The Monovalent Cation Content and Adenosine Triphosphatase Activity of Human Normal and Leukemic Granulocytes and Lymphocytes: Relationship to Cell Volume and Morphologic Age

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RELATIVELY LITTLE STUDY has been directed to membrane transport function as a determinant or as a reflection of disordered maturation in leukemic cells. Studies by Lubin and co-workers have suggested that a reduction in intracellular potassium concentration may reduce the rate of nucleic acid and protein synthesis in bacterial1 and mammalian2 cells. Quastel and Kaplan3 have demonstrated that incorporation of radioactive precursors into nucleic acids by lymphocytes stimulated with phytohemagglutinin is markedly inhibited in the presence of ouabain; and that the effect of ouabain is prevented by increasing the extracellular concentration of potassium. More recently, Jung and Rothstein4 have shown intricate patterns of monovalent cation flux during cell division of parasynchronized mouse lymphoblasts; and, Mayhew and Levinson5 have produced inhibition of monovalent cation transport and cell division in ascites tumor cell cultures exposed to ouabain. Such observations suggest that the qualitative distribution of intracellular monovalent cations may be important in supporting vital cell functions that facilitate cell maturation and mitosis; and, that dynamic alterations in pumps and leaks of monovalent cations are essential concomitants of mitosis and its related changes in cell volume.

Studies of leukocyte intracellular cations by Rigas6 have suggested that some human leukemic blast cells have a relatively low intracellular potassium and high intracellular sodium concentration, resulting in a potassium:sodium ratio of less than one. Observations by Block and Bonting7 have indicated that major differences in adenosine triphosphatase (ATPase) activity distinguish leukemic from normal leukocytes.

The aforementioned observations raise the consideration that an abnormality
in control of intracellular monovalent cations could play a role in the disordered mitotic rate and maturation sequence of some leukemic cells. It is not clear, however, whether the differences in cation content or ATPase are related to cell age, the leukemic state or other considerations, such as the effects of cell isolation. No studies have been published comparing cation content and the sodium-potassium activated ATPase of normal and leukemic cells of similar morphologic age, studied by similar methods.

The present studies were performed to reexamine the monovalent cation content of human normal and leukemic granulocytes and lymphocytes to determine the relationship of cation content to cell volume, cell age and the leukemic state. In addition, studies were performed to compare the sodium-potassium activated, ouabain-inhibitable ATPase in human leukemic and normal leukocytes.

**METHODS**

**Leukocyte Isolation**

Normal human polymorphonuclear granulocytes (PMNG) were obtained from patients without hematologic disease who had leukocytosis and differential counts with more than 90 per cent PMNG's. Chronic granulocytic leukemic (CGL) granulocytes were obtained from untreated patients. Acute granulocytic leukemia (AGL) blast cells were obtained from patients with differential leukocyte counts containing greater than 85 per cent myeloblasts. Mixed granulocyte-lymphocyte and lymphocyte preparations were obtained from healthy donors. Bone marrow cells from subjects without hematologic disease were obtained at the time of diagnostic examination.

PMNG's, AGL blast cells, normal mixed leukocytes, CGL and bone marrow granulocytes were prepared by exposing 2 to 100 ml. of blood or marrow to 5 to 10 volumes of 150 mM NH₄Cl for 10 minutes at 25 C.³ The resultant hemolysate was centrifuged 150 × g. for 6 minutes at 4 C. and the sediment of leukocytes washed twice with Hanks balanced salt solution (HBSS).

Normal lymphocytes were prepared by mixing 300 to 400 ml. of heparinized whole blood with 4 per cent polyvinylpyrrolidone in HBSS in a separatory funnel. After sedimentation for 45 minutes the lower erythrocyte layer was allowed to drain slowly. The remaining plasma was diluted with HBSS and filtered at 37 C. through a 50 ml. syringe packed with glass wool. The filtrate was centrifuged at 150 × g. at 4 C. and resuspended in 150 mM NH₄Cl for 10 minutes. The hemolysate was centrifuged at 150 × g. and the sedimented lymphocytes washed twice with HBSS. Lymphocytes composed 95 to 98 per cent of the isolated leukocytes and erythrocytes composed less than 5 per cent of the total cells. Since on occasion, platelet contamination was excessive defibrinated blood rather than heparinized blood was subsequently used to isolate normal lymphocytes. No difference in results was noted when heparinized or defibrinated blood was used; however, the yield of lymphocytes was reduced with the latter method. In some cases, the leukocytes of patients with AGL with PMNG's composing greater than 10 to 15 per cent of cells were prepared in a similar manner as normal lymphocytes. Filtration through glass wool removed the PMNG, and the residual proportion of myeloblasts was greater than 95 per cent.

**Leukocyte Incubation**

Leukocytes were incubated in 15 to 20 ml. of HBSS containing glucose at pH 7.50 for up to 150 minutes. Leukocyte cation measurements were made after cells were incubated in HBSS at 37 C., HBSS at 2 C., or HBSS at 37 C. containing 5 × 10⁻⁴ M ouabain. Samples of cell suspensions were obtained at zero time and various intervals thereafter for measurement of leukocyte potassium, sodium, water, weight, volume and morphology. All materials were plastic or siliconized glassware.
Leukocyte Cation and Water

After incubation, samples were removed and placed in weighed tubes, the cells sedimented by centrifugation at 200 × g, and washed with 145 mM choline chloride buffered at pH 7.50 with 10 mM tris (hydroxymethyl) aminomethane. When the number of leukocytes permitted, duplicate or triplicate samples were measured. Parallel samples were washed in tris-buffered choline chloride containing sucrose-14C and 5 mM non-radioactive sucrose. The washed cells were weighed immediately, dried at 90 C. for 18 hours, cooled to room temperature and reweighed. The dried cell button was digested with 25 μL of nitric acid for 60 minutes at 37 C. Prior studies had shown that nitric acid digestion did not release carbon-14 from labelled sucrose. Leukocyte digests were brought to 1 mL with 0.975 mL of distilled deionized water and aliquots were counted in a liquid scintillation counter. Quenching was corrected with an external standard. Cell water was calculated as (wet weight minus sucrose-14C space) minus dry weight. Acid digests were also diluted with distilled deionized water transferred to a 10 mL volumetric containing 15 mM lithium chloride and measured for potassium and sodium by flame photometry using lithium chloride as an internal standard. Nitric acid blanks were measured with each experiment. Erythrocyte sodium and potassium were measured by flame photometry so that monovalent cation content per μ3 cell volume could be compared to that of leukocytes.

Cell Counts

Leukocyte cell counts were made in duplicate using a Bright Line hemocytometer and phase contrast microscope. Erythrocyte cell counts were made in triplicate on a Model B Coulter counter.

Cell Volume

The relative volume of lymphocytes and granulocytes was measured in a 400 channel analyzer with a Coulter counter 100μ diameter orifice. The effect of graded tonicity solutions of phosphate buffered saline on leukocyte volume was studied. Absolute cell volume in μ3 was measured by performing leukocyte counts and simultaneously measuring packed leukocyte volume in thrombocytocrit tubes.* Because of their increased ease of handling, absolute cell volume measurements of lymphocytes were made. Erythrocyte mean cell volume was calculated from cell counts and microhematocrit determinations.

Adenosine Triphosphatase

Leukocytes previously washed in a 300 mosm tris-histidine buffer, pH 7.2, were suspended in 0.25 M sucrose containing 5 × 10⁻⁴ M disodium ethylene dinitrilotetraacetic acid and 0.02 per cent deoxycholate and homogenized at 4 C. using a motor-driven teflon pestle. One-tenth mL of leukocyte homogenate was incubated in 0.2 mL of 80 mM tris-histidine buffer, pH 7.2, containing 0.1 mL of 2 mM tris-adenosine triphosphate and 0.1 mL of the chloride salt of cofactors Mg; or Na, K, and Mg; or Na, K, Mg and ouabain 5 × 10⁻⁴ M. Previously determined optimal co-factor concentrations of 2 mM Mg, 80 mM Na and 15 mM K were used. The enzyme reaction mixture, at a final volume of 0.5 mL, was allowed to proceed at 37 C. for 45 minutes when the reaction was stopped with 11 per cent perchloric acid and release of inorganic phosphorus measured by the method of Fiske-Subbarow. Enzyme activity was expressed as total ATPase (cofactors Mg, Na, K), residual ATPase (co-factor Mg), or sodium-potassium ATPase (total ATPase minus residual ATPase). The protein content of leukocyte homogenate was measured by the biuret method.

RESULTS

Leukocyte Isolation and Morphology

The effect of 150 mM NH₄Cl on erythrocyte volume was observed with

Fig. 1.—(Left) The effect of 150 mM NH₄Cl on erythrocyte volume. (Center) The volume distribution of isolated PMNG’s and normal lymphocytes as compared to their respective mode and distribution in the bimodal pattern of normal blood leukocytes. (Right) The bimodal volume distribution of normal blood leukocytes is shown, as is the absence of volume change in 150 mM NH₄Cl. The swelling of lymphocytes (left-hand mode) and PMNG’s (right-hand mode) in hypotonic phosphate buffered saline (PBS) is demonstrated. Machine settings and orifice size were different for erythrocytes and leukocytes and hence relative volumes shown are not comparable from one panel to another.

the use of a 400 channel analyzer (Fig. 1). Erythrocyte swelling began immediately; however, the critical hemolytic volume was not reached for 3 to 4 minutes, and all cells did not lyse until 6 to 8 minutes. Reswelling took place over the succeeding minutes; and by 10 minutes, near maximal swelling occurred. Leukocytes placed in 150 mM NH₄Cl did not undergo any volume change over a 30 minute period of observation, whereas leukocytes swelled in hypotonic phosphate buffered saline. In saline concentrations less than 0.3 per cent cell lysis could be observed by volume measurement and by phase microscopy, unless tonicity was restored in 20 seconds (Fig. 1). Lysis was manifest microscopically by cell swelling, loss of nuclear chromatin pattern, loss of normal rate of granule oscillations and loss of varying amounts of cytoplasmic contents. Bizarre extensions and protrusions of the cytoplasmic membrane and fragments of cytoplasm containing granules and surrounded by membrane were seen. Apparent resealed ruptures in cytoplasmic membranes were noted. Little difference was noted in the propensity of normal and leukemic lymphocytes and PMNG’s to swell in response to hypotonicity.

All leukocyte preparations were examined by phase contrast microscopy and had less than 5 per cent and usually less than 2 per cent erythrocytes. Platelet contamination was trivial. At 37 C. isolated PMNG’s could be shown to have active motility, and avid phagocytosis. Leukocytes, resuspended in autologous serum, were stained with Wright’s stain and the extent of their morphologic preservation is shown in Figure 2. When examined at 2 C.
the cells appeared contracted and morphological distinctions were difficult whether by phase microscopy or on examination of stained smears. At 25 to 37°C, the PMNG's were spread and formed firm adhesions to the slide and morphologic details became evident (Fig. 3). Morphologic details of immature granulocytes and lymphocytes also were inapparent at 2°C but became evident on warming. This occurred rapidly and suggested a change in the physio-chemical state of the cell structure in relation to temperature. The decreased adhesiveness of PMNG's as ambient temperature is lowered toward 0°C is an interesting parallel to the apparently contracted appearance of the cell at low temperature. It has been suggested that the contracted appearance and reduced adhesiveness of cells at cold temperatures are the consequence of changes in surface properties which are related to an inhibition of relaxation of surface contractile proteins.12

Fig. 2.—The morphologic integrity of isolated A) normal PMNG's B) normal lymphocytes C) leukemic myeloblasts D) CGL granulocytes. Original magnification 1250 X.
Effect of Isolation Procedure

The potassium: sodium ratio of leukocytes was found to be between 0.70 and 1.70 after isolation. As shown in Figure 4, when leukocytes, suspended in HBSS, were incubated at 37 C., a significant and invariable influx of
potassium and efflux of sodium occurred leading to a potassium:sodium ratio of over 3.0. The restoration of higher intracellular potassium and lower sodium was accomplished against a concentration gradient and was prevented at 4 C. and at 37 C. in the presence of ouabain. Total leukocyte cation content did not undergo significant change during the 2 hours incubation, and leukocyte volume was unchanged or slightly reduced (< 10 per cent change) at 1 and 2 hours in each of the three incubation systems. Influx of potassium and efflux of sodium was also inhibited when extracellular potassium was reduced to 1.5 mM and the medium maintained isosmotic with sodium chloride. Marked alterations in pH were also shown to influence cation movement with an optimum for sodium and potassium movement in the range of 7.3 to 7.5. Although the observations in Figure 3 were made on mixed PMNG (85 per cent)-lymphocyte (15 per cent) preparations, observations using normal and leukemic granulocytes and lymphocytes were similar. Therefore, leukocyte cation was measured in individual leukocyte types after the cells had been incubated in HBSS for 90 to 100 minutes.

Monovalent Cation, Water, Weight and Volume

Small lymphocyte. As seen in Table 1, the monovalent cation concentration per Kg. cell water and the cation content per cell was similar in leukemic and normal small lymphocytes, and in both cases the potassium:sodium ratio was approximately 3.5 after 90 minutes of incubation in vitro. The proportion of total cell weight contributed by water and the cell dry weight were also similar in leukemic and normal lymphocytes (Table 2). Cell volume was not significantly different when the two cell types were compared (Table 3).

Granulocytes. Potassium concentration was similar in granulocytes from

| Table 1.—Intracellular Monovalent Cation Concentration and Content of Human Lymphocytes |
|---------------------------------|-----------------|------------------|
|                                  | Normal Lymphocytes (N = 9) | CLL Lymphocytes (N = 14) |
| Potassium Millimoles/Kg. H₂O   | 120 ± 2.6        | 124 ± 3.2        |
| Moles/cell × 10⁻¹⁵                 | 28 ± 4.3         | 29 ± 1.1         |
| Sodium Millimoles/Kg. H₂O        | 34 ± 3.3         | 33 ± 1.6         |
| Moles/cell × 10⁻¹⁵                 | 7.9 ± 3.2        | 7.6 ± 1.1        |

Values are expressed as mean and standard error.

| Table 2.—Water Content and Dry Weight of Human Lymphocytes |
|---------------------------------|-----------------|------------------|
|                                  | Normal Lymphocytes (N = 9) | CLL Lymphocytes (N = 14) |
| Per cent of weight as water     | 79 ± 1.2        | 79 ± 0.26        |
| Dry weight Gm./cell × 10⁻¹²     | 61 ± 2.9        | 63 ± 2.4         |

Values are expressed as mean and standard error.
Table 3.—Relative Cell Volume of Human Leukocytes

<table>
<thead>
<tr>
<th></th>
<th>PMNG (N = 7)</th>
<th>Normal Lymphocyte (N = 9)</th>
<th>CLL Lymphocyte (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel number</td>
<td>72 ± 4.1</td>
<td>40 ± 2.5</td>
<td>45 ± 3.1</td>
</tr>
<tr>
<td>Volume ratio to normal lymphocyte</td>
<td>1.8</td>
<td>1.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard error.

Table 4.—Intracellular Monovalent Cation Concentration and Content of Human Granulocytes

<table>
<thead>
<tr>
<th></th>
<th>Leukemic Myeloblasts (N = 6)</th>
<th>CGL Granulocytes (N = 9)</th>
<th>Normal Bone Marrow Granulocytes (N = 4)</th>
<th>Normal PMNG (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>Millimoles/KgH₂O</td>
<td>Moles/cell × 10⁻¹⁵</td>
<td>Millimoles/KgH₂O</td>
<td>Moles/cell × 10⁻¹⁵</td>
</tr>
<tr>
<td></td>
<td>117 ± 4.2</td>
<td>49 ± 1.7</td>
<td>114 ± 2.8</td>
<td>51 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>40 ± 3.8</td>
<td>16 ± 1.7</td>
<td>35 ± 1.3</td>
<td>16 ± 0.8</td>
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</table>

Values are expressed as mean and standard error.

leukemic and normal subjects (Table 4). The sodium concentration was 25 per cent lower in normal PMNG than in leukemic myeloblasts. The sodium concentration of bone marrow granulocytes and CGL granulocytes was similar and intermediate between myeloblasts and PMNG’s. The expected sodium concentration in a sample composed of 70 per cent PMNG’s and 30 per cent immature granulocytes would be 33 mM/Kg. cell water, a value similar to that observed in CGL and marrow granulocytes.

Blast cells had a higher proportion of cell weight as water than did PMNG’s (Table 5). CGL and normal bone marrow granulocytes were similar in their water content.

Detailed analyses of the volume distribution of leukemic granulocytes were not carried out. The distribution of samples from the blood of subjects with CGL and from the bone marrow of normal subjects were unimodal and their modes were similar to normal PMNG’s; however, a greater tendency to positive skewing of the volume distribution was present in the former two which was felt to be due to the presence of promyelocytes and closely related large cells noted on phase contrast microscopic examination of cell suspensions.

Table 5.—Water Content and Dry Weight of Human Granulocytes

<table>
<thead>
<tr>
<th></th>
<th>Leukemic Myeloblasts (N = 6)</th>
<th>CGL Granulocytes (N = 9)</th>
<th>Normal Bone Marrow Granulocytes (N = 4)</th>
<th>Normal PMNG (N = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent of weight</td>
<td>81 ± .58</td>
<td>78 ± .70</td>
<td>77 ± 2.8</td>
<td>76 ± .73</td>
</tr>
<tr>
<td>as water</td>
<td>(N = 6)</td>
<td>(N = 9)</td>
<td>(N = 4)</td>
<td>(N = 11)</td>
</tr>
<tr>
<td>Dry weight</td>
<td>100 ± 3.8</td>
<td>130 ± 3.9</td>
<td>140 ± 4.3</td>
<td>140 ± 3.5</td>
</tr>
<tr>
<td>Gm./cell × 10⁻¹²</td>
<td>(N = 6)</td>
<td>(N = 11)</td>
<td>(N = 4)</td>
<td>(N = 14)</td>
</tr>
</tbody>
</table>

Values expressed as mean and standard error.
Acute granulocytic leukemic cells also showed a unimodal distribution; and, although the mode of the distribution was similar to or slightly less than that of PMNG's, the distribution was often very broad indicating an increased frequency of smaller and larger cells. In occasional cases of AGL cytologic examination of stained smears suggested two populations of cells because of the prominence of small and large blast cells. However, the volume distributions were unimodal, and hence the presence of different populations of blast cells suggested by cytologic observations could not be confirmed by observing modality of the frequency distribution curve. Nevertheless, it is possible that the broad distribution curves could have been composed of at least two populations of almost equal size with mean values not different enough to produce bimodality. Studies have suggested different populations of blast cells based on patterns of nucleic acid synthesis and mitotic capacity and evidence has been presented that cell volume and synthetic capacity of myeloblasts may be correlated.

The relative cell volume of the PMNG is shown in Table 3. It can be seen that the mature granulocyte is approximately 1.8 times the volume of the small lymphocyte. The estimate of the absolute cell volume of the PMNG is shown in Figure 5. The ratio of cell volume of the PMNG to the small lymphocyte and erythrocyte is compared to the ratio of total monovalent cation per cell and it can be seen that the differences in monovalent cation (and accompanying anion) content closely parallels differences in cell volume.

**Adenosine triphosphatase (ATPase)** Studies of leukocyte ATPase indicated co-factor optima for magnesium between 2 and 5 mM, for sodium between 80 and 100 mM, and for potassium between 3 and 15 mM. Half-maximal sodium-potassium ATPase activity was observed with 1 mM KCl in the presence of 80 mM NaCl. The sodium-potassium activated ATPase activity was shown to increase linearly over 45 minutes of incubation. The release of inorganic phosphate was linear and proportional over a range of .25 to 1.0

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**CELL VOLUME AND TOTAL MONOVALENT CATION CONTENT**

<table>
<thead>
<tr>
<th>Volume (μm²)</th>
<th>Total Monovalent Cation (moles/Cell x 10⁻¹⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERYTHROCYTE</td>
<td>90</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>269</td>
</tr>
<tr>
<td>PMNG</td>
<td>484*</td>
</tr>
</tbody>
</table>

*ESTIMATED FROM PMNG TO LYMPHOCYTE VOLUME RATIO OF 1.8:1 AS MEASURED ON 400 CHANNEL ANALYZER.*

**Fig. 5.—**Comparative cation content and volume of erythrocytes, small lymphocytes and PMNG's.
Table 6.—Sodium-Potassium Adenosine Triphosphatase Activity of Human Lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>Normal Lymphocytes (N = 5)</th>
<th>CLL Lymphocytes (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium-potassium ATPase</td>
<td>27 ± 3.6</td>
<td>32 ± 2.5</td>
</tr>
</tbody>
</table>

Values expressed as mean and standard error.
Moles P$_i$ \( \times 10^{-8} \) released/45 min./mg. protein.

mg./ml. of leukocyte protein per incubation tube. The sodium-potassium activation was inhibited by \( 5 \times 10^{-4} \) M ouabain.

No difference in sodium-potassium activated ATPase was present when normal or leukemic small lymphocytes were compared (Table 6). Myeloblasts had higher sodium-potassium ATPase than PMNG; however, the activity of these enzymes per mg. protein in the myeloblast was similar to that of the lymphocyte (Table 7). CGL and normal bone marrow granulocytes had similar activities and, in the case of sodium-potassium ATPase, were intermediate between myeloblasts and PMNG (Table 7). The protein content per leukocyte was not measured. Expression of the activity of leukocyte ATPase per mg. protein does not allow comparison of this enzyme activity per cell between different leukocyte types. The volume and dry weight of the small lymphocyte is approximately 60 per cent that of the myeloblast. Protein content is presumably proportionately reduced since leukocyte nitrogen has been shown to be proportional to cell weight. Hence the sodium-potassium ATPase activity per lymphocyte is approximately three times that per PMNG and half that of the myeloblast. If the sodium-potassium activated ATPase is expressed per milligram of cellular protein the lymphocyte has six times the activity of the PMNG and approximately the same activity as the myeloblast. The ATPase measured represented the total cellular activity and not that of the cell plasma membrane. In addition even if activity were limited to the plasma membrane, the leukocyte surface is highly invaginated and hence a close estimate of cell surface area cannot be made. For these reasons, a comparison of cell membrane ATPase per mg. membrane protein or per unit surface area cannot be made from our data. Nevertheless, the similarity between small lymphocytes and myeloblasts suggests that leukocytes with potential for mitosis and maturation have as one of their characteristics, a high ATPase activity.

Table 7.—Sodium-Potassium Adenosine Triphosphatase Activity of Human Granulocytes

<table>
<thead>
<tr>
<th></th>
<th>Leukemic Myeloblasts (N = 6)</th>
<th>CCL Granulocytes (N = 4)</th>
<th>Normal Bone Marrow Granulocytes (N = 1)</th>
<th>Normal PMNG (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium-potassium ATPase</td>
<td>33 ± 9.0</td>
<td>11 ± 2.1</td>
<td>13</td>
<td>5.4 ± 1.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard error.
Moles P$_i$ \( \times 10^{-8} \) released/45 min./mg. protein.
**DISCUSSION**

The fact that little consistent information has accrued regarding regulation of monovalent cations in human leukocytes is related, in part, to the difficulties involved in obtaining human leukocytes, free of erythrocytes, in quantities sufficient for chemical studies. In addition, it is currently impossible to isolate leukocytes without physical or chemical trauma, and this has been shown to affect the cation concentration of animal\textsuperscript{8} and human leukocytes.\textsuperscript{7} In our studies a low or reversed potassium-sodium ratio was present in isolated leukocytes. This reversal was present in normal as well as leukemic cells and was corrected to a high potassium, low sodium steady-state in every case by incubation of the leukocytes in a physiologic medium. The extent to which the alteration in cations was related to the use of ammonium chloride as an erythrolytic agent is uncertain, although it is clear that hypotonic saline lysis produces similar changes.\textsuperscript{7}

Baron and Roberts\textsuperscript{9} examined the monovalent cation concentration of normal mixed human leukocytes at the time of isolation and observed a potassium:sodium ratio of 1.7. They studied the possible effect of sedimentation and distilled water lysis of erythrocytes and concluded their results were not affected by the separatory methods used. Block and Bonting\textsuperscript{7} using methods similar to Baron and Roberts\textsuperscript{1} to separate leukocytes observed that the potassium:sodium ratio of isolated leukocytes increased after 60 minutes of incubation in vitro and then fell thereafter. We observed that potassium:sodium ratio continued to increase until a steady state potassium:sodium ratio of 3 or greater was reached in 90 to 100 minutes. It seems most likely, therefore, that the cation content at the conclusion of the leukocyte isolation procedure did not represent the steady-state in vivo pattern. While it is not certain that the concentrations obtained after incubation in vitro reflect in vivo values the restoration of a steady-state makes the post-incubation values a more likely reflection of in vivo concentrations. It is evident that studies of leukocyte cation concentrations in human subjects must take into consideration alterations of intracellular cations induced by cell isolation.

Previous studies which have examined the cation content of leukemic granulocytes and lymphocytes have not examined normal cells. Block and Bonting\textsuperscript{7} found sodium and potassium per kilogram dry weight of cells to be significantly greater in AGL myeloblasts as compared to CGL granulocytes; however, cell water and weight were not measured; and, therefore the differences could have been related to differences in cell dry weight and not to differences in cation concentration. Our observations indicate that the number of AGL myeloblasts per kilogram dry weight is 45 per cent greater than CGL granulocytes (Table 5). If Block and Bonting’s data are expressed as cation content per cell, the two cell types have identical sodium and potassium content. This in itself is not conclusive since the cation content per volume of cell and more specifically per volume of distribution of monovalent cation per cell would have to be known to determine with certainty whether a real difference in cation concentration existed. Our observations indicate that CGL granulocytes and AGL myeloblasts are similar in monovalent cation content per cell or in monovalent cation concentration per kilogram cell water.
In a study of the electrolyte, nitrogen and water content of human leukemic leukocytes Rigas suggested that immature lymphoid cells (leukemic "lymphoblasts") had higher cell sodium than mature lymphoid cells (small lymphocyte of CLL). This was due to the high cell sodium and low cell potassium in the cells from patients with acute lymphatic leukemia (potassium:sodium ratio < 1.0). An inversion of the potassium:sodium ratio during the isolation of acute leukemic cells could have produced such an effect. In 2 cases of acute lymphatic leukemia, we observed a potassium:sodium ratio less than one at the time of isolation which was significantly increased by incubation in vitro. Furthermore, a difference between acute lymphatic leukemic blast cells and CLL lymphocytes can no longer be accepted a priori as the result of maturation since there is no certainty that the small lymphocyte is the result of the maturation of a lymphoblast. Indeed, current schemes of lymphopoiesis make it difficult to correlate size or morphology of the lymphocyte with chronologic age or degree of maturity. In such situations only cohort studies will be able to demonstrate relationship of maturation to cellular characteristics.

Under the condition of our experiments, leukocyte cation content was similar when leukemic and normal cells of similar morphologic type were compared. The higher cell sodium observed in immature granulocytes and lymphocytes when compared to PMNG's may be a characteristic of younger cells or may be related to the greater tendency of PMNG's to undergo cytologic changes over short periods in vitro. We would have anticipated however, an increased cell sodium and decreased cell potassium as a result of cell damage. A difference in the quantity of other intracellular molecules contributing to the osmotic contents, or differences in monovalent cation binding in PMNG's could explain the variation in total monovalent cation content as compared to other leukocyte types. Similar variations in total intracellular monovalent cations in Ehrlich ascites carcinoma cells have been described recently.

Leukocyte sodium-potassium ATPase activity was identifiable in all leukocyte types. The proportion of leukocyte ATPase that was sodium-potassium activated was similar to that found in human leukocyte homogenates by Block and Bonting, and less than that observed by Yunis and coworkers. It is of interest that Larinefelt has observed that small amounts of sodium and potassium retained in cell preparations may produce factitiously reduced monovalent cation adenosine triphosphatase in vitro. Also, evidence has been obtained which indicates that a variety of nucleated mammalian cells contain material capable of inhibiting monovalent cation activated ATPase and that these inhibitors reside in the histone fraction of the cell nucleus. ATPase activity may be facilitated by countering such inhibitors with low concentrations of desoxycholate. Therefore the ATPase activity of cell homogenates may reflect varying interactions between enzyme and natural inhibitors as well as exogenous factors introduced during preparation. The latter are known to influence the proportion of cation activated ATPase activity of cells prepared in vitro.

Normal leukemic leukocytes of similar morphologic type had similar ATPase activity, although normal blast cells and leukemic blast cells could not be compared. The ATPase activity was similar in CGL and normal bone marrow granulocytes and in normal and leukemic small lymphocytes. The sodium-potassium
activated ATPase of CGL and bone marrow granulocytes was intermediate between PMNG's and myeloblasts. The difference in the ATPase activity observed between leukemic myeloblasts and PMNG is more likely a reflection of differences in stage of maturity rather than a "leukemic lesion" as previously suggested.7

The precise roles of the monovalent cation activated ATPase in the function or maintenance of the leukocyte have been explored, in part. The monovalent cation activated ATPase in PMNG may be an important site of action of endotoxin in the latter's induction of the release of endogenous pyrogen. Berlin and Wood24 have observed that release of endogenous pyrogen from rabbit leukocytes occurred when the cells were incubated in a potassium-free isotonic sodium chloride medium. The presence of potassium or closely related alkali metal ions in the medium prevented release of pyrogen. In the presence of potassium, ouabain was capable of inducing pyrogen release. These findings suggested that the intracellular transport of potassium and a process dependent on such transport inhibited pyrogen release. More recently Tenney and Rafter25 have observed that endotoxin inhibits the monovalent cation activated ATPase of leukocytes from rabbit and human blood and that the extent of the inhibition is similar to that produced by ouabain. Hence, the release of leukocyte pyrogen may be related to a ouabain-like inhibition of sodium-potassium activated ATPase by endotoxin. The possible relationship of altered cell volume and cell integrity to the inhibition of ATPase by endotoxin has not been elucidated. The monovalent cation activation and ouabain inhibition of leukocytes by both ouabain and reduced extracellular potassium indicates a similar relationship of the monovalent cation activated ATPase to monovalent cation movement in human normal and leukemic leukocytes as in erythrocytes26 and other cells studied.27 Cell swelling after prolonged inhibition of monovalent cation active transport by endotoxin might be an integral factor in the fragmentation of PMNG and the release of pyrogen.

It is now appreciated that an extracellular to intracellular sodium concentration gradient is required for the optimal movement of certain substrates into cells. Yunis and co-workers21 have indicated that optimal amino acid transport in CLL lymphocytes and CCL granulocytes is related to a sodium-potassium activated, cardiac glycoside inhibited, ATPase and that lymphocytes show a greater rate of amino acid uptake than granulocytes. This observation correlates with the increased activity of ATPase in lymphocytes as compared to CCL granulocytes in our studies and those of Block and Bonting.7 The need of lymphocytes, like the myeloblast, for more rapid availability of substrates and co-factors to support the increased rate of synthesis of structural and functional macromolecules during cell division, cell maturation and cell function may require the more abundant ATPase activity. The increased sodium-potassium ATPase activity of immature granulocytes and lymphocytes may also reflect an increased pump activity required to balance the increased rate of cation leak across membranes of immature leukocytes. These characteristics may be necessary for the rapid and complex permeability changes during mitosis.4

The inhibition of incorporation of radioactive precursors into nucleic acid by human lymphocytes incubated in the presence of ouabain,8 and the inhibition
of cell division by ouabain\(^3,5\) may, as mentioned above, be related to the role of the sodium-potassium ATPase in maintaining intracellular substrate pools. However, interference with transmembrane ion movements by ouabain with subsequent alterations in control of cell volume or intracellular synthetic processes with monovalent cation requirements must also be considered among the possible explanations for an inhibition of cell division after inhibition of sodium-potassium activated ATPase.

Although these data suggest a potentially important correlation between sodium-potassium activated ATPase and granulocyte maturation, they also indicate that impaired mitotic capacity of human blast cells in established leukemic states is not likely to be due to a deficiency of intrinsic sodium-potassium ATPase activity or a resultant alteration in intracellular monovalent cation content.

**Summary**

Differences in volume, water content and weight of leukocytes of different morphologic type are described. These differences make it mandatory that the influence of those factors which contribute to the denominator in comparative studies of leukocyte characteristics be considered if different cell types are being compared. The influence of cell isolation on monovalent cation content is emphasized. Restoration of a high potassium, low sodium steady-state occurred at 37 °C. after incubation in physiologic solutions. Inhibition of this cation restoration by ouabain, or deviations from optimal extracellular pH, optimal ambient temperature and optimal extracellular potassium concentration coupled with the presence of ouabain inhibitable monovalent cation activated adenosine triphosphate hydrolysis in vitro support the presence of active cation transport in all types of human leukocytes studied. It is likely that discrepant values for leukocyte cation content and absence of monovalent cation activated ATPase previously reported are related to technical factors.

Leukemic and normal granulocytes and lymphocytes of similar morphologic type do not differ in their monovalent cation content or in sodium-potassium ATPase activity. Cell volume of leukemic and normal small lymphocytes and PMNG’s parallels their cation content. Immature granulocytes and small lymphocytes have higher sodium-potassium ATPase activities than mature granulocytes. Sodium concentration and water content were also slightly higher in immature granulocytes and lymphocytes as compared to PMNG’s. The data are consistent with the hypothesis that increased sodium-potassium ATPase activity is a biochemical feature of leukocytes with the potential to divide and differentiate. The impaired mitotic capacity of human leukemic blast cells does not appear to be related to a deficiency in sodium-potassium ATPase activity or a resultant alteration in intracellular monovalent cation content.

**SUMMARIO IN INTERLINGUA**

Es describite differentias de volumine, del contento de aqua, e del peso del leucocytos de varie typos morphologic. Iste differentias require que le influentia del factores contribuiente al denominator in studios comparative de caracteristicas de leucocytos es considerate quando differente typos cellular es comparete. Le influentia del isolation cellular super le contento de cationes monovalente es sublineate. Restauration del stato stabile a
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alte valores de kalium e basse valores de natrium occurreva a 37 C post incubation in solutiones physiologic. Inhibition de iste restauration catonic per ouabaina o deviationes ab le optime pH extracellular, optime temperaturas ambiental, e optime concentrationes extracellular de kalium associate con le presentia in vitro del hydrolyse de triphosphato de adenosina activate per cationes monovalente de typo inhibibile per ouabaina supporta le these del presentia de un active transporto de cationes in omne le typos de leucocytus human studiate. Il es probable que valores divergente pro le contento leucocytic de cationes e le absentia de ATPase activate per cationes monovalente previemente reportate es relacionate a factores technic.

Granulocytos e lymphocytos leucemic e normal de simile typos morphologic non differe in lor contento de cationes monovalente o in lor activitate de ATPase de natrium-kalium. Le volume cellular de micre lymphocytos leucemic e normal e le PMNGes es parallel al contento cellular de cationes. Granulocytos immatur e micre lymphocytos ha plus alte activitates de ATPase de natrium-kalium que granulocytos matur. Le concentration de natrium e le contento de aqua esseva etiam levemente plus alte in granulocytos e lymphocytos immatur in comparation con PMNGes. Le datos es compatible con le hypothese que un augmento del activitate de ATPase de natrium-kalium es un caracteristica biochimic de leucocytos con le potentialitate de divider se e differentiar se. Le defective capacitate mitotic de leucemic blastocytos human non pare esser relationate a un carentia in activitate de ATPase de natrium-kalium o un resultante alteration in le contento intracellular de cationes monovalente.

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The Monovalent Cation Content and Adenosine Triphosphatase Activity of Human Normal and Leukemic Granulocytes and Lymphocytes: Relationship to Cell Volume and Morphologic Age

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