each sample is handled in the same manner with respect to time.

Flux coefficients were calculated on the basis of changes in specific activity and assumed a steady state in the intracellular concentration of cations.10 They cannot, therefore, be applied to the measurement of fluxes after adding ouabain. A decrease in the rate of equilibration of the isotope was always evident in the presence of ouabain. Its magnitude, however, varied greatly and was apparently dependent on the length of time interval between adding ouabain.

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*This term is used here because flux coefficients could not be measured in this case, as will be discussed later, as a result of the changes in intracellular concentration of cations during the experiment.
and measuring the fluxes, and the resultant change in concentration of intracellular cations (Fig. 7). The magnitude of the ouabain-inhibitable flux is probably represented by the initial net change in concentrations of sodium and potassium upon incubation with ouabain (Fig. 2). It apparently accounts for about half of the total cation fluxes in leukocytes. Active fluxes were also inhibited by low temperature, but not by ethacrynic acid. The cation shifts which occur in WBC washed with chilled buffers are due to these very rapid fluxes. They demand rigid control of environmental conditions for accurate measurement of intracellular cations.

It is of interest to note that one aspect of granulocyte function, the capacity to ingest particles and mount an oxidative attack against them, is entirely unaffected by marked intracellular monovalent cation shifts induced by ouabain and decreased temperature. Whether other aspects of granulocyte physiology, such as life span in the circulation, are markedly affected by such shifts remains to be determined.

REFERENCES

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